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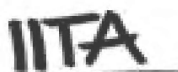
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# Challenges and Opportunities for Enhancing Sustainable Cowpea Production

Edited by

C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò



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## About IITA

The International Institute of Tropical Agriculture (IITA) was founded in 1967 as an international agricultural research institute with a mandate for improving food production in the humid tropics and to develop sustainable production systems. It became the first African link in the worldwide network of agricultural research centers known as the Consultative Group on International Agricultural Research (CGIAR), formed in 1971.

IITA's mission is to enhance the food security, income, and well-being of resource-poor people primarily in the humid and subhumid zones of sub-Saharan Africa, by conducting research and related activities to increase agricultural production, improve food systems, and sustainably manage natural resources, in partnership with national and international stakeholders. To this end, IITA conducts research, germplasm conservation, training, and information exchange activities in partnership with regional bodies and national programs including universities, NGOs, and the private sector. The research agenda addresses crop improvement, plant health, and resource and crop management within a food systems framework and targetted at the identified needs of three major agroecological zones: the savannas, the humid forests, and the midaltitudes. Research focuses on smallholder cropping and postharvest systems and on the following food crops: cassava, cowpea, maize, plantain and banana, soybean, and yam.

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## Foreword

Cowpea is an important food legume and an essential component of cropping systems in the drier regions and marginal areas of the tropics and subtropics covering parts of Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America. It is particularly important in West Africa with over 9.3 million hectares and 2.9 million tonnes annual production. With about 25% protein in its grains, cowpea is an important source of quality nourishment to the urban and rural poor who cannot afford meat and milk products. Cowpea haulms contain over 15% protein and constitute a valuable source of fodder. The International Institute of Tropical Agriculture (IITA) has the global mandate for cowpea improvement. In collaboration with the regional and national research programs, IITA has developed a range of improved cowpea breeding lines combining multiple disease and insect resistance with early maturity and preferred seed types, and has distributed these to over 65 countries.

From 1970 to 1988, the research concentrated on developing cowpea varieties for sole crop only. However, from 1989, cowpea breeding has been diversified to include systematic improvement of local varieties as well as development of a range of improved dual-purpose cowpea varieties which can produce higher grain as well as fodder yields under sole cropping and in traditional intercropping systems. In 1996, IITA decided to further broaden the objectives by including improvement of cowpea-cereals systems and involving crop-livestock integration rather than variety improvement alone. The specific objectives are to develop improved cowpea varieties and improved cropping systems with integrated pest management appropriate for adoption in the Sudan savanna and the Sahel.

An integral part of IITA's cowpea research is an active technology and information exchange system among cowpea researchers worldwide through cowpea international trials, special workshops, individual and group training, and periodic world cowpea conferences. IITA organized the first World Cowpea Research Conference in 1984 and the second in 1995. The selected papers presented in these conferences were collated and published in two books: Cowpea research, production, and utilization (1985) and Advances in cowpea research (1997). Both books have become very popular among cowpea researchers the world over.

In view of the rapid developments in cowpea research, the delegates of the second World Cowpea Conference felt that a gap of 10 years was too long and recommended that in future, the World Cowpea Conferences should be held every 5 years. IITA agreed with the recommendation and organized the World Cowpea Conference III in September 2000. This book contains selected papers presented in the conference. It is hoped that this will be a useful supplement to the earlier two books and help to enhance collaborative work among cowpea researchers, leading to innovative approaches and improved technologies in the years to come.

**L. Brader**

*Director General*

*International Institute of Tropical Agriculture*

## Preface

Cowpea researchers from different parts of the world came together to participate in the World Cowpea Research Conference III which took place from 4 to 8 September 2000 at the headquarters of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Two previous world cowpea research conferences had been held: the first in 1984 at Ibadan, Nigeria and the second in 1995 at Accra, Ghana. The interval between the first and second world cowpea research conferences was eleven years while that between the second and the third, was only five years. The shorter interval between the second and third conferences attests to an increase in the number of researchers focusing on cowpea. These conferences provide opportunities for cowpea researchers to interact and exchange scientific information resulting from their research activities. At the same time plans for the future are made.

The conference featured both oral presentations and posters displays. Most of the oral paper presentations are included in this proceedings volume which is divided into five sections: (a) cowpea genetics and breeding, (b) cowpea integrated pest management, (c) biotechnology for cowpea, (d) cowpea contributions to farming systems, and (e) cowpea postharvest and socioeconomic studies.

The reports presented indicate that appreciable progress has been made in cowpea research during the past five years and cowpea research has impacted positively on the productivity of the crop especially in sub-Saharan Africa. At the Accra meeting of 1995, a limited number of reports were presented in the area of socioeconomic studies in cowpea. However, at this conference, a section was devoted to postharvest and socioeconomic studies and a number of papers were presented. There were presentations on the economics of cowpea in West Africa and cowpea supply prospects in Nigeria. The adoption of improved technologies that will enhance the productivity of cowpea by farmers in Ghana as well as the industrial potential of the crop in the subregion were also discussed.

In addition, a session was specifically devoted to cowpea biotechnology during which reports were presented on the isolation and sequencing of resistance gene analogs from cowpea and the placement of these in the cowpea linkage map. Seed lectins obtained from some leguminous plants, particularly the African yam bean (*Sphenostylis stenocarpa*), were reported to have adverse effects on the important postflowering insect pests of cowpea such as the pod sucking bugs and the cowpea bruchid (*Callosobruchus maculatus*). The detection of the adverse effects of these lectins on some cowpea pests is an indication that there are potential candidate genes that can be used for the transformation of cowpea for resistance to these pests which are capable of causing extensive grain yield loss in the crop. When they become available, transgenic cowpea varieties with resistance to postflowering insect pests will boost the productivity of the crop in African farmers' fields.

The contributions of cowpea to the farming systems in the dry savanna regions of sub-Saharan Africa were also highlighted. Apart from contributing to soil fertility through nitrogen fixation and production of organic matter, cowpea fodder provides quality feed for livestock in the subregion. Ruminants fed cowpea fodder as supplement are known to gain weight appreciably.

It is hoped that cowpea researchers will find the contents of this book useful and stimulating.

**C.A. Fatokun**

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## **Section I**

# **Cowpea genetics and breeding**





# 1.1

## Recent genetic studies in cowpea

B.B. Singh<sup>1</sup>

### Abstract

A number of recent studies have added further information on the genetics of important traits in cowpea. These include inheritance of qualitative traits such as plant pigmentation; flower color; seed color; seed coat texture; resistance to rust, scab, smut, nematode, severe mosaic virus, *Striga*, *Alectra*, aphid, bruchid, heat; drought tolerance; and male sterility, and quantitative traits such as protein content, seed size, seed yield, and fodder quality. A few studies on linkage and mapping have also been conducted. The gene symbols from recent studies and earlier reports have been collated in a classified and trait-based gene index for easy reference. While reviewing the past genetic work, obvious gaps needing further studies have been indicated.

### Introduction

The first comprehensive review of cowpea genetics was published in 1980 (Fery 1980) and subsequent supplements were published in 1985 (Fery 1985) and in 1997 (Fery and Singh 1997). This paper complements the earlier literature by reviewing some recent work on cowpea genetics and pointing out some gaps needing further research.

### Species relationship

Cowpea is a variable species composed of wild perennials, wild annuals, and cultivated forms. Genetic variation in 199 germplasm lines of nine subspecies and two botanical varieties of wild and cultivated cowpea were evaluated by Pasquet (1999) using allozyme analysis to characterize the genepool. The allozyme data confirmed that perennial out-crossers are primitive and more remote from each other and from perennial out-inbreds. Within the large genepool, mainly made of perennial taxa, the cultivated cowpea form a genetically coherent group and are closely related to annual wild cowpea which may include the likely progenitor of cultivated cowpea.

Cardinali et al. (1995) analyzed 32 accessions of cultivated and wild cowpea for phenolic content using HPLC to better characterize wild species of *Vigna*. The cultivated cowpea always contained three flavonoid aglycones: quercetin, kaempferol, and isorhamnetin. These were lacking in the wild relatives. They also observed that resistance to aphid in cultivated cowpea was related to high flavonoid levels. In a similar study, Sonnante et al. (1996) examined the isozyme variation in 25 accessions of wild and cultivated *Vigna unguiculata*, 49 accessions of seven wild species belonging to section *Vigna*, and 11 accessions of *Vigna vexillata* to assess genetic relationship within and among species. They observed that *Vigna unguiculata* was closer genetically to *Vigna vexillata* than to the species belonging to section *Vigna*. Venora and Padulosi (1997) carried out a karyotypic analysis of mitotic chromosomes of 11 wild taxa of *Vigna unguiculata* and

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found a low degree of karyological variability. The results indicate that despite high morphological variability in cowpea, such diversity is not evident at the chromosomal level. Gomathinayagam et al. (1998) reported a successful cross between *Vigna vexillata* and *Vigna unguiculata* using embryo culture. They obtained 13 hybrid plants which showed intermediate morphological traits between the parents for leaf shape, pod color, and seed coat color. However, the stem and leaf types and pod hairiness of hybrid plants were like those of *Vigna vexillata*, the maternal parent. Electrophoresis studies of the hybrid plants for peroxidase and esterase and cytological studies confirmed that they were true hybrids. However, the same cross has not been successful at IITA (see Fatokun in this volume). Another report of an attempted wide cross between cowpea (*Vigna unguiculata*) and bambara groundnut (*Vigna subterranea*) was published by Begemann et al. (1997). The cowpea line TVu 13677 was crossed as a female parent with bambara groundnut variety TVsu-501. The crossed flowers produced an abnormally short pod (1 cm) with only one seed. The  $F_1$  seed gave rise to a plant which had a longer growth period (80 days) compared to the female parent TVu 11677 (60 days). Tyagi and Chawala (1999) reported a successful cross between *Vigna radiata* and *Vigna unguiculata* using in vitro culture method.

The above-mentioned studies may indicate that wide crosses are possible between *Vigna unguiculata* and other *Vigna* species, however, none of the authors have followed up with the hybrid populations, indicating that further work needs to be done to verify these reports.

## Genetics of plant pigmentation

Because of the great diversity in pigmentation of cowpea stem, leaf, flower, peduncle, petiole, and pod, this trait has been studied by a large number of researchers from 1919 to date. However, since most of the studies involved pigmentation of one or the other plant parts at a time, there seems to be several gene symbols assigned for the same trait or similar traits. A summary of gene symbols assigned for pigmentation of different plant parts by previous workers is presented in Table 1, which clearly indicates the overlap and confusion. For example, seven gene symbols (*Pp-1*, *Pp-2*, *pg*, *Pb*, *Pbr*, *Pu*, and *X*) have been assigned to plant pigmentation covering the plant, petiole base, branch base, stem-pod-petiole, and all the vegetative parts, which obviously have overlaps. Similar overlaps and confusion about the gene symbols are evident for flower color, calyx color, and pod color (Table 1). None of the reports have endeavored to study plant pigmentation on a holistic basis, explaining the relationship between pigmentation in different plant parts. Even the recent studies reported here have not clarified the situation (Table 2). Joshi et al. (1994) reported that  $P_1$  is a pleiotropic gene for pigmentation in axil, calyx, corolla, pod tip, and seed with localized genes conditioning coloration on individual parts. Uguru (1995) showed that petal color is governed by one allelic pair WW, while pod and shoot colors appear to be determined pleiotropically by two allelic pairs, PrPr and GrGr. Calyx color was reported to be controlled by three duplicate genes and standard petal color is controlled by a single dominant gene. Biradar et al. (1997) reported three genes for calyx color, three genes for seed coat color, four genes for pod tip pigmentation, and four genes for flower color with some genes showing pleiotropic effects. Venugopal (1998) observed that 1–5 pairs of genes were involved in the inheritance of plant pigmentation in cowpea. Sangwan and Lodhi (1998) studied the inheritance of flower color and pod color.

Table 1. Gene index for plant pigmentation in cowpea.

| Trait                           | Gene symbol | References                     |
|---------------------------------|-------------|--------------------------------|
| <b>Plant pigmentation</b>       |             |                                |
| Purple plant                    | <i>Pp-1</i> | Venugopal and Goud 1997*       |
| Purple plant                    | <i>Pp-2</i> | Venugopal and Goud 1997*       |
| Pale green plant                | <i>pg</i>   | Saunders 1960a                 |
| Purple petiole base             | <i>Pb</i>   | Sen and Bhowal 1961            |
| Purple branch base              | <i>Pbr</i>  | Sen and Bhowal 1961            |
| Purple stem, pod, petiole       | <i>Pu</i>   | Sen and Bhowal 1961            |
| Anthocyanin in vegetative parts | <i>X</i>    | Harland 1919b                  |
| <b>Flower color</b>             |             |                                |
| Purple flower                   | <i>Pf</i>   | Kolhe 1970                     |
| Pale flower                     | <i>L</i>    | Harland 1919a                  |
| Dark flower color               | <i>D</i>    | Harland 1919a                  |
| Tinged flower                   | <i>G</i>    | Harland 1920                   |
| Yellow strips on petals         | <i>Ystp</i> | Kolhe 1970                     |
| <b>Calyx color</b>              |             |                                |
| Brown calyx color vs green      | <i>Bcy</i>  | Kolhe 1970                     |
| Purple calyx color              | <i>P</i>    | Harland 1920                   |
| Purple calyx color              | <i>Pv</i>   | Sen and Bhowal 1961            |
| Purple calyx color              | <i>E</i>    | Harland 1920                   |
| <b>Pod color</b>                |             |                                |
| Black pod vs white              | <i>Bk</i>   | Capinpin 1935*                 |
| Brown pod vs straw              | <i>Bp</i>   | Saunders 1960b*                |
| Cocoa brown pod                 | <i>Cbr</i>  | Krishnaswamy et al. 1945       |
| Reddish (cerise)                | <i>Ce</i>   | Saunders 1960a*                |
| Green pod vs cream              | <i>Gp</i>   | Kolhe 1970                     |
| Green pod vs white              | <i>Gnp</i>  | Singh and Jindla 1971*         |
| Purple pod                      | <i>Pp</i>   | Mortensen and Brittingham 1952 |
| Dark pod color                  | <i>k</i>    | Mortensen and Brittingham 1952 |
| Light green pod                 | <i>lg</i>   | Krishnaswamy et al. 1945       |
| Purple pod                      | <i>P</i>    | Harland 1920                   |
| Purple pod, stem, petiole       | <i>Pu</i>   | Sen and Bhowal 1961            |
| Speckled pod                    | <i>Sk</i>   | Saunders 1960a*                |
| Straw yellow pod-1              | <i>Sy-1</i> | Krishnaswamy et al. 1945*      |
| Straw yellow pod-2              | <i>Sy-2</i> | Krishnaswamy et al. 1945*      |
| Red tip pod                     | <i>Pb</i>   | Mortensen and Brittingham 1952 |
| Purple pod with green sutures   | <i>Pg</i>   | Sen and Bhowal 1961            |
| Purple tip pod                  | <i>Pt</i>   | Sen and Bhowal 1961            |
| Purple sutures on green pod     | <i>Ps</i>   | Sen and Bhowal 1961            |

\*Symbols by Fery (1980).

They observed that purple flower color is dominant over white flower and black pod color is partially dominant over white pod color with monogenic inheritance for both traits.

The confusion about the genetics of plant pigmentation arises due to the fact that most of the published reports do not give specific details of the pigmentation pattern and pigmented parts. For example, purple flower color does not mean much because pigmentation in cowpea flowers may be restricted only to standard, wing, or keel petals or a combination of two or all the three parts. A close examination (by the author) of several cowpea varieties has revealed very interesting and contrasting combinations of

**Table 2. Index of new gene symbols.**

| Gene symbol | Character  | Reference                   |
|-------------|--|-----------------------------|
| <i>bcm</i>  | Resistance to black-eye cowpea mosaic virus                        | Arshad et al. (1998)        |
| <i>Bk-2</i> | Black pod color  | Aliboh et al. (1996)        |
| <i>fa</i>   | Fasciated plant  | Adu-Dapaah et al. (1999)    |
| <i>Dhp</i>  | Dehiscent pod  | Aliboh et al. (1996)        |
| <i>Gr</i>   | Green shoot color  | Uguru (1995)                |
| <i>P1</i>   | Pleiotropic gene for axil, calyx, corolla pod tip, and seed colors | Joshi et al. (1994)         |
| <i>pms</i>  | Partial male sterility   | Singh and Adu-Dapaah (1998) |
| <i>ps</i>   | Photosensitivity   | Ishiyaku and Singh (2001)   |
| <i>Ptc</i>  | Calyx pigmentation   | Biradar et al. (1997)       |
| <i>Pt</i>   | Calyx pigmentation   | Biradar et al. (1997)       |
| <i>Pc</i>   | Calyx pigmentation   | Biradar et al. (1997)       |
| <i>Rds1</i> | Resistance to drought  | Mai-Kodomai et al. (1999)   |
| <i>Rds2</i> | Resistance to drought  | Mai-Kodomai et al. (1999)   |
| <i>Rt1</i>  | Rough seed coat texture  | Singh and Ishiyaku (2000)   |
| <i>Rt2</i>  | Rough seed coat texture  | Singh and Ishiyaku (2000)   |
| <i>Vsm</i>  | V-shaped mark on leaves  | Aliboh et al. (1996)        |

plant pigmentation (Table 3). It has also been observed (by the author) that all the cowpea varieties with brown rough seed have no pigmentation on any plant part except for a faint purple tinge on the inner margins of the standard petal. Also all the cowpea varieties with white rough seed have purple pigmentation on the joints (bases of the branch, peduncle, petiole, and leaflets), which are always inherited as one gene. However, the pigmentation of whole stem, petiole, peduncle, and pod is independent of the pigmentation on the joints. It has also been observed that whenever the calyx is pigmented, the pod tips are also pigmented and this is independent of other pigmentation. Another interesting pigmentation pattern is present in the cowpea variety Kamboinse local. It has dark purple pigmentation on the stem, petiole, peduncle, joints, calyx, and pod, but the flowers are completely white except for a purple dash in the back of the standard. The cowpea varieties listed in Table 3 represent a good set of differentials for different pigmentation patterns and efforts are under way to use them in planned genetic studies to elucidate inheritance pattern and interaction, if any, of specific plant pigmentations.

## Genetics of disease resistance

Inheritance of, resistance to several cowpea diseases has been reported between 1995 and 2000. Vale et al. (1995) studied the inheritance of resistance to cowpea severe mosaic comovirus (CpSMV) using cowpea variety Macaibo as the resistant parent and Pitiuba as the susceptible parent. The  $F_1$  plants were uniformly susceptible and  $F_2$  segregated into a ratio of three susceptible to one resistant, indicating involvement of a single recessive gene pair for resistance. The authors have mentioned that Macaibo is immune to CpSMV. Arshad et al. (1998) studied the inheritance of resistance to blackeye cowpea mosaic (BICMV) in six cowpea varieties: IT86F-2089-5, IT86D-880, IT90K-76, IT86D-1010, IT86F-2065-5, and PB1CP3. The segregation pattern in  $F_2$ , and backcross populations

**Table 3. Pigmentation of different parts in selected cowpea varieties.**

| Genetic type    | Pigmentation in various plant parts |                |                |                |                |                |                |                |                |                | Seed color   |
|-----------------|-------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|--------------|
|                 | Stem                                | Jts            | Pet            | Ped            | Clx            | s              | FL<br>w        | Pd<br>k        | Pdt            |                |              |
| TVx 3236-OC-1   | —                                   | —              | —              | —              | —              | —              | —              | —              | —              | —              | —            |
| IT87D-941-1     | —                                   | —              | —              | —              | +              | —              | —              | —              | —              | —              | Brown rough  |
| TVx 3236-OC-2   | —                                   | —              | —              | —              | —              | —              | +              | —              | —              | —              | Brown rough  |
| IT98K-628-2     | +                                   | —              | —              | +              | —              | —              | —              | +              | +              | —              | White rough  |
| IT90K-277-2     | —                                   | +              | —              | —              | —              | —              | —              | —              | —              | —              | White rough  |
| IT98K-598       | —                                   | +              | —              | —              | —              | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | —              | —              | Brown smooth |
| IT97K-1101-5    | +                                   | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | Black smooth |
| Kamboinse local | + <sup>2</sup>                      | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | b              | —              | —              | + <sup>2</sup> | + <sup>2</sup> | + <sup>2</sup> | White rough  |
| IT86D-719       | +                                   | +              | —              | +              | + <sup>2</sup> | —              | —              | —              | + <sup>2</sup> | + <sup>2</sup> | White rough  |
| IT95K-1491      | —                                   | +              | —              | —              | +              | +              | +              | —              | +              | +              | White smooth |

Jts = joints, Pet = petiole, Ped = peduncle, Clx = calyx, Fl = flower, Pd = pod, Pdt = pod tip, s = standard, w = wing, k = keel, b = a purple dash at the back of the standard petal.

suggested that the resistance to BICMV is controlled by single recessive gene pair in each cowpea line. They designated *bcm* as the gene symbol.

Ryerson and Heath (1996) studied the inheritance of resistance to rust *Uromyces vignae* in cowpea cultivar Calico Crowder. The segregation pattern in  $F_2$  generation and subsequent progeny suggested the presence of multiple genes and also the presence of dominant and recessive resistance components. Rangaiah (1997) also reported the inheritance of rust (*Uromyces vignae*) resistance in cowpea in eight  $F_2$  populations. He observed that a minimum of two genes control resistance to rust in cowpea. Nakawuka and Adipala (1997) screened 75 cowpea lines against scab of which 10 were resistant. These were then used to study the genetics of resistance to scab by Tumwegamire et al. (1998) using a half-diallel cross set. Broad-sense heritability for foliar resistance was 93.8% and for pod resistance it was 97% and 84.5%, respectively. This indicates major gene inheritance, which had earlier been reported by Abadassi et al. (1987) in TVx 3236.

## Genetics of resistance to nematodes

Roberts et al. (1996) identified IT84S-2049 cowpea line from IITA to be completely resistant to diverse populations of the root-knot nematodes, *Meloidogyne incognita* and *M. javanica*. The resistance in this variety was effective against nematode isolates that are virulent to the resistance gene *Rk* present in commercial cultivars in California such as CB5 and CB46. Systematic genetic studies indicated that the resistance in IT84S-2049 was conferred by a single dominant gene which was either allelic to *Rk* gene or a different gene very closely linked to *Rk*. Therefore, the symbol *Rk2* was proposed to designate this new resistance factor. Rodriguez et al. (1996) screened nine cowpea varieties for resistance to the root-knot nematode *Meloidogyne incognita*. They observed that IITA-3, Habana 82, Incarita-1, IT86D-364, IT87D-1463-8, Vinales 144, P902, and IITA-7 were highly resistant whereas the local variety Cancharro was highly susceptible.

## Genetics of new mutants

Singh and Adu-Dapaah (1998) reported a partial sterile mutant controlled by a single recessive gene *pms*. The mutant plants remained green for a longer period than the wild type and they had thick, leathery leaves with a few fleshy 1–3 seeded pods with gaps and

about 77% viable pollen indicating partial male as well as partial female fertility. The homozygous recessive plants (*pms pms*) bred true for partial sterility. Adu-Dapaah et al. (1999) also reported a fasciated mutant, which was observed in an  $F_4$  population of a cross TVu 3000  $\times$  IT82D-604. The mutant plants were both male and female sterile and exhibited crumpled petals and sepals, rosette branching, and abnormal stigmas ranging in number from zero to two. Genetic study showed that this trait was controlled by a single recessive gene, which was designated as *fa*. Odeigah et al. (1996) reported several induced mutants of which four were male sterile and female fertile and two mutants were completely sterile. All the six mutants showed a monogenic recessive inheritance.

## Genetics of leaf, pod, and seed types

Aliboh et al. (1996) studied the inheritance of inverted V-shaped marks on leaves, pod dehiscence, and dry pod color in crosses involving wild, weedy, and cultivated varieties of cowpea. The segregation pattern in  $F_2$  and backcross generations indicated monogenic dominant inheritance for all the three traits. The gene symbols *Vsm*, *Dhp*, and *Bk-2* were assigned for the V-shaped leaf marks, pod dehiscence, and black dry pod color, respectively. Kehinde and Ayo-Vaughan (1999) and Singh and Ishiyaku (2000) reported inheritance of seed coat texture in cowpea and indicated the involvement of two pairs of genes for this trait. The crosses between smooth and rough seed texture segregated into three smooth:one rough. However, the crosses involving white rough seed  $\times$  brown rough seed showed a complementary gene action. The  $F_1$  was smooth and  $F_2$  segregated into a nine smooth:seven rough ratio. This was supported by the backcross data. The gene symbols *rt<sub>1</sub>* and *rt<sub>2</sub>* were assigned for rough testa. Rough seed coat texture is an important trait in West and Central Africa because it facilitates removal of the seed coat for certain food preparations.

## Genetics of photosensitivity and drought tolerance

Ishiyaku and Singh (2001) observed that the photosensitive cultivars not only flower early but also become extremely dwarfed when day lengths are less than 12.5 hours. The dwarfing under short-day length was observed to be a pleiotropic effect of the photosensitivity gene as it showed monogenic recessive inheritance that is completely associated with photosensitivity. The gene symbol *ps* was assigned to it. This is the first report indicating the effect of photoperiod on vegetative growth of plants. All earlier reports linked photosensitivity with reproductive stage only.

Mai-Kodomi et al. (1999) reported simple inheritance of drought tolerance in cowpea. Using a box screening method, they identified two types of shoot drought tolerance. Type 1 plants stayed green for a long time after withholding water and the whole plant died with continued dry conditions. In contrast, the Type 2 plants stayed alive for a much longer period, but the whole plant did not die with continued dry conditions. They mobilized moisture from the lower leaves to keep the growing tips alive for longer and so the plants dropped the lower leaves first and dried upward slowly such that when watering was resumed, they recovered. Both Type 1 and Type 2 drought tolerance are inherited as monogenic dominant traits. The  $F_1$  crosses between them showed dominance of Type 1 and  $F_2$  segregated into three Type 1:one Type 2, suggesting that these are alleles at the same locus. The gene symbols *Rds1* (resistance to drought stress) and *Rds2* were assigned for these traits. This is the first report of monogenic inheritance of drought tolerance in plants. The simple inheritance was observed probably because of simplified screening methods

and selective screening for shoot drought tolerance only. The details are further presented elsewhere in this volume (Singh). Menendez and Hall (1996) studied the heritability of carbon isotope discrimination (DELTA) which may be a useful selection criterion for drought adaptation in cowpea. Broad-sense heritability for DELTA in two crosses (TVx 309 × Prima and TVx 309 × CB 46) was 0.47 and 0.33, respectively, indicating an intermediate level of genetic variability for this trait. Ten cDNAs of genes that were induced by dehydration stress were cloned by differential screening from drought tolerant cowpea variety (Luchi et al. 1996). The clones were collectively named CPRD (cowpea clones responsive to dehydration). A dehydrin gene involved in chilling tolerance during seedling emergence has been identified (Ismail et al. 1997, 1999) and mapped using recombinant inbreds (Menendez et al. 1997).

## Genetics of quantitative traits and heterosis

Damarany (1994) published information on heritability and genetic advance for 13 characters in cowpea. Broad-sense heritability for seed weight/plant was 94.4%, 85.9% for pods/plant, and 83.3% for 100 seed weight. Genetics of pod yield and its components were studied in  $F_2$  and backcross populations of a cross involving two vegetable cowpea varieties, UCR 193 and IT81D-1228-14 by Pathmanathan et al. (1997). The broad-sense heritability for pod weight was 84% and the narrow-sense heritability was 75% indicating good genetic variability for effective selection. Menendez and Hall (1996) studied the heritability of harvest index in two crosses (TVx 309 × Prima and TVx 309 × CB 46). The broad-sense heritability for this trait was 38% and 58% in the two crosses, respectively.

Sangwan and Lodhi (1995) studied heterosis for yield and yield components in 25 crosses involving 11 cowpea varieties. Better parent heterosis ranged from 28.8% to 84.0% for seed yield/ha. Heterosis up to 81.6% over better parent was observed for pod/plant, 35.6% for pod length, 20.4% for seed/pod, and 36% for seed weight/plant. Hybrid Fos-1 × Co1, Fos-2 × EC 4216, and EC 4216 × C28 were most promising. Arvindhan and Das (1996) reported 215% heterosis for seed yield in the cross CS 55 × CO4. Bhor et al. (1997) studied  $P_1$ ,  $F_1$ , and  $F_2$  populations of 14 crosses and observed 63.8% better parent heterosis for seed yield in the cross V240 × VCM8. They further observed that the heterosis was 4.3% for plant height and 91.52% for days to maturity. They observed that progeny derived from crosses showing high heterosis also showed high inbreeding depression indicating the importance of nonadditive gene action. Bhushana et al. (2000) estimated heterosis for several traits in 36 hybrids. They observed a midparental heterosis of 171.5% for number of secondary branches/plant, 11.5% for pods/plant, 105.3% for seed yield/plant, 75.5% for primary branches/plant, 30.31% for pod length, and 20% for 100 seed weight. They also observed -15.9% heterosis for days to 50% flowering. Heterosis for fodder yield was reported by Ponmariam and Das (1996) and Arvindhan and Das (1996) and the highest heterosis (121%) was recorded for the hybrid UPC9201 × CO5.

High values for heterosis indicates good genetic diversity among cowpea varieties used in these studies indicating the possibility of isolating high yielding transgressive segregates from hybrid populations. However, the estimates for heterosis in most cases is from space planted  $F_1$  hybrids, which may not be a true index of performance under normal plant populations used for commercial crops. Therefore, there is a need to estimate heterosis under recommended plant population for maximum yield of cowpea. This will, of course, require



making a large number of pollinations to obtain enough  $F_1$  seeds to test under normal density.

## Linkage and mapping

The first report of linkage in cowpea was published by Uguru and Ngwuta (1995). From the genetic analysis of  $F_1$ ,  $F_2$ , and  $F_3$  populations derived from the crosses involving cowpea variety AN-14-D with purple calyx, purple petal, and purple pod and varieties An-36-F and AE-36-W which were nonpigmented. They observed linkage between the three traits with calyx and petal color most tightly linked ( $0.576 \pm 0.009$  cm). Another report on linkage was published by Githiri et al. (1996) who identified genes on four linkage groups as indicated below:

| Linkage group  | Trait involved   |
|--|--|
| 1. $Sw \rightarrow Fbc$<br>$41 \pm 4.8$  | $Sw$ = swollen base, $Fbc$ = cream flower bud  |
| 2. $Pus \rightarrow Pub \rightarrow Cbr$<br>$4 \pm 1.5 \quad 30 \pm 5.7$                                 | $Pus$ = purple stem, $Cbr$ = cocoa brown pod color, $Pub$ = purple pods                            |
| 3. $Pod \rightarrow Ndt \rightarrow Hg \rightarrow Bpd$<br>$26 \pm 28 \quad 26 \pm 2.8 \quad 24 \pm 9.5$ | $Pd$ = purple peduncle,<br>$Ndt$ = nondeterminate, $Hg$ = erect plant<br>$Bpd$ = branched peduncle |
| 4. $Put \rightarrow Bk$<br>$19 \pm 2.4$  | $Put$ = purple pod tips, $Bk$ = grey black pod   |

They used  $F_2$  data from four crosses to estimate the recombination frequencies, which need further confirmation using backcross and  $F_3$  data. Kehinde et al. (1997) studied the segregation pattern of 12 loci in  $F_2$  and backcross populations and identified five linkage groups. Linkage group 1 comprised of five genes,  $Pg$  (nodal pigmentation),  $Pf$  (purple flower),  $Pc$  (smooth seed coat),  $Na$  (narrow eye), and  $Br$  (brown seed coat) with the probable order  $Pg \rightarrow Na \rightarrow Br \rightarrow Pc \rightarrow Pf$ . The second linkage group was  $Bpd$  (branched peduncle)  $\rightarrow Bp$  (brown dry pod)  $\rightarrow Dhp$  (pod dehiscence). The third linkage group consisted of  $Crl$  (crinkled leaf)  $\rightarrow Pt$  (sessile leaf). The hastate leaf ( $Ha$ ) and septafoleate leaf ( $spt$ ) showed independent segregation from others showing different linkage groups (4 and 5). A DNA marker based (RFLP and RAPD analysis) genetic map of cowpea was first reported by Fatokun et al. (1993). This contained 92 markers with a span of 717 cM of the genome from a cross between IT84S-2246-4 and TVNu 1963. Recently, Menendez et al. (1997) published another genetic map consisting of 181 loci, comprising 133 RAPDs, 19 RFLPs, 25 AFLPs, three morphological or classical markers, and a biochemical marker (dehydrin). These markers identified 12 linkage groups spanning 972 cM with an average distance of 6.4 cM between markers. Myers et al. (1996) identified one RFLP marker, to be tightly linked to the aphid resistance gene ( $Raci$ ). Recently, Ouedraogo et al. (2001) have identified three AFLP markers tightly linked to the *Striga* resistance gene  $Rsg\ 2-1$  and six AFLP markers linked to the *Striga* resistance gene  $Rsg\ 4-3$  setting the stage for marker-assisted selection (MAS) in cowpea. However, a lot of work is needed to saturate the genetic map of cowpea and identify more markers before routine MAS can be practised.

## References

- Abadassi, J.A., B.B. Singh, T.A.O. Ladeinde, S.A. Shoyinka, and A.M. Emechebe. 1987. Inheritance of resistance to brown blotch, *Septoria* leaf spot and scab in wild *Vigna* (*Vigna vexillata*). Indian Journal of Genetics 47: 299–303.
- Adu-Dapaah, H.K., B.B. Singh, and C.A. Fatokun. 1999. A fasciated mutant in cowpea (*Vigna unguiculata* (L.). Acta Agronomica Hungarica 47: 371–376.
- Aliboh, V.O., O.B. Kehinde, and I. Fawole. 1996. Inheritance of leaf mark, pod dehiscence and dry pod color in crosses between wild and cultivated cowpeas. African Crop Science Journal 5(2): 283–288.
- Arshad, M., M. Bashir, A. Sharif, and B.A. Malik. 1998. Inheritance of resistance in cowpea (*Vigna unguiculata* [L.] Walp) to blackeye cowpea mosaic potyvirus. Pakistan Journal of Botany 30(2): 263–270.
- Arvindhan, S. and L.D.V. Das. 1996. Heterosis and combining ability in fodder cowpea for green fodder and seed yield. Madras Agricultural Journal 83: 11–14.
- Begemann, F., J. Heller, and J. Mushanga. 1997. An experiment to cross bambara groundnut and cowpea. Pages 135–137 in Bambara groundnut. Proceedings of Workshop on conservation and improvement of Bambara groundnut, 14–16 November 1995, Harare, Zimbabwe.
- Bhor, T.J., N.S. Kute, A.D. Dumbre, and N.D. Sarode. 1997. Heterosis and inbreeding depression in cowpea. Indian Journal of Agricultural Research 31: 122–126.
- Bhushana, H.O., K.P. Viswanatha, P.A. Runachala, and G.K. Halesh. 2000. Heterosis in cowpea for seed yield and its attributes. Crop Research (Hisar) 19: 277–280.
- Biradar, B.D., J.V. Goud, and S.S. Patil. 1997. Differential expression of pleiotropic genes for pigmentation in cowpea (*Vigna unguiculata* [L.] Walp). Crop Research (Hisar) 14(2): 233–242.
- Capinpin, J.M. 1935. A genetic study of certain characters in varietal hybrids of cowpea. Philippine Journal of Science 57: 149–164.
- Cardinali, A., V. Linsalata, P. Perriono, V. Lattanzio, R. Brouillard, M. Jay, and A. Scalbert. 1995. Pages 375–376 in Chemotaxonomy of wild *Vigna* species as potential sources of resistance to insects. Polyphenols 94: 17th International Conference, Palma de Mallorca, Spain.
- Damarany, A.M. 1994. Estimates of genotypic and phenotypic correlation, heritability and potency of gene set in cowpea (*Vigna unguiculata* [L.] Walp). Assuit Journal of Agricultural Science 25: 1–8.
- Fatokun, C.A., D. Darinsh, D.I. Menancio-Hautea, and N.D. Young. 1993. A linkage map for cowpea (*Vigna unguiculata*) [L.] Walp.) based on DNA markers. Pages 256–258 in Genetic maps, edited by S.J. O'Brien. Locus Maps of Complex Genomes. VI Edition. Cold Spring Harbor Laboratory Press, USA.
- Fery, R.L. 1981. Genetics of *Vigna*. Pages 311–394 in Horticultural reviews, edited by J. Janick. AVI Publishing, Westport, CT, USA.
- Fery, R.L. 1985. The genetics of cowpea. A review of the world literature. Pages 25–62 in Cowpea research, production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Fery, R.L. and B.B. Singh. 1997. Cowpea genetics: a review of recent literature. Pages 13–29 in Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell and L.E.N. Jackai. Copublication of IITA and JIRCAS. IITA, Ibadan, Nigeria.
- Githiri, S.M., P.M. Kimani, and R.S. Pathak. 1996. Linkage relationships among loci controlling morphological traits in cowpea (*Vigna unguiculata* [L.] Walp.) Euphytica 92(3): 307–311.
- Gomathinayagam, P., S.G. Ram, R. Rathnaswamy, and N.M. Ramaswamy. 1998. Interspecific hybridization between *Vigna unguiculata* (L.) Walp. and *V. vexillata* (L.). A. Rich, through in vitro embryo culture. Euphytica 102(2): 203–209.

- Harland, S.C. 1919a. Inheritance of certain characters in the cowpea (*Vigna sinensis*). Journal of Genetics 8: 101–132.
- Harland, S.C. 1919b. Notes on inheritance in cowpea. Agricultural News, Barbados 18: 20.
- Harland, S.C. 1920. Inheritance of certain characters in the cowpea (*Vigna sinensis*). Journal of Genetics 10: 193–205.
- Ishiyaku, M.F. and B.B. Singh. 2001. Inheritance of shortday-induced dwarfing in photosensitive cowpea. African Crop Science Journal 9(2): 1–8.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1997. Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. Crop Science 37: 1270–1277.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1999. Allelic variation of a dehydrin gene co-segregates with chilling tolerance during seedling emergence. Proceedings of National Academy of Science 96: 13566–13570.
- Joshi, S.S., R. Sreekantharadhy, K.G. Shambulingappa, D.P. Jagannatha, and C.V. Jayaramu. 1994. Inheritance of a few qualitative characters in cowpea (*Vigna unguiculata* [L.] Walp.) Crop Research (Hisar) 8(2): 330–336.
- Kehinde, O.B., G.O. Myers, and I. Fawole. 1997. Analysis of genetic linkage in the cowpea *Vigna unguiculata*. Pertanika Journal of Tropical Agricultural Science 20(1): 75–82.
- Kehinde, O.B. and M.A. Ayo-Vaughan. 1999. Genetic control of seed coat texture in cowpea, *Vigna unguiculata* (L.) Walp. Tropical Agricultural Research and Extension 2(1): 7–9.
- Kolhe, A.K. 1970. Genetics studies in *Vigna* sp. Poona Agricultural College Magazine 59: 126–137.
- Krishnaswamy, N., K.K. Nambiar, and A. Mariakulandai. 1945. Studies in cowpea (*V. unguiculata* [L.] Walp). Madras Agricultural Journal 33: 145–160.
- Luchi, S., K. Yamaguchi-Shinozaki, T. Urao, T. Terao, and K. Shinazaki. 1996. Novel drought inducible genes in the highly drought-tolerant cowpea: cloning of DNAs and analysis of the expression of the corresponding genes. Plant and Cell Physiology 37: 1073–1082.
- Mai-Kodomi, Y., B.B. Singh, O. Jr. Myers, J.H. Yopp, J.P. Gibson, and T. Terao. 1999. Inheritance of drought tolerance in cowpea. India Journal of Genetics and Plant Breeding 59: 317–323.
- Menendez, C.M. and A.E. Hall. 1996. Heritability of carbon isotope discrimination and correlations with harvest index in cowpea. Crop Science 36(2): 233–238.
- Menendez, C.M., A.E. Hall, and P. Gepts. 1997. A genetic linkage map of cowpea developed from a cross between two inbred domesticated lines. Theoretical and Applied Genetics 95: 1210–1217.
- Mortensen, J.A. and W.H. Brittingham. 1952. The inheritance of pod color in the southern pea, *Vigna sinensis*. Proceedings of the American Society of Horticultural Science 59: 451–456.
- Myers, G.O., C.A. Fatokun, and N.D. Young. 1996. RELP mapping of an aphid resistance gene in cowpea. Euphytica 91: 181–187.
- Nakawuka, C.K. and E. Adipala. 1997. Identification of sources and inheritance of resistance to *Sphaceloma* scab in cowpea. Plant Disease 81: 1395–1399.
- Odeigah, P.G.C., A.O. Osanyin Peju, and G.O. Myers. 1996. Induced male sterility in cowpea. Journal of Genetics and Plant Breeding 50: 171–175.
- Ouedraogo, J.T., V. Maheshwari, D.K. Berner, C.A. St-Pierre, F. Belize, and M.P. Timko. 2001. Identification of AFLP markers linked to resistance of cowpea to parasitism by *Striga gesnerioides*. Theoretical and Applied Genetics 102: 1029–1036.
- Pasquet, R.S. 1999. Genetic relationship among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. Theoretical and Applied Genetics 98: 1104–1119.
- Ponmariammal, T. and L.D. Vijendra Das. 1996. Heterosis for fodder yield in cowpea. Madras Agricultural Journal 83: 658–659.
- Rangaiah, S. 1997. Inheritance of resistance to *Uromyces phaseoli* in *Vigna unguiculata* (L.) Walp. Crop Improvement 24(2): 251–252.

- Roberts, P.A., W.C. Matheswand, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to *Rk* locus in cowpea. *Crop Science* 36: 889–894.
- Rodriguez, I., M.G. Rodriguez, L. Sanchez, and A. Iglesias. 1996. Expression of resistance to *Meloidogyne incognita* in cowpea cultivars. *Revista de Proteccion Vegetal* 11: 63–65.
- Ryerson, D.E. and M.C. Heath. 1996. Inheritance of resistance to the cowpea rust fungus in cowpea cultivar Calico Crowder. *Canadian Journal of Plant Pathology* 18(4): 384–391.
- Sangwan, R.S. and G.P. Lodhi. 1995. Heterosis for grain characters in cowpea (*Vigna unguiculata* [L.] Walp.). *Legume Research* 18: 75–80.
- Sangwan, R.S. and G.P. Lodhi. 1998. Inheritance of flower and pod color in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica* 102(2): 191–193.
- Saunders, A.R. 1960a. Inheritance in the cowpea 2: seed coat color pattern; flower, plant and pod color. *South African Journal of Agricultural Science* 3: 141–142.
- Saunders, A.R. 1960b. Inheritance in the cowpea 3: mutations and linkages. *South African Journal of Agricultural Science* 3: 327–348.
- Sen, N.K. and J.G. Bhowal. 1961. Genetics of *Vigna sinensis* (L.) savi. *Genetics* 32: 247–266.
- Singh, B.B. and H.K. Adu-Dapaah. 1998. A partial male sterile mutant in cowpea. *African Crop Science Journal* 6: 97–101.
- Singh, B.B. and M.F. Ishiyaku. 2000. Genetics of rough seed coat texture in cowpea. *Journal of Heredity* 91: 170–174.
- Singh, K.B. and L.N. Jindla. 1971. Inheritance of bud and pod color, pod attachment and growth habit in cowpeas. *Crop Science* 11: 928–929.
- Sonnante, G., A.R. Piergiovanni, Q. Ng, and P. Perrino. 1996. Relationship of *Vigna unguiculata*, *Vigna vexillata* and species of section *Vigna* based on isozyme variation. *Genetic Resources and Crop Evolution* 43: 157–165.
- Tumwegamire, S., P.R. Rubaihayo, and E. Adipala. 1998. Genetics of resistance to *Sphaceloma* scab of cowpea. *African Crop Science Journal* 6(3): 227–240.
- Tyagi, D.K. and H.S. Chawala. 1999. Effect of season and hormones on crossability barriers and in vitro hybrid development between *Vigna radiata* and *Vigna unguiculata*. *Acta Agronomica Hungarica* 47: 147–154.
- Uguru, M.I. 1995. Inheritance of color patterns in cowpea (*Vigna unguiculata* [L.] Walp.). *Indian Journal of Genetics and Plant Breeding* 55(4): 379–383.
- Uguru, M.I. and A.A. Ngwuta. 1995. Genetics and linkage relationships of anthocyanin genes in vegetable cowpea. *Biologisches Zentralblatt* 114: 273–278.
- Pathmanathan, U., R.P. Ariyanayagam, and S.O. Haque. 1997. Genetic analysis of yield and its components in vegetable cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica* 96(2): 207–213.
- Vale, C.C. do, J.A. Lima, do Vale, C.C. 1995. The inheritance of immunity in *Vigna unguiculata* Macaibo to cowpea severe mosaic virus. *Fitopatologia Brasileira* 20(1): 30–32.
- Venugopal, R. 1998. Inheritance in cowpea (*Vigna unguiculata* [L.] Walp. V.) pod characters. *Crop Research (Hisar)* 15(1): 77–84.
- Venugopal, R. and J.V. Goud. 1996. Inheritance in cowpea (*Vigna unguiculata* [L.] Walp.). III Floral characters. *Mysore Journal of Agricultural Sciences* 30(1): 14–20.
- Venugopal, R. and J.V. Goud. 1997. Inheritance of pigmentation of cowpea. *Current Science* 3: 141–142.
- Venora, G. and S. Padulosi. 1997. Karyotypic analysis of wild taxa of *Vigna unguiculata* (L.) Walpers. *Caryologia* 50: 125–138.

## 1.2

# Breeding cowpea for tolerance to temperature extremes and adaptation to drought

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### Abstract

Cowpea exhibits incomplete emergence when soil temperatures are below 19 °C. Chilling tolerance at emergence appears to be conferred by a dominant gene encoding a dehydrin protein. Seed immunoblot assays facilitate breeding for this trait. Cowpea can exhibit floral bud suppression and low pod set when night temperatures are higher than 17 °C. Heat-tolerance genes enhanced flowering, pod set, and grain yield under hot subtropical conditions but with no difference between tolerant and susceptible lines in hot tropical conditions. In glasshouse studies, heat-tolerant lines had high yields under both long and short days but heat-susceptible lines only exhibited low yields in long days. Delayed leaf senescence can enhance drought adaptation of early cowpea cultivars by enabling them to produce a greater second pod flush if the first flush is damaged by drought. Genetic studies demonstrated that combining the delayed leaf senescence and heat tolerance traits could breed cultivars with enhanced yield stability.

### Introduction

In subtropical zones, such as the San Joaquin Valley of California, cowpea is sown in the spring (Hall and Frate 1996). Early sowing can result in high grain yields if it enables the crop to escape hot summer weather that can hinder reproductive development (Hall 1992). If sowing is too early, however, and the soil is cooler than 19 °C, chilling damage can cause slow and incomplete emergence (Ismail et al. 1997). This paper will discuss research showing that breeding cowpea for both chilling tolerance at emergence and heat tolerance at flowering can partially solve these problems for subtropical zones. We also will discuss studies of whether genes that confer heat tolerance during reproductive development in subtropical zones have any adaptive value for cowpea grown in tropical zones in West Africa. Cowpea in the Sahelian (annual rainfall of about 200 to 500 mm) and dry savanna (annual rainfall of about 500 to 700 mm) zones of West Africa can experience both heat and drought stress (Hall et al. 1997a). Cowpea cultivars that begin flowering early can escape drought in some locations and years and produce useful yields of grain. Unfortunately, the early cowpea cultivars tend to be very sensitive to droughts that occur during early stages of reproductive development (Thiaw et al. 1993). A delayed-leaf-senescence (DLS) trait has the potential to enhance the drought adaptation of cowpea in the dry savanna

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zone and wetter part of the Sahelian zone. In California, the DLS trait had been shown to enhance the ability of early flowering cowpea to recover after an early drought and produce a compensatory second flush of pods in some field conditions (Gwathmey and Hall 1992). The DLS trait also had been shown to enhance the second flush of pods in one tropical location where lines were tested in the wetter part of the Sahelian zone (Hall et al. 1997b). We will discuss research on whether there is an interaction between the DLS and heat-tolerance genes because they have contrasting effects on the partitioning of carbohydrate in the plant.

### **Chilling tolerance during emergence**

Warm-season annual crops exhibit slow and incomplete emergence when subjected to cool soils. The threshold soil temperature where cowpea exhibits incomplete emergence is quite high at about 19 °C (Ismail et al. 1997). Soil temperatures below 19 °C often occur in spring in the San Joaquin Valley of California where cowpea is grown (soil and air temperature data for many locations in California can be obtained at the web site [www.ipm.ucdavis.edu](http://www.ipm.ucdavis.edu)). A cowpea line with chilling tolerance was found and it was hypothesized that the chilling tolerance is due to two independent and additive factors (Ismail et al. 1997). The factors are a specific dehydrin protein with a dominant nuclear effect and a maternal effect associated with slow electrolyte leakage from seed under chilling conditions. Slower electrolyte leakage indicates greater plasma membrane integrity. The dehydrin protein has been purified and partially characterized (Ismail et al. 1999a). The hypothesis concerning the contribution to chilling tolerance during emergence of the dehydrin protein has been confirmed using near isogenic lines and it was shown that the maternal electrolyte leakage effect is not cytoplasmically inherited (Ismail et al. 1999b). The phenotypic expression of the dehydrin has been mapped (Menéndez et al. 1997) and the structural gene encoding the dehydrin maps to the same location (Ismail et al. 1999b).

The dehydrin protein can be readily manipulated by classical breeding. An immunoblot assay of a chip taken from a single seed is used to detect the presence of the dehydrin protein and the seed still retains its ability to germinate (Ismail et al. 1999b). Using this assay technique, cowpea lines that combine the dehydrin and chilling tolerance during emergence with heat tolerance during reproductive development have been developed.

### **Heat tolerance during reproductive development**

Six genetically similar pairs of lines that either have or do not have heat tolerance during reproductive development were bred at the University of California (UCR). A pedigree breeding approach was used with field screening for flower production and pod set in a very hot field environment (average maximum and minimum daily air temperatures in a weather station shelter of 43 and 24 °C, respectively, for the first 60 days after sowing) as described by Hall (1992). The performance of these six pairs of lines has been evaluated in eight field environments in the subtropical zone of California that have contrasting temperatures but similar high levels of solar radiation and optimal management with complete irrigation (Ismail and Hall 1998). A subset of the data from this study is presented in Table 1. All of the heat-susceptible lines had much lower grain yields in the environments with average night temperatures higher than 17 °C at flowering. The heat-tolerant lines had 394 to 554 kg/ha greater average grain yields than

**Table 1.** Grain yields of six pairs of heat-tolerant and heat-susceptible cowpea lines grown with complete irrigation at three locations in the subtropical zone of California, USA, over 2 years with high levels of solar radiation and optimal management.

|  | Riverside with<br>early sowing<br>1995 & 1996 | Shafter |      | Coachella<br>valley<br>1995 & 1996 |
|--|---|---------|------|------------------------------------|
|  |   | 1995    | 1996 |                                    |
|  |   | kg/ha   |      |                                    |
| Heat-tolerant lines                              | 3086  | 3357    | 2492 | 894                                |
| Heat-susceptible lines                           | 2976  | 3310    | 2098 | 340                                |
| Significance                                     | NS  | NS      | **   | ****                               |
| Daily minimum<br>air temperature °C <sup>†</sup> | 14.6  | 16.4    | 17.4 | 23.7                               |

\*\* and \*\*\*\* are significant at the 0.01 and 0.0001 levels whereas NS is not significant at the 5% level.

<sup>†</sup>Average for the 3-week period beginning one week prior to the start of flowering.

Source: From Tables 2 and 3 in Ismail and Hall 1998.

the heat-susceptible lines in the hotter environments, but similar grain yields in the cooler environments. One of these heat-tolerant lines has been released as California Blackeye No. 27 (CB27) for use as a dry grain cultivar in California (Ehlers et al. 2000). Heat-tolerant lines were much shorter compared with heat-susceptible lines and this effect was more pronounced in hotter environments (Ismail and Hall 1998). In California, the semi-dwarf cultivar CB27 has a greater yield advantage over current standard height cultivars, such as CB5, when grown on rows 51 to 76 cm apart rather than the wide rows (about 102 cm apart) that are used by some growers (Ismail and Hall 2000). Row spacing in this study was 10 cm between plants in the row.

In the relatively high night temperatures experienced in tropical zones, the heat-tolerant lines developed in California experience even more dwarfing than in subtropical zones. We have studied whether the heat-tolerance genes shown to be effective in the subtropical zone of California are also effective in tropical zones of West Africa where there is substantial cowpea production. Daily minimum air temperatures were substantially higher in the savanna and Sahelian zones (Table 2) than the threshold of 17 °C for causing damage to flower development and pod set of the heat-susceptible lines indicated by the studies of Ismail and Hall (1998) in Table 1. In all of the trials in West Africa, however, there was no significant difference in grain yield between the averages of the six heat-tolerant and six heat-susceptible lines (Table 2).

The contrasting performance of the 2 sets of lines may be explained by the longer day lengths experienced by plants in California compared with West Africa. Controlled-environment studies had shown that high night temperatures could be more damaging to cowpea in long-day than in short-day conditions (Mutters et al. 1989). Studies in which the heat tolerance of contrasting cowpea lines were evaluated in greenhouses with high night temperature and either long or short days (Ehlers and Hall 1998) have provided some explanations for the contrasting performance of the lines in different field conditions. A subset of the data is presented in Table 3. Heat-tolerant California lines had greater grain yields than heat-susceptible California lines in hot long-day greenhouse conditions (Table 3) as had been observed in hot long-day field conditions in Shafter in 1996 and in the Coachella Valley in both 1995 and 1996 (Table 1). In contrast, in hot, short-day greenhouse conditions, heat-tolerant and heat-susceptible California lines had similar

**Table 2.** Grain yields of the same six pairs of heat-tolerant and heat-susceptible cowpea lines used for the study in Table 1 when grown under rainfed conditions at three locations in the dry Savanna zone (northern Ghana) and three environments in the Sahelian zone (peanut basin of Senegal) with optimal management.

|  | Northern Ghana    |                 |               | Senegal        |                   |                   |
|--|-------------------|-----------------|---------------|----------------|-------------------|-------------------|
|  | Nyankpala<br>1998 | Damongo<br>1998 | Manga<br>1998 | Bambey<br>1998 | Thilmakha<br>1999 | Thilmakha<br>1998 |
|  | kg/ha             |                 |               | kg/ha          |                   |                   |
| Heat-tolerant lines  | 1241              | 869             | 564           | 1165           | 1839              | 1370              |
| Heat-susceptible lines   | 1351              | 895             | 559           | 1101           | 1894              | 1369              |
| Significance in all cases differences between sets of lines were not significant at the 5% level |                   |                 |               |                |                   |                   |
| Air temperature °C <sup>†</sup>  | 25.7              | 20.7            | 22.5          | 24.4           | 23.5              | 25.3              |
| Sowing date  | 29 July           | 28 June         | 2 August      | 5 August       | 14 July           | 29 July           |

<sup>†</sup>Average daily minimum air temperature for the three-week period beginning one week prior to the start of flowering, using Louga data for Thilmakha.



grain yields and they were high (Table 3). In hot, short-day field conditions in Africa also heat-tolerant and heat-susceptible California lines had similar grain yields but they were moderate (Table 2). The moderate level of the grain yields may be explained by the fact that these California lines are not well adapted to West Africa. For example, the California lines are highly susceptible to wet and dry pod rots. The heat-tolerant parents (Prima and TVu4552) used in developing the heat-tolerant California lines have been shown to have high pod set under hot, short-day conditions. Prima was shown to have higher grain yield than IT84S-2246 due to its greater pod set in studies in hot growth chambers with 12-hour days (Craufurd et al. 1998). In hot, short-day conditions in screenhouses at Kano, West Africa, TVu4552 has exhibited much greater pod set and grain production than many other cowpea accessions (personal communication B. B. Singh, March 2001). The genes in Prima, TVu4552, and the California lines that confer heat tolerance during pod set probably can enhance pod set in tropical conditions but they need to be combined with additional genes that confer local adaptation. Also effects of these genes on grain yield may not be as large in hot, short-day tropical environments as has been observed in hot subtropical zones.

Some of the African cultivars and lines that were studied in greenhouses by Ehlers and Hall (1998) also had heat tolerance in that they exhibited high grain yields in hot, short-day conditions (Table 3). These heat-tolerant materials included landraces that evolved in the hot Sahelian zone (58-57 and Suvita 2) and cultivars (Mouride and TN88-63) and breeding lines (B89-600 from Senegal and the IT lines developed by IITA at Kano, Nigeria) that had been selected based on grain yield in hot tropical conditions. The heat-susceptible African lines in Table 3 also include some that had been selected for high grain yield in hot, short-day conditions (Melakh, N'diambour, and Bambey 21). These data suggest that selecting for high grain yield in hot parts of Africa is not always effective in incorporating

**Table 3. Grain yields of contrasting cowpea cultivars and lines in a greenhouse with high night temperature (day/night 36/27 °C) at Riverside, CA, USA under summer (long-day) or spring (short-day) conditions.**

|  | Long days     | Short days    |
|--|---------------|---------------|
|  | g/plant       |               |
| Heat-tolerant California lines (4)<br>H8-9-3, H8-14-13, 518-2, H8-8-4 <sup>†</sup>   | 26 (22 to 32) | 47 (42 to 54) |
| Heat-susceptible California cultivars and lines (7)<br>H14-10-10, H8-8-31, H35-5-6, H8-14-18, CB5, CB46,<br>H8-14-19-1   | 1 (0 to 3)    | 40 (36 to 48) |
| Heat-tolerant African cultivars and lines (10)<br>Mouride, B89-600, IT89KD-252, IT89KD-245,<br>IT88DM-400, IT89KD-107-5, TN88-63, 58-57,<br>IT89KD-355, Suvita 2 <sup>†</sup>                          | 1 (0 to 6)    | 44 (38 to 53) |
| Heat-susceptible African cultivars and lines (12)<br>Melakh, IT82D-889, N'diambour, IT84S2049,<br>Bambey 21, TVx12-01e, IT86D-719, KN-1,<br>Bambey 23, IT82D-375, Sumbrisogle, IT85F-2614 <sup>†</sup> | 2 (0 to 9)    | 21 (10 to 30) |

<sup>†</sup>Cultivars and lines listed in rank order with the first having the highest yield in short days.

Source: From Table 6 in Ehlers and Hall 1998.

heat tolerance. Field screening for heat tolerance is difficult in the Sahelian and savanna zones because of biotic stresses, such as flower thrips, that damage floral development and pod set in a manner that is similar to the effects of heat stress. Progress has been made, however, in screening cowpea for reproductive-stage heat tolerance in Africa by growing them in screenhouses during the dry season in Kano, Nigeria where daily minimum air temperatures vary from 24 to 27 °C and daily maximum air temperatures vary from 38 to 42 °C (Singh 1998).

Methods for screening to detect reproductive-stage, heat-tolerance genes that are more efficient than field screening have been sought. Ismail and Hall (1999) have suggested that measurements of plasma membrane thermostability based upon electrolyte leakage from leaf disks has the potential to be used for screening for reproductive-stage, heat-tolerance genes. Recent studies by S. Thiaw and A.E. Hall indicate, however, that effective screening for plasma membrane thermostability may require that plants be grown in long-day conditions. Also it may be necessary to put leaf disks in aerated solutions when measuring electrolyte leakage and the differences in electrolyte leakage between genotypes differing in reproductive-stage heat tolerance may be small.

The overall conclusion is that the reproductive-stage, heat-tolerance genes discovered by Hall and associates can be effective in subtropical conditions and may be effective in the tropics under either long-day or short-day conditions providing, other stresses do not damage plant growth and development. Also note that with sowing prior to late June in the Sahelian and dry savanna zones of West Africa, day lengths may be long enough to enhance the detrimental effects of heat on reproductive development. Empirical breeding studies indicate that heat-tolerance genes may be useful in West Africa. For example, Marfo has bred a cultivar for northern Ghana, Sul 518-2, using a heat-tolerant line from California as one of the parents with some initial screening for heat tolerance but the heat-tolerance of Sul 518-2 under Ghanaian conditions has not yet been confirmed.

### **Heat tolerance interaction with delayed-leaf-senescence**

Reproductive-stage, heat-tolerance genes cause greater partitioning of carbohydrates to pods (Ismail and Hall 1998), whereas, the delayed-leaf-senescence (DLS) trait is associated with greater partitioning of carbohydrate to stem bases (Gwathmey et al. 1992) and also probably to roots. Studies were conducted to test: (1) whether the DLS trait reduces first-flush yields and thus reduces the beneficial effects of the heat-tolerance trait; and (2) whether the heat-tolerance trait enhances senescence after the first flush of pods is produced thereby reducing the expression of the DLS trait (Ismail et al. 2000). A cross was made between a heat-tolerant parent and a DLS parent and then four sets of lines were selected that either have or do not have the DLS and heat-tolerance traits (Ismail et al. 2000). It was shown that the DLS trait can be effectively selected beginning with  $F_3$  families providing a field nursery is used that has a senescence inducing soil environment. There is a tendency for senescence inducing soil conditions to develop in fields where cowpea has been grown for several years, even with alternate year rotation to other crop species, due possibly to the build up of a soilborne disease (Ismail et al. 2000). Individual plants with DLS were selected from families where most plants exhibited DLS. Selection for heat tolerance was done in field nurseries in extremely hot field and greenhouse environments and involved selecting plants for flower production and pod set (Hall 1992).

Performance of the four sets of lines in a hot field environment is described in Table 4. The heat-tolerance trait enhanced grain yield by a substantial amount, 886 kg/ha, whereas

presence of the DLS trait did not have a significant effect on the first-flush grain yield of the heat-tolerant lines (there was a nonsignificant decrease of 295 kg/ha). If this decrease in yield is real, it does not represent a large penalty in that the DLS trait has the potential to increase second-flush grain yield by up to 2000 kg/ha (Ismail and Hall 1998). Performance of the four sets of lines was evaluated in a soil environment where there was substantial death of non-DLS lines after producing the first flush of pods (73% of the plants died compared with < 1% for DLS lines). The presence of the heat-tolerance genes did not have a significant effect on the proportion of plants that died (nonsignificant increases of 10 percentage points in non-DLS lines and 1 percentage point in non-DLS lines occurred as shown in Table 5).

The overall conclusions are that (1) the DLS trait can greatly enhance plant survival after the first flush of pods is produced and may only cause a small decrease in first-flush grain yield; and (2) the heat-tolerance trait can substantially increase first-flush grain yield and may only slightly enhance the tendency for premature plant death in non-DLS lines with no effect on lines having the DLS trait.

**Table 4. First-flush grain yields of four sets of lines with and without heat-tolerance and with and without the delayed-leaf-senescence trait.**

|                        | Delayed-leaf-senescence lines | Senescent lines | Average |
|------------------------|-------------------------------|-----------------|---------|
|                        |                               | kg/ha           |         |
| Heat-tolerant lines    | 3168                          | 3463            | 3316    |
| Heat-susceptible lines | 2248                          | 2613            | 2430    |
| Average                | 2708                          | 3038            |         |

The heat-tolerance effect was very highly significant whereas the delayed-leaf-senescence and interaction effects were not significant at the 5% level and the CV was 19.3%.

Note: Data are average values for 10 lines per set from an experiment at Shafter, CA, USA in 1998 (Ismail et al. 2000).

**Table 5. Percentage of plants that died after producing the first flush of pods for four sets of lines with and without heat-tolerance and with and without the delayed-leaf-senescence trait.**

|                        | Delayed-leaf-senescence lines  | Senescent lines |
|------------------------|--------------------------------|-----------------|
|                        | Percentage of plants that died |                 |
| Heat-tolerant lines    | 1                              | 78              |
| Heat-susceptible lines | 0                              | 68              |

The delayed-leaf-senescence effect was very highly significant whereas the heat-tolerance and interaction effects were not significant at the 5% level.

Note: Data are average values for 10 lines per set from an experiment at Riverside, CA, USA in 1998 (Ismail et al. 2000).

Conclusions

Cowpea cultivars can be bred that combine chilling tolerance at emergence with heat tolerance during flowering and pod set. These cultivars could have enhanced yield stability in subtropical zones such as those in California. Cowpea cultivars can be bred that combine early flowering with heat tolerance during flowering and pod set and

delayed-leaf-senescence. These cultivars might have enhanced yields and yield stability in subtropical zones and tropical zones, such as the drier part of the savanna zone and the wetter part of the Sahelian zone in West Africa.

## References

- Craufurd, P.Q., M. Bojang, T.R. Wheeler, and R.J. Summerfield. 1998. Heat tolerance in cowpea: effect of timing and duration of heat stress. *Annals of Applied Biology* 133: 257–267.
- Ehlers, J.D. and A.E. Hall. 1998. Heat tolerance of contrasting cowpea lines in short and long days. *Field Crops Research* 55: 11–21.
- Ehlers, J.D., A.E. Hall, P.N. Patel, P.A. Roberts, and W.C. Matthews. 2000. Registration of California Blackeye 27. *Crop Science* 40: 854–855.
- Gwathmey, C.O. and A.E. Hall. 1992. Adaptation to midseason drought of cowpea genotypes with contrasting senescence traits. *Crop Science* 32: 773–778.
- Gwathmey, C.O., A.E. Hall, and M.A. Madore. 1992. Pod removal effects on cowpea genotypes contrasting in monocarpic senescence traits. *Crop Science* 32: 1003–1009.
- Hall, A.E. 1992. Breeding for heat tolerance. *Plant Breeding Reviews* 10: 129–168.
- Hall, A.E. and C.A. Frate. 1996. Blackeye bean production in California. University of California Division of Agricultural Science Publications 21518. 23 pp.
- Hall, A.E., B.B. Singh, and J.D. Ehlers. 1997a. Cowpea breeding. *Plant Breeding Reviews* 15: 215–274.
- Hall, A.E., S. Thiwaw, A.M. Ismail, and J.D. Ehlers. 1997b. Water-use efficiency and drought adaptation of cowpea. Pages 87–98 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Ismail, A.M. and A.E. Hall. 1998. Positive and potential negative effects of heat-tolerance genes in cowpea. *Crop Science* 38: 381–390.
- Ismail, A.M. and A.E. Hall. 1999. Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. *Crop Science* 39: 1762–1768.
- Ismail, A.M. and A. E. Hall. 2000. Semidwarf and standard-height cowpea responses to row spacing in different environments. *Crop Science* 40: 1618–1623.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1997. Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. *Crop Science* 37: 1270–1277.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1999a. Purification and partial characterization of a dehydrin involved in chilling tolerance during seedling emergence of cowpea. *Plant Physiology* 120: 237–244.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1999b. Allelic variation of a dehydrin gene co-segregates with chilling tolerance during seedling emergence. *Proceedings of the National Academy of Science* 96: 13566–13570.
- Ismail, A.M., A.E. Hall, and J.D. Ehlers. 2000. Delayed-leaf-senescence and heat-tolerance traits mainly are independently expressed in cowpea. *Crop Science* 40: 1049–1055.
- Menéndez, C.M., A.E. Hall, and P. Gepts. 1997. A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. *Theoretical and Applied Genetics* 95: 1210–1217.
- Mutters, R.G., A.E. Hall, and P.N. Patel. 1989. Photoperiod and light quality effects on cowpea floral development at high temperatures. *Crop Science* 29: 1501–1505.
- Singh, B.B. 1998. Screening for heat tolerance. Page 42 *in* Project 11 Cowpea–cereals systems improvement in the dry savannas. Annual Report 1998, IITA, Ibadan, Nigeria.
- Thiaw, S., A.E. Hall, and D.R. Parker. 1993. Varietal intercropping and the yields and stability of cowpea production in semiarid Senegal. *Field Crops Research* 33: 217–233.

## Recent progress in cowpea breeding

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### Abstract

Considerable progress has been made in breeding improved cowpea varieties in the last five years. The major breeding objectives were to develop high yielding cowpea varieties for sole cropping as well as intercropping with acceptable seed types and resistance to major diseases, insect pests, nematodes, and the parasitic plants *Striga* and *Alectra* and tolerance to heat and drought. Good progress was also made in breeding early maturing grain type, dual purpose, and fast growing fodder type cowpea varieties. The informal network of world cowpea researchers catalyzed by IITA and the Bean/Cowpea Collaborative Research Support Program has been very effective in evaluating and selecting improved cowpea varieties for a wide range of environments. As a consequence, total world cowpea production has substantially increased.

### Importance

Cowpea is an important food legume and an essential component of cropping systems in the drier regions of the tropics covering parts of Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America. Being a fast growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen, and its decaying residues contribute to soil fertility. Cowpea is consumed in many forms: the young leaves, green pods, and green seeds are used as vegetables; dry seeds are used in various food preparations; and the haulms are fed to livestock as nutritious supplement to cereal fodder. In West and Central Africa, cowpea is of major importance to the livelihoods of millions of people providing nourishment and an opportunity to generate income. Trading fresh produce and processed food and snacks provide rural and urban women with the opportunity for earning cash income and, as a major source of protein, minerals, and vitamins in daily diets, it positively impacts on the health of women and children. The bulk of the diet of rural and urban poor Africa consists of starchy food made from cassava, yam, plantain and banana, millet, sorghum, and maize. The addition of even a small amount of cowpea ensures the nutritional balance of the diet and enhances the protein quality by the synergistic effect of high protein and high lysine from cowpea and high methionine and high energy from the cereals. This nutritious and balanced food ensures good health and enables the body to resist infectious diseases and slow down their development.

### World production of cowpea

Singh et al. (1997) estimated a world total of about 12.5 million ha grown to cowpea with a production of 3 million tonnes (t). The exact statistics are still not available but there

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seems to be an increase in the area as well as production since 1997. The available data on area, production, and average yield of cowpea in 11 selected countries (Table 1) totals 11.3 million ha and 3.6 million t. The estimated area and production in over 50 other countries in Asia, Africa, and Central and South America that grow cowpea would make a world total of over 14 million ha and 4.5 million t. Nigeria is the largest producer and consumer of cowpea with about 5 million ha and over 2 million t production annually. Each Nigerian eats cowpea and the per capita consumption is about 25 to 30 kg per annum. Niger Republic is the next largest producer with 3 million ha and over 650 000 t production. Northeast Brazil grows about 1.5 million ha of cowpea with about 491 558 t production that provides food to about 25 million people. In Brazil as a whole, per capita consumption of cowpea is about 20 kg annually. In southern USA, about 40 000 ha of cowpea is grown with an estimated 45 000 t annual production of dry cowpea seed and a large amount of frozen green cowpeas. India is the largest cowpea producer in Asia and together with Bangladesh, Indonesia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, and other Far Eastern countries, there may be over 1.5 million ha under cowpea in Asia. There is a need to make concerted efforts to collect accurate statistics on cowpea area and production in different countries.

## Progress in cowpea breeding

Recent reviews by Singh et al. (1997) and Hall et al. (1997) have described progress in cowpea breeding in different regions of the world. The aim of this paper is to update both articles. The International Institute of Tropical Agriculture (IITA) continues to be the center for cowpea research. However, recently, cowpea improvement programs at the University of California, Riverside (USA) and Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil have been strengthened and expanded. Significant research on various aspects of cowpea improvement is also being done in Burkina Faso, India, Mali, Nigeria, and Senegal, and to a lesser extent in a number of other countries. A brief review of the progress made is presented.

## Breeding methods

Singh (1996) reported the results of an experiment conducted to ascertain whether segregating populations such as  $F_2$ ,  $F_3$ ,  $F_4$ ,  $F_5$ , and others should be grown under intercrop or sole

**Table 1. Major cowpea growing countries in the world (1999–2000).**

| Country      | Area under cowpea (ha) | Production (t) | Yield (kg/ha) |
|--------------|------------------------|----------------|---------------|
| Nigeria      | 5 050 100              | 2 108 000      | 417           |
| Niger        | 3 800 000              | 650 000        | 171           |
| Brazil       | 1 500 000              | 491 558        | 324           |
| Mali         | 512 455                | 113 000        | 220           |
| Tanzania     | 145 000                | 46 000         | 317           |
| Myanmar      | 105 000                | 100 000        | 952           |
| Uganda       | 64 000                 | 64 000         | 1000          |
| Haiti        | 55 000                 | 38 500         | 700           |
| USA          | 40 000                 | 45 000         | 1000          |
| Sri Lanka    | 15 000                 | 12 120         | 808           |
| South Africa | 13 000                 | 5600           | 430           |
| Total        | 11 299 555             | 3 669 778      | 324           |

Source: FAOSTAT and national reports.

crop for selecting high yielding lines for intercropping. Two crosses involving IT89KD-374 and IT89KD-288 as local improved parents and IT90K-48-1, which is resistant to aphid, bruchid, thrips, and *Striga* and *Alectra*, were made in 1990 and  $F_2$  seeds from the two populations were subdivided into two sets each. One set was grown in sole crop with two insecticide sprays and the other set was grown under intercropping with millet, without insecticide spray in 1991. The  $F_3$  progenies selected from these populations were grown in sole crop and intercrop, respectively, maintaining separate sole crop and intercrop streams in 1992. Likewise  $F_4$  progenies were grown in separate streams in 1993,  $F_5$  progenies in 1994, and  $F_6$  progenies in 1995. The standard pedigree method was followed to select desirable plant/progenies while evaluating  $F_2$  to  $F_6$  generations. The promising  $F_6$  progenies were bulk harvested in 1995 and multiplied in the dry season for a yield trial under intercrop and sole crop in the 1996 crop season. A total of 52  $F_6$  lines selected from the segregating progenies of the two crosses advanced in sole crop and intercrop streams were yield tested along with eight checks, including the original parents as well as best local and improved checks. The trial included sole crop and a combination of 1-row millet with 1-row cowpea intercropped with and without spray of insecticide. The grain and fodder yields of the breeding lines selected under intercropping were significantly better than those selected under sole crop averaged over the two crosses. The mean grain yield of all the lines derived from the sole crop was 1149 kg/ha in sole-crop sprayed and 190 kg/ha in intercrop with no spray, compared to 1328 kg/ha and 265 kg/ha, respectively, of the lines derived from intercrop. This indicated that selection under intercropping without spray is more effective for higher yield than selection under sole crop. This may be due to greater stress and selective pressure under intercropping.

In a comparative study of different breeding methods, the mean performance of  $F_3$  progenies derived from single seed descent method was better than that of progenies developed via single plant selection for yield and yield components (Mehta and Zaveri 1997). Also, the broad-sense heritability was higher in the population developed through the single seed descent selection method. Vishwanathan and Nadarajan (1996) conducted  $G \times E$  analysis of several cowpea varieties and they observed IT86D-1056 and C04 cowpea varieties to be the most stable. Singh (2000) showed that by testing and selection of varieties at known hot spots for different diseases, insect-pests, and *Striga/Alectra*, the genotype  $\times$  environment interaction can be minimized to ensure stable performance of improved varieties over a wider range of environments. He also showed that by simultaneously testing and selecting under sole crop with only two sprays of insecticide, sole crop without spray and intercrop without spray, high yielding varieties with stable performance with little or no insecticide could be identified (Singh 1999a, 2000). Diallel analysis of six cowpea genotypes and their  $F_1$  hybrids revealed additive gene action for most of the quantitative traits including green fodder and total dry matter (Ponmariammal and Das 1996).

## Interspecific crosses

Gomathinayagam et al. (1998) reported successful crosses between *Vigna vexillata* and *Vigna unguiculata* using embryo culture. They grew the  $F_1$  hybrids and harvested  $F_2$  seeds that were planted and then backcrossed to *V. unguiculata*. However, the resulting backcross seeds looked closer to *Vigna vexillata*. Therefore, there is a need to further examine the progenies obtained from this cross before ascertaining whether this was a true hybrid. Tyagi and Chawla (1999) also reported successful crosses between *Vigna radiata* and

*Vigna unguiculata* using in vitro culture techniques. Gibberellic acid treatment sustained the pods for 9–10 days, which were then used for embryo culture. About 10% of total embryos cultured resulted in plantlet formation. However, the authors did not report further growth and culture of these plantlets and therefore, it is not certain whether the crosses were true hybrids.

Extreme wide crosses have been possible in other crop species using large numbers of pollinations along with newer techniques and perseverance. For example, Knyast et al. (2000) successfully crossed oat (var. Seneca 60 hexaploid) with maize pollen and added maize chromosomes to oat genome. This involved pollinating 60000 oat spikelets by maize pollen 48 hours after emasculation. The spikelets were sprayed with 100 ppm 2-4-D about 48 hours after pollination. A total of 4300 embryos were isolated and cultured on modified M.S medium 14 days after pollination. From these only 379  $F_1$  plantlets developed successfully and these were transferred to pots of which 135 plants survived and had retained one or two maize chromosomes in addition to the complete oat haploid genome. From these four fertile disomic and two fertile monosomic oat-maize addition lines were developed, which are now being used to widen the genetic base of barley and to breed improved varieties with completely new traits. This study indicates that a very large number of pollinations and application of new embryo culture techniques along with a lot of patience is needed to achieve success in wide hybridization. Therefore, there is a need to continue efforts to cross *Vigna vexillata* and other *Vigna* species with cowpea to broaden its genetic base using new emerging techniques.

## Mutations

Adu-Dapaah et al. (1999) reported a fasciated mutant and Singh and Adu-Dapaah (1998) reported a partial sterile mutant, both of which originated spontaneously. The fasciated mutant does not have much breeding value but the partial sterile mutant can be used for facilitating hybridization in cowpea. John (1999) reported 50 Kr of gamma rays to be most effective for inducing mutations in cowpea and Odeigah et al. (1996) obtained several male sterile mutants using gamma rays, ethyl methane sulphonate (EMS), and sodium azide. Saber and Hussein (1998) reported induced mutants using gamma rays showing resistance to rust. Gunasekaran et al. (1998) treated seeds of the cowpea variety C04 with gamma rays and ethidium bromide and analyzed  $M_1$  and  $M_2$  progenies for different agronomic traits. They observed a great deal of variation in  $M_2$  population for different traits and further noticed that gamma rays were more effective in inducing mutation than ethidium bromide.

## Disease resistance

Latunde-Dada et al. (1999) studied the mechanism of resistance to anthracnose in TVx 3236 cowpea. In this variety the initially injected epidermal cells underwent a hypersensitive response restricting the growth of the pathogen. The phytoalexins “kievitone” and “phaseollidin” accumulated more rapidly in the stem tissue of TVx 3236 compared to the susceptible variety. Lin et al. (1995) screened 131 cowpea varieties by artificially inoculating with *Cercospora cruenta* (*Mycosphaerella cruenta*) from which 15 varieties were identified immune and seven resistant. Singh et al. (1997), Singh (1998), and Singh (1999a) developed several cowpea lines with resistance to *Cercospora*, smut, rust, *Septoria*, scab, *Ascochyta* blight, and bacterial blight (Table 2). Some of the varieties, which showed multiple resistance



**Table 2. Sources of resistance to major diseases in cowpea.**

| Diseases           | Sources of resistance  |
|--------------------|--|
| Anthraxnose        | TVx 3236   |
| <i>Cercospora</i>  | IT89KD-288, IT97K-1021-15<br>IT97K-463-7, IT97K-478-10<br>IT97K-1069-8, IT97K-556-4  |
| Smut               | IT97K-556-4, IT95K-1090-12<br>IT95K-1091-3, IT95K-1106-6<br>IAR-48, IT97K-506-6  |
| Rust<br>(Uromyces) | IT97K-1042-8, IT97K-569-9<br>IT97K-556-4, IT97K-1069-8<br>IT95K-238-3, IT97K-819-118<br>IT90K-277-2, IT97K-1021-15<br>IT96D-610, IT86D-719                           |
| <i>Septoria</i>    | TVu 12349, TVu11761, IT95K-398-14<br>IT90K284-2, IT95K-1090-12<br>IT97K-1021-15, IT98K-205-8<br>IT98K-476-8, IT97K-819-118, IT95K-193-12<br>TVu 1234, IT95K-1090-12, |
| Scab               | IT98K-476-8, IT97K-1069-8<br>TVx 3236, IT95K-398-14<br>IT97K-1021-15, IT95K-1133-6   |
| <i>Ascochyta</i>   | TVu 11761  |
| Bacterial blight   | IT95K-398-14, IT95K-193-12<br>IT81D-1228-14, IT95K-1133-6<br>IT97K-556-4, IT97K-1069-8, IT90K-284-2,<br>IT91K-93-1, IT91K-118-20                                     |

were IT97K-1021-15, IT97K-556-4, and IT98K-476-8. Wydra and Singh (1998) screened 90 cowpea breeding lines and identified IT90K-284-2, IT91K-93-10, and IT91K-118-20 to be completely resistant to three virulent strains of bacterial blight. Eight varieties were resistant to two strains and two varieties were resistant to one strain. All the remaining varieties were susceptible to bacterial blight. Santos et al. (1987) screened 156 cowpea varieties under field infestation with smut and identified three highly resistant ones. Nakawuka and Adipala (1997) identified Kvu 46, Kvu 39, and Kvu 454 to be resistant to scab in Uganda. Rodriguez et al. (1997) found L-198 and CNx 377-1E to be resistant to *Macrophomina*. Uday et al. (1996) identified V-265 also to be resistant to *Macrophomina*. In an interesting study, Zohri (1993) artificially inoculated 16 cowpea varieties with *Aspergillus flavus* to monitor aflatoxin production. He found that two cowpea varieties from IITA, IT82E-16 and IT81D-1032, did not support *Aspergillus* growth and therefore no aflatoxin production was observed on these varieties. This indicates the possibility of breeding for resistance to *Aspergillus flavus* in cowpea.

## Resistance to nematodes

Several sources of resistance to nematodes were identified including some of the improved breeding lines with high yield potential (Rodriguez et al. 1996; Roberts et al. 1996, 1997;

Fery and Dukes 1995a; Ehlers et al. 2000a; and Singh 1998). Some of the varieties with high yield and nematode resistance are IT849-2049, IT89KD-288, IT86D-634, IT87D-1463, IT95K-398-14, IT96D-772, IT96D-748, IT95K-222-5, IT96D-610, IT87K-818-18, and IT97K-556-4. Among these varieties, IT89KD-288 was found to be resistant to four strains of *Meloidogyne incognita* in USA (Ehlers et al. 2000a). Singh et al. (1996, 1998a) found IT89KD-288 to be high yielding and highly resistant to nematodes in the trials conducted at Kano (Nigeria), where nematode attack is very severe in the dry season planting with irrigation. IT89KD-288 was taken by one farmer in 1994 and through farmer to farmer diffusion, it has become a popular variety because of its nematode resistance and high yield in the dry season. Cowpea cultivation in the dry season was not possible before because all the local cowpea varieties were susceptible to nematodes.

## Resistance to viruses

Singh and Hughes (1998, 1999) reported several cowpea breeding lines to be completely resistant to cowpea yellow mosaic, blackeye cowpea mosaic, and cowpea aphid borne mosaic. Of these IT96D-659, IT96D-660, IT97K-1068-7, and IT95K-52-34 were most promising in terms of resistance and yield potential. Bashir et al. (1995) screened several cowpea varieties from IITA and observed that IT86F 2089-5, IT86D-880, IT90K-284-2, IT90K-76, IT86D-1010, and IT87D-611-3 were immune to blackeye cowpea mosaic. Van-Boxtel et al. (2000) artificially screened 14 cowpea varieties with three isolates of blackeye cowpea mosaic and 10 isolates of cowpea aphid borne mosaic virus in order to identify lines with multiple strain resistance. They observed that cowpea breeding lines IT86D-880 and IT86D-1010 were resistant to all the three isolates of blackeye cowpea mosaic and five strains of cowpea aphid borne mosaic. IT82D-889, IT90K-277-2, and TVu 201 showed resistance to one or the other of the five remaining isolates and thus by using the abovementioned five cowpea varieties as parental lines, it is possible to breed new cowpea varieties with combined resistance to all the 13 strains of the viruses.

The most important factors that constrain cowpea production in the northeastern region of Brazil are the virus diseases, caused mainly by cowpea severe mosaic virus (CSMV) of the group Comovirus, cowpea aphid borne mosaic virus (CABMV) of the group Potyvirus, cucumber mosaic virus (CMV) of the group Cucumovirus, and cowpea golden mosaic virus (CGMV) of the group Geminivirus (Lima and Santos 1988). Substantial efforts have been made in breeding for resistance to viruses and progress has been made. Lima and Nelson (1977) identified the cultivar Macaibo as having immunity to CSMV while Vale and Lima (1995) showed that inheritance of this resistance is conditioned by a recessive gene. Rios and Neves (1982) confirmed the immunity of Macaibo and a new source of resistance to CSMV in line FP 7733-2, from which the variety CNC 0434 was developed (Rios et al. 1982). This variety was recommended for cultivation in the state of Maranhão (EMBRAPA 1986). Lima et al. (1986), in a study that involved 248 genotypes, identified four new genotypes (TVu 379, TVu 382, TVu 966, and TVu 3961) as being immune to CSMV and CABMV. Cultivars Cowpea 535, Dixiecream, Bunch Purple Hull, Lot. 7909-Purple, V-17, and TVu 612 were immune only to CABMV. Lima et al. (1998), in another study that involved 44 genotypes, confirmed the immunity of genotypes TVu 379, TVu 382, TVu 966, and TVu 3961 to three strains of CSMV. Santos and Freire Filho (1986) screened 450 genotypes for resistance to CGMV. Of those genotypes, 57 were classified as highly resistant, among these being CNC 0434, TVu 612, CE-315 (TVu 2331), and

BR 1-Poty. Three lines from the EMBRAPA cowpea breeding program, TE87-98-8G, TE87-98-13G, and TE87-108-6G and two lines introduced from IITA, IT84S-2135 and IT84S-1627, were found to be resistant to CABMV and immune to CMV by the Laboratory of Virology of the Center of Agrarian Sciences of the Federal University of Ceará. Two other lines from IITA, IT85F-2687 and IT86D-716, were immune to both viruses (Rocha et al. 1996). These resistance sources have been used in cowpea improvement in Brazil. Several varieties that have been released commercially, and breeding lines that are still under evaluation were developed from crosses with the varieties CNC 0434, Macaíbo, and TVu 612. Resistance to CSMV, CABMV, and CGMV has already been incorporated in some of the released varieties like BR 10-Piauí (Santos et al. 1987), BR 12-Canindé (Cardoso et al. 1988), BR 14-Mulato (Cardoso et al. 1990), BR 17-Gurguéia (Freire Filho et al. 1994), EPACE 10 (Barreto et al. 1988), Setentão (Paiva et al. 1988), IPA 206 (IPA, 1989), and BR 16-Chapeo-de-couro (Fernandes et al. 1990b). Presently, crosses are being made to improve resistance to CMV.

## Resistance to *Striga* and *Alectra*

Cowpea suffers considerable damage due to *Striga gesnerioides* in West and Central Africa and to *Alectra vogelii* in West and Central Africa as well as in eastern and southern Africa. Good progress has been made in breeding improved cowpea varieties with combined resistance to *Striga* and *Alectra* (Atokple et al. 1995, Berner et al. 1995, Singh and Emechebe 1997, Singh et al. 1997, Singh 2000). The most promising new cowpea varieties are IT93K-693-2, IT95K-1090-12, IT97K-499-39, IT97K-497-2, and IT97K-819-154 with combined resistance to *Striga* and *Alectra* and major diseases. The details of breeding for *Striga* and *Alectra* resistance are presented in this volume by B.B. Singh.

## Insect resistance

Insect pests are a major constraint in cowpea production. Considerable progress has been made in the last four years in developing cowpea varieties resistant to several insects. Pandey et al. (1995) reported TVu 908 to be resistant to leaf beetles. Singh et al. (1996) reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Of these, IT90K-76, IT90K-59, and IT90K 277-2 are already popular varieties in several countries. Among the new varieties IT97K-207-15, IT95K-398-14, and 98K-506-1 have a high level of bruchid resistance (Singh 1999c). Nkansah and Hodgeson (1995) confirmed resistance of TVu 801 and TVu 3000 to the Nigerian aphid strain but found that the two lines were susceptible to aphids from the Philippines. Similar differential reactions to aphids has been observed in the USA (A.E. Hall, personal communication) indicating the existence of different aphid strains. Shade et al. (1999) also reported a virulent strain of bruchid (*Callosobruchus maculatus*) which was able to cause severe damage to TVu 2027, which is otherwise resistant to the bruchid strain in Nigeria. Yunes et al. (1998) observed that the 7s-storage protein, “vicillin” is responsible for bruchid resistance in cowpea lines related to TVu 2027. Only low levels of resistance have been observed for *Maruca* pod borer and pod bugs, which cause severe damage and yield reduction in cowpea. Jagginavan et al. (1995) observed cowpea lines P120 and C11 to be least damaged by *Maruca* and Veeranna and Hussain (1997) found TVx 7 to be most resistant to *Maruca* and has a high density of trichomes (21.41/mm<sup>2</sup>). Veerappa (1998) screened 45 cowpea lines for resistance to *Maruca* pod borer and observed that the tolerant lines

had higher phenol and tannin contents compared to susceptible lines. This is in line with the general observation that cowpea varieties with pigmented calyx, petioles, pods, and pod tips suffer less damage due to *Maruca*.

As indicated earlier, a distant wild relative of cowpea *Vigna vexillata* has shown high levels of resistance to *Maruca* pod borer and bruchid but all the efforts made at IITA to transfer *Maruca* resistance genes from *Vigna vexillata* to cowpea have not been successful (Fatokun in this volume). Gomathinayagam et al. (1998) reported a successful susceptible cross between *Vigna vexillata* and cowpea and also made a backcross in F<sub>2</sub> generation but the resulting seeds looked like the wild parent (personal communications). This work is not being followed further raising the question whether the original cross and the backcross seeds were true hybrids. Over the last 10 years concerted efforts were made by IITA in collaboration with advanced laboratories in the USA and Italy to transform cowpea with the *Bt* gene for *Maruca* resistance. However, no success has been achieved as yet.

While the wide crosses and transformation of cowpea with the *Bt* gene have not been successful, considerable progress has been made in pyramiding minor genes for field resistance to *Maruca* pod borer and pod bugs through conventional breeding. Singh (1999a) screened new improved cowpea breeding lines for field resistance to major insect pests without insecticide sprays and he observed several cowpea lines with grain yield of 500 kg/ha to 856 kg/ha without any chemical protection. The local variety yielded 0 to 48 kg/ha in the same trials. The most promising varieties are IT90K-277-2, IT93K-452-1, IT94K-437-1, IT97K-569-9, IT95K-222-3, IT97K-837, and IT97K-499-38. These lines are resistant to major foliar diseases, aphid, thrips, and bruchid with pods at a wide angle and suffer less damage due to *Maruca*. IT94K-437-1 and IT97K-499-38 also have combined resistance to *Striga* and *Alectra*. Developed through conventional breeding approaches, the new field resistant lines require only one or two sprays of insecticide for a normal yield of 1.5 to 2.5 t compared to four to six sprays needed for the susceptible varieties.

## Drought, heat, and cold tolerance

Since cowpea is grown in varied environments it encounters different types of stresses including drought, heat, and cold. Good progress has been made at IITA on breeding for enhanced drought and heat tolerance, and at the University of California, Riverside on water use efficiency, heat tolerance, and chilling tolerance (Okosun et al. 1998a, 1998b, Singh et al. 1999a, 1999b; Mai-Kodomi et al. 1999a, 1999b; Hall et al. 1997; Ismail and Hall 1998; Singh 1999e). Simple, cheap, and nondestructive screening methods for drought tolerance have been developed and used to identify and breed for drought tolerant cowpea varieties.

Heat tolerant lines have been developed and heat tolerance is now better understood in cowpea than any other crop (Singh 1999b, Ismail and Hall 1998). Recently the effectiveness of heat tolerance has been quantified using pairs of genetically related and unrelated lines with and without heat tolerance genes (Ismail and Hall 1998). This work is reviewed in detail in this volume by Hall et al. Singh (1999b) grew 102 cowpea breeding lines at IITA Kano Station from March to May when the temperatures ranged from 24 to 27 °C in the night and from 38 to 42 °C during the day. Most of the lines showed severe flower abortion with little or no pods and these were rated as heat susceptible. The most susceptible lines, IT97K-461-2 and IT97K-461-4, showed complete sterility with no development of pollen beyond the microspore stage. These lines are otherwise normal and very high yielding in

the regular crop season (July–October) when day temperatures are below 35 °C and night temperatures below 24 °C. In contrast to the heat susceptible lines, the heat tolerant lines had normal pollen, good pod set, and normal grain yield. The best heat tolerant lines were IT97K-472-12, IT97K-472-25, IT97K-819-43, and IT97K-499-38.

The details of work on chilling tolerance are reviewed in this volume by Hall et al. A dehydrin gene involved in chilling tolerance during seedling stage has been identified (Ismail et al. 1997, 1999) and mapped using recombinant inbred lines (Menendez et al. 1997). The role of the dehydrin in chilling tolerance has been confirmed using near-isogenic lines (Ismail et al. 2000) and efforts are underway to understand the mechanism involved in the control of its expression.

## **Enhanced N-fixation and efficient use of phosphorus**

Significant variation in cowpea rhizobium strains has been observed for nodulation in cowpea (Mandal et al. 1999) but the local rhizobia invariably outpopulate the introduced strains. Therefore, in recent years, major efforts have concentrated on exploiting genetic variability in cowpea as a host for effective nodulation and nitrogen fixation (Buttery et al. 1992). Graham and Scott (1983) observed major genetic differences for nodulation and dry matter and N accumulation among 12 cowpea varieties. They also observed a significant relationship between total N and seed yield and nodule weight. Mandal et al. (1999) also observed significant varietal differences in cowpea for nodule number and nodule weight as well as for nitrogenase activity indicating a good possibility of breeding improved cowpea varieties with enhanced N-fixation. Sanginga et al. (2000) screened 94 cowpea lines and observed major varietal differences in cowpea for growth, nodulation, and arbuscular mycorrhizal fungi root infection as well as for performance under low and high phosphorus. The improved cowpea variety IT86D-715 showed equally good growth under low as well as high phosphorus levels. It also showed better N-fixation than others. Based on its adaptability to grow in low P soils and overall positive N balance, they recommended cultivation of IT86D-715 cowpea variety in soils with low fertility. Kolawale et al. (2000) screened 15 cowpea varieties for tolerance to aluminum and to determine the effect of phosphorus addition on the performance of Al-tolerant lines. The results indicated IT91K-93-10, IT93K-2046-1, and IT90K-277-2 cowpea varieties to be tolerant to aluminum and they gave a higher response to phosphorus fertilization when grown in soils with aluminum toxicity problems. Singh et al. (1998) evaluated improved cowpea varieties under low and high fertility and they also observed major varietal differences. They found IT96D-772, IT96D-739, IT96D-740, and IT96D-666 cowpea varieties to be good performers under low as well as high fertility whereas most other varieties were poor in poor fertility and good in good fertility. These studies further indicate a good possibility of developing improved cowpea varieties with enhanced nitrogen fixation and higher yields under low phosphorus as well as in soils with aluminum toxicity. There is a need for closer interactions between cowpea breeders and soil scientists and soil microbiologists.

## **Improved nutritional quality**

Cowpea is a major source of protein, minerals, and vitamins in the daily diets of the rural and urban masses in the tropics, particularly in West and Central Africa where it complements well with the starchy food prepared from cassava, maize, millet, sorghum, and yam. Systematic efforts have just begun at IITA and a few other institutions to develop

improved cowpea varieties with enhanced levels of protein and minerals combined with faster cooking and acceptable taste. Singh (1999d) screened 52 improved and local cowpea varieties to estimate the extent of genetic variability for protein, fat, minerals etc. On a fresh weight basis (about 10% moisture), the protein content ranged from 20 to 26%, fat content from 0.36% to 3.34%, iron content from 56 ppm to 95.8 ppm, and manganese content from 5 ppm to 18 ppm. The improved cowpea varieties IT89KD-245, IT89KD-288, and IT97K-499-35 had the highest protein content (26%) whereas the local varieties like Kanannado, Bauchi early, and Bausse local had the lowest protein content (21 to 22%). One of the local varieties, IAR 1696, had high protein content (24.78%) and high fat content (3.28%) as well as high iron content (81.55 ppm). Similarly an improved variety, IT95K-686-2, had high protein (25%), high fat content (3.3%), and high iron content (76.5 ppm). Appropriate crosses have been made to study the inheritance of protein, fat, and iron contents and to initiate a breeding program for improving these quality traits. In another experiment, various physical properties of selected cowpea varieties were determined. The relative density of cowpea seed ranged from 1.01 to 1.09, and hardness (crushing weight) ranged from 3.96 kg for IT89KD-288 to 8.4 kg for Aloka local. The seed hardness was positively correlated with cooking time. There have been earlier reports on the extent of genetic variability for quality traits in cowpea. Hannah et al. (1976) reported high methionine content in TVu 2093 and Bush Sitao (3.24–3.4 mg/g) dry seeds compared to 2.75–2.88 mg/g seeds of the check variety G-81-1. Rosario et al. (1980) observed the highest trypsin inhibitor activity in winged bean and lima bean and the lowest activity in mung bean and rice bean whereas the trypsin inhibitor values for cowpea were intermediate. Fashakin and Fasanya (1988) analyzed 10 cowpea varieties and observed a range for protein content from 21.5 to 27% and for iron from 8 to 15 mg/100g dry seeds. Nout (1996) evaluated five newly released cowpea varieties used to make popular snack food, *koose* (also called *akara* and *kosai* in Nigeria). They found that *akara* prepared from high yielding new cowpea varieties Ayiyi (IT83S-728-13) and Bengpla (IT83S-818) were the best. Similarly Singh (1999d) in collaboration with the Women in Agriculture (WIA) section of the Kano Agricultural and Rural Development Authority KNARDA (Nigeria) evaluated three improved cowpea varieties, IT98D-867-11, IT89KD-288, and IT90K-277-2 and one local variety Dan Ila for four popular local dishes—*kosai*, *danwake*, *alale*, and *dafaduka*. These were subjected to an independent taste panel of over 50 persons of different economic status and background. The improved variety IT90K-277-2 was rated as the best and others were as good as the local variety. None of the varieties was rated as unacceptable. IT90K-277-2 has already become very popular in Nigeria and Cameroon as a high yielding variety. These observations indicate that high yield is not negatively correlated with improved nutritional and food quality traits and that sufficient genetic variability exists to improve these traits in cowpea.

## Development and release of cowpea varieties

A large number of cowpea varieties have been released in several countries around the world and the collaborative interactions between the IITA cowpea breeding program and national program scientists have been very effective. A total of 68 countries have identified and released improved cowpea varieties from IITA for general cultivation. The countries and the name of breeding lines released are presented in Table 3. The availability of high yielding disease and insect resistant varieties with desired seed and growth types is quietly

**Table 3. Countries that have released IITA developed improved cowpea varieties.**

| Country                     | Variety released  | Country                        | Variety released   |
|-----------------------------|---|--------------------------------|--|
| Angola                      | TVx 3236  | Argentina                      | IT82D-716  |
| Australia                   | IT82E-18 (Big Buff)   | Belize                         | VITA-3, IT82D-889, IT82E-1   |
| Benin Republic              | VITA-4, VITA-5,<br>IT81D-1137,<br>IT84S-2246-4  | Bolivia                        | IT82D-889, IT83D-442   |
| Botswana                    | ER-7, TVx 3236  | Brazil                         | VITA-3, VITA-6, VITA-7,<br>TVx 1836-01J  |
| Burkina Faso                | TVx 3236, VITA-7<br>(KN-1)  | Burma                          | VITA-4 (Yezin-1)   |
| Cameroon                    | IT81D-985 (BR1),<br>IT81D-994, (BR2),<br>TVx 3236,<br>IT88D-363 (GLM-92),<br>IT90K-277-2 (GLM-93)   | Central<br>African<br>Republic | VITA-1, VITA-4, VITA-7,<br>VITA-5, TVx 1948-01F,<br>IT81D-1137, IT83S-818,<br>IT82E-18, IT81D-994                        |
| Costa Rica                  | VITA-1, VITA-3,<br>VITA-6, VITA-7   | Colombia                       | IT83S-841  |
| Cuba                        | IT84D-449 (Titan)<br>IT84D-666 (Cubinata-666)<br>IT86D-314 (Mulatina-314)<br>IT86D-368, (IITA-Precoz)<br>IT86D-782 (Tropico-782)<br>IT86D-792 (Yarey-792)<br>IT88S-574-3 (OR 574-3) | Côte<br>d'Ivoire               | IT88D-361, IT88D-363   |
| Democratic<br>Rep. of Congo | VITA-6, VITA-7<br>IT89KD-349,<br>IT89KD-349,<br>IT89KD-389,<br>IT89KD-355   | Cyprus                         | IT85D-3577   |
| Egypt                       | TVu 21, IT82D-716<br>IT82D-709, IT82D-812,<br>IT82E-16  | El Salvador                    | TVx 1836-013J<br>(Castilla deseda),<br>VITA-3 (TECPAN V-3),<br>VITA-5 (TECPAN V-5)                                       |
| Ghana                       | IT82E-16 (Asontem)<br>IT83S-728-13 (Ayiya)<br>IT83S-818 (Bengpla)<br>TVx 1843-1C (Boafa)<br>TVx 2724-01F (Soronko)  | Ecuador                        | VITA-3   |
| Guinea Bisau                | IT82E-9, IT82D-889  | Ethiopia                       | TVx 1977-01D, IT82E-16,<br>IT82E-32  |
| Guatemala                   | VITA-3  | Equatorial<br>Guinea           | IT87D-885  |
|                             |   | Fiji                           | VITA-1, VITA-3   |
|                             |   | Gambia                         | IT84S-2049, (Sosokoyo)<br>IT83S-728-13   |
|                             |   | Guinea<br>Conakry              | IT81D-879, IT83D-340-5,<br>IT82E-16, IT85F-867-5<br>(Pkoku Togboi)<br>IT85F-2805, IT83S-990,<br>IT87S-1463, IT84S-2246-4 |
|                             |   | Guyana                         | ER-7, TVx 2907-02D,<br>TVx 66-2H,<br>VITA-3, IT87D-611-3   |
|                             |   | Haiti                          | VITA-4, IT87D-885  |

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**Table 3 (continued)**

| Country      | Variety released  | Country     | Variety released  |
|--------------|---|-------------|---|
| India        | VITA-4, TVx 1502,<br>IT85E2020 (Vamban 1)   | Jamaica     | VITA-3, ER-7, IT84S-2246-4,<br>IT82E-124  |
| Lesotho      | IT82E-889, IT87D-885<br>IT82E-16, IT82E-32  | Liberia     | IT82D-889, TVx 3236,<br>VITA-5, VITA-4, VITA-7  |
| Malawi       | IT82D-889, IT82E-16<br>IT82E-25   | Mali        | TVx 3236, IT89KD-374<br>(Korobalen)<br>IT89KD-245 (Sangaraka)   |
|              |   | Mozambique  | IT82D-812, IT83S-18,<br>IT85F-2020  |
| Mauritius    | TVx 3236  |             |   |
| Namibia      | IT81D-985, IT89KD-245-1, IT87D-453-2  | Nicaragua   | VITA-3  |
| Nepal        | IT82D-752 (Aakash)<br>IT82D-889 (Prakash)   | Nigeria     | TVx 3236, IT81D-994,<br>IT86D-719, IT88D-867-11,<br>IT89KD-349, IT86D-721,<br>IT88D-867-11, IT82E-60,<br>IT89KD-374, IT90K-277-2, |
| Niger        | IT89KD-374,<br>IT90K-372-1-2<br>IT90K-82-2,<br>IT89KD-288   |             |   |
| Pakistan     | VITA-4  | Paraguay    | IT86D-1010, IT87D-378-4,<br>IT87D-697-2, IT87D-2075   |
| Panama       | VITA-3  | Philippines | IT82D-889   |
| Peru         | VITA-7  | Senegal     | TVx 3236  |
| Sierra Leone | TVx 1990-01E,<br>IT86D-721,<br>IT86D-719, IT86D-1010,<br>IT82E-32, TVx 3236,<br>TVu 1990,<br>VITA-3 | Somalia     | TVx 1502, IT82D-889<br>IT82E-32   |
| South Yemen  | VITA-5, VITA-7  | South Korea | VITA-5, IT83S-852,<br>IT82D-889   |
| South Africa | IT90K-59,<br>IT82E-16 (Pannar 311)  | Sudan       | IT84S-2163<br>(Daha ElGoz = Gold<br>from sand)  |
|              |   | Swaziland   | IT82D-889 (Umtilane),<br>IT82E-18, IT82E-27,<br>IT82E-71  |
| Sri Lanka    | IT82D-789 (Wijaya)<br>IT82D-889 (Waruni)<br>TVx 309-01EG, VITA-4<br>TVx 930-01B, (Lita)             | Thailand    | VITA-3, IT82D-889   |
|              |   | Uganda      | TVx 3236, IT82E-60  |

.../continued



**Table 3 (continued)**

| Country  | Variety released   | Country   | Variety released                                |
|----------|--|-----------|---|
| Suriname | IT82D-889, IT82-D789<br>(for nematode resistance)                                | USA       | IT84S-2246-4, IT84S-2049,<br>IT89KD-288         |
| Tanzania | TKx 9-11D (Tumaini)<br>TVx 1948-01F (Fahari)<br>IT82D-889 (Vuli-1)<br>IT85F-2020 | Yemen     | TVx 3236, IT82D-789,<br>VITA-5                  |
| Togo     | VITA-5, TVx 3236,<br>IT81D-985, (VITOCO)   | Venezuela | VITA-3, IT81D-795,<br>IT82D-504-4 TVx 1850-01E, |
|          |  | Zambia    | TVx 456-01F, TVx 309-01G,<br>IT82E-16 (Bubebe)  |
|          |  | Zimbabwe  | IT82D-889                                       |

catalyzing rapid increase in cowpea cultivation including its extension in nontraditional areas. Many countries where new cowpea varieties are making a difference, have given specific names to the new varieties and, in some areas, farmers themselves have given names and facilitated farmer to farmer diffusion of seeds. A few examples are Big Buff in Australia; BR-1 in Cameroon; Titan and Cubinata in Cuba; Asontem and Bengpla in Ghana; Akash (sky) and Prakash (light) in Nepal; Sosokoyo in Gambia; Pkoko Togboi in Guinea Conakry; Korobalen and Sangaraka in Mali; Dan IITA (son of IITA) and Dan Bunkure in Nigeria; Pannar 31 in South Africa; Vuli-1 in Tanzania; Dahal Elgoz (gold from the sand) in Sudan; Umtilane in Swaziland; and Bubebe in Zambia.

The US Vegetable Laboratory at Charleston, South Carolina, has released several cowpea cultivars in the past five years. These include the “snap” cultivar Bettersnap (Fery and Dukes 1995b), the cream type cultivar Tender Cream (Fery and Dukes 1996), and the persistent-green cultivars Charleston Greenpack, (Fery 1998), Petite-N-Green (Fery 1999), Green Pixie (Fery 2000), and Green Dixie, (USDA 2000). The persistent-green varieties are an important new market class of cowpea for the freezing industry in the US (Ehlers, Fery, Hall in this volume) because they are virtually identical in appearance to fresh-shelled cowpeas after they are imbibed with water, but the harvesting costs are much lower because persistent-green grains may be harvested dry with fast, efficient combines, and cleaned and stored dry. With the appearance of a freshly harvested vegetable product, low product cost, and ease of storage and handing, the persistent-green cowpea is attractive to vegetable processors for use in new products or blends with other vegetables. This could help increase cowpea consumption in the US and elsewhere. California Blackeye No. 27 (CB27) is a new blackeye cowpea cultivar for producing dry grain that was released by the University of California, Riverside in 1999. CB27 has high yield, heat tolerance, strong, broad-based resistance to root-knot nematodes, resistance to two races of *Fusarium* wilt, excellent canning quality, and a brighter white seed, compared to the standard blackeye variety in California, CB46 (Ehlers et al. 2000b).

Brazil has released 18 varieties in the last 12 years for the northern region. Two of these, Monteiro (Freire Filho et al. 1998) and Riso do Ano (Fernandes et al. 1990a) were obtained through collection and selection in local populations. Sixteen varieties were developed using pedigree breeding methods. Most of these have been mentioned in the

virus resistance section. Dry grain yields during the rainy season typically range from 1000 to 1200 kg/ha, while the production under irrigation during the dry season is from 1500 to 2000 kg/ha. All these varieties were selected under the rainfed system. Therefore, it is possible that varieties can be developed with much higher yields under irrigation if selection is conducted under these conditions. It is worth noting that even with these low yield levels, positive economic returns are realized. To overcome local constraints, varieties are needed with resistance to a wide spectrum of diseases and pests.

Several other varieties have been released in different countries such as Charodi-1 (Sreekumar et al. 1993) and Vamban 1 (IT85F-2020) (Viswanathan et al. 1997) in India; Big Buff (IT82E-18 Imrie, 1995) and Ebony PR (ADTA 1996) in Australia; IT83S-852 and IT82D-889 (Lee et al. 1996) in South Korea; Melakh and Mouride (Cisse et al. 1997) in Senegal; IT87D-611-3 (Singh et al. 1994) in Guyana; Cream 7 (Hassan 1996) in Egypt; IT90K-76, IT90K-277-2, IT90K-82-2 in Nigeria; Sangaraka (IT89KD-374-57) and Korobalen (IT89KD-245) in Mali; INIFAT 93 (Diaz et al. 1997) in Cuba; and GLM 93 (IT90K-277-2) in Cameroon. This is not an exhaustive list as the information from all countries is not available.

## References

- Adu-Dapaah, H.K., B.B. Singh, and C. Fatokun. 1999. A fascinated mutant in cowpea (*Vigna unguiculata* [L.] Walp.). *Acta Agronomica Hungarica* 47: 371–376.
- Atokple, I.D.K., B.B. Singh, and A.M. Emechebe. 1995. Genetics of resistance to *Striga* and *Alectra* in cowpea. *Journal Heredity* 86: 45–49.
- Australia Division of Tropical Agriculture (ADTA). 1996. Variety: “EbonyPR” sny Line 4 A. Application No:96/159. *Plant Varieties Journal* 9(4): 25.
- Berner, D.K., J.G. Kling, and B.B. Singh. 1995. *Striga* research and control—a perspective from Africa. *Plant Disease* 79: 652–660.
- Bashir, M., Z. Ahmed, R. Zafar, and B.A. Malik. 1995. Sources of immunity in cowpea against blackeye cowpea mosaic potyvirus. *Pakistan Journal of Phytopathology* 7(2): 94–97.
- Buttery, B.R., S.J. Partk, and D.J. Hume. 1992. Potential for increasing nitrogen fixation in grain legumes. *Canadian Journal of Plant Science (Canada)*. V. 72 (2): 323–349.
- Barreto, D.P.D., A.A. dos Santos, M.A.W. Quindere, J.C. Vidal, J.P.P. Araujo, E.E. Walt, G.P. Rios, e, B.P. Neves. Epace-10: Nova Cultivar DE Caupi PARA O CEARÁ. Fortaleza: EPACE, 1988. Folder.
- Cardoso, M.J., F.R. Freire Filho, e, C. Athayde Sobrinho. BR I4-Mulato: nova cultivar de feijão macassar para o estado do Piauí. Teresina: Embrapa-Uepae de Teresina, 1990. 4 pp. (Embrapa-Uepae de Teresina. Comunicado Técnico 48.)
- Cardoso, M.J., A.S.A. dos Santos, F.R. Freire Filho, e, A.B. Frota. “BR I2-Canindé”: cultivar de feijão macassar precoce com resistência múltipla a vírus. Teresina: Embrapa-Uepae de Teresina, 1988. 3 pp. (Embrapa-Uepae de Teresina. Comunicado Técnico 39.)
- Cisse, N., M. Ndiaye, S. Thiaw, and A.E. Hall. 1997. Registration of “Melakh” cowpea. *Crop Science* 37(6): 1978.
- Diaz, M., T. Shagarodsky, N. Lastres, F. Canet, and G. Puldon. 1997. INFAT 93, a new variety of cowpea (*Vigna unguiculata*). *Agrotecnia-de-Cuba*. 27(1): 148.
- Ehlers, J.D., W.C. Matthews, A.E. Hall, and P.A. Roberts. 2000a. Inheritance of a broad-based form of nematode resistance in cowpea. *Crop Science* 40: 611–618.
- Ehlers, J.D., A.E. Hall, P.N. Patel, P.A. Roberts, and W.C. Matthews. 2000b. Registration of ‘California Blackeye 27’ Cowpea. *Crop Science* 40: 854–855.
- Embrapa. Centro Nacional de Pesquisa de Arroz e Feijão (Goiânia, GO). 1986. Cultivares de arroz, feijão caupi lançadas em cooperação com o Centro Nacional de Pesquisa de Arroz e Feijão. Goiânia, EMBRAPA-CNPAP Documentos 15: 43–68.

- Fashakin, J.B. and J.I. Fasanya. 1988. Chemical composition and nutritive changes of some improved varieties of cowpea (*Vigna unguiculata*). I. Some selected varieties from the International Institute of Tropical Agriculture, Ibadan, Nigeria. *Tropical Science (UK)* 28 (2): 111–118.
- Fernandes, J.B., J.S. de Holanda, A.A. Simplicio, F. Bezerra Neto, J. Torres, e, J. Rego Neto. 1990a. Comportamento ambiental e estabilidade produtiva de cultivares de caupi no Rio Grande do Norte. *Pesquisa Agropecuária Brasileira* 25(11): 1555–1560.
- Fernandes, J.B., N.A. de Sousa, e, J.S. de Holanda. 1990b. BR I6-Chapéu-de-couro: nova cultivar de feijão macassar para o sertão do Rio Grande do Norte. Natal: EMPARN. Folder.
- Fery, R.L. and P.D. Dukes. 1995a. Registration of US-566, US-567, and US-568 root-knot nematode resistant cowpea germplasm lines. *Crop Science* 35: 1722.
- Fery, R.L. and P.D. Dukes. 1995b. ‘BetterSnap’ southernpea. *HortScience* 30: 1318–1319.
- Fery, R.L. and P.D. Dukes. 1996. ‘Tender Cream’ southernpea. *HortScience* 31: 1250–1251.
- Fery, R.L. 1998. ‘Charleston Greenpack’, a pinkeye-type southernpea with a green cotyledon phenotype. *HortScience* 33: 907–908.
- Fery, R.L. 1999. ‘Petite-N-Green’, a small-seeded, full-season, green cotyledon, pinkeye-type southernpea. *HortScience* 34: 938–939.
- Fery, R.L. 2000. ‘Green Pixie’, a small-seeded, green cotyledon, cream-type southernpea. *HortScience* 35: (in press).
- Freire Filho, F.R., V.Q. Ribeiro, P.H.S. da Silva, e, P.A.C. Carvalh. 1998. Monteiro: cultivar de caupi de tegumento branco para cultivo irrigado, Teresina: Embrapa-Cpamn. Embrapa-Cpamn, Comunicado Técnico 85: 1–3.
- Freire Filho, F.R., A.A. dos Santos, A.G. de Araujo, M.J. Cardoso, P.H.S. da Silva, e, V.Q. Ribeiro. 1994. BR 17-Gurguéia: nova cultivar de caupi com resistência a vírus para o Piauí. Teresina: Embrapa-Cpamn, 6 pp. Embrapa-Cpamn. Comunicado Técnico 61.
- Gomathinayagam, P., S.G. Ram, R. Rathnaswamy, and N.M. Ramaswamy. 1998. Interspecific hybridisation between *Vigna unguiculata* (L.) Walp. and *V. vexillata* (L.) A. Rich. through in vitro embryo culture. *Euphytica* 102(2): 203–209.
- Graham, R.A. and T.W. Scott. 1983. Varietal characteristics and nitrogen fixation in cowpea. *Tropical Agriculture (Trinidad and Tobago)* 60(4): 269–271.
- Gunasekaran, M., U. Selvaraj, and T.S. Raveendram. 1998. Induced polygenic mutations in cowpea (*Vigna unguiculata* L. Walp). *South-Indian Horticulture* 46: 1–2, 13–17.
- Hall, A.E., B.B. Singh, and J.D. Ehlers. 1997. Cowpea breeding. *Plant Breeding Reviews* 15: 215–274.
- Hannah, L.C., J. Ferrero, and D.W. Dessauer. 1976. High methionine lines of cowpea. *Tropical Grain Legume Bulletin (Nigeria)* 4: 9.
- Hassan, H.M. 1996. New selected strains of the cowpea cultivar “Cream 7”. *Alexandria Journal of Agricultural Research* 41(2): 399–406.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1997. Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. *Crop Science* 37: 1270–1277.
- IPA (Recife, PE). Caupi-IPA-206: nova cultivar de feijão macassar (*Vigna unguiculata* [L.] Walp.) tipo moita para Pernambuco. Recife, 1989.
- Ismail, A.M. and A.E. Hall. 1998. Positive and potential negative effects of heat-tolerance genes in cowpea. *Crop Science* 38: 381–390.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1999. Allelic variation of a dehydrin gene co-segregates with chilling tolerance during seedling emergence. *Proceedings of the National Academy of Science* 96: 13566–13570.
- Ismail, A.M., A.E. Hall, and J.D. Ehlers. 2000. Delayed-leaf-senescence and heat tolerance traits mainly are independently expressed in cowpea. *Crop Science* 40: 1049–1055.
- Imrie, B.C. 1995. Register of Australian grain legume cultivars. *Vigna unguiculata* L. (cowpea) cv. Big Buff. *Australian Journal of Experimental Agriculture* 35(5): 678.
- Jagginavan, S.B., K.A. Kulkarni, and S. Lingappa. 1995. Reaction of cowpea genotypes to the damage of pod borer complex. *Karnataka Journal of Agricultural Science* 89(1): 90–93.

- John, S.A. 1999. Mutation frequency and chlorophyll mutations in parents and hybrid of cowpea following gamma irradiation. *Indian Journal of Genetics and Plant Breeding* 59(3): 357–361.
- Knyast, R.G., W.E. Odland, R.J. Okagaki, O. Riera-Lizarazu, S.M. Maguieira, C.D. Russell, H.W. Rines, and R.L. Phillips. 2000. Complete set of maize individual dual-chromosome additions in oat. *Agronomy Abstracts* 2000: 188.
- Kolawale, G.O., G. Tian, and B.B. Singh. 2000. Differential response of cowpea varieties to aluminum and phosphorus application. *Journal of Plant Nutrition* 23: 731–740.
- Latunde-Dada, A.O., R.J. O'Connell, P. Bowyer, and J.A. Lucas. 1999. Cultivar resistance to anthracnose disease of cowpea caused by *Colletotrichum destructivum* O'Gara. *European Journal of Plant Pathology* 105: 445–450.
- Lee, S.M., K.J. Yun, J.B. Tae, S.M. Lee, J.Y. Koo, and B.T. Jeon. 1996. Studies on the growth characteristics and yield of cowpea cultivars for silage. *Journal of the Korean Society of Grassland Science* 16(2): 105–112.
- Lima, J.A. de A., R.C.A. Lima, M.F.B. Gonçalves, and I.M. Sittolin. 1998. Biological and serological characteristics of a genetically different cowpea severe mosaic virus strain. *Virus Reviews and Research* 3(12): 57–65.
- Lima, J.A. de A. and M.R. Nelson. 1977. Etiology and epidemiology of mosaic of cowpea in Ceará Brasil. *Plant Disease Report* 61(10): 864–867.
- Lima, J.A. de A., C.D.G. Santos, e, L.F.S. Silveira. 1986. Comportamento de genótipos de caupi em relação aos dois principais vírus que ocorrem no Ceará. *Fitopatologia Brasileira* 11: 151–161.
- Lima, J.A. de A. e, A.A. Santos. 1988. Vírus que infestam o caupi no Brasil. Pages 507–545 in J.P.P. de Araújo and E.E. Watt. *org O Caupi no Brasil*. Goiânia: EMBRAPA-CNPAP/Ibadan, IITA.
- Lin, M.C., H.P. Chen, Y.F. Wang, W.Z., Zhang, M.C., Lin, H.P., Chen, and Y.F. Wang. 1995. Evaluation of cowpea varieties resistant to cowpea leaf mould (*Cercospora cruenta* Sacc.). *Crop Genetic Resources* 4: 36–37.
- Mai-Kodomi, Y., B.B. Singh, O. Myers Jr., J.H. Yopp, P.J. Gibson, and T. Terao. 1999a. Two mechanisms of drought tolerance in cowpea. *Indian Journal of Genetics* 59: 309–316.
- Mai-Kodomi, Y., B.B. Singh, T. Terao, O. Myers Jr., J.H. Yopp, and P.J. Gibson. 1999b. Inheritance of drought tolerance in cowpea. *Indian Journal of Genetics* 59: 317–232.
- Mandal, J., A. Chattopadhyay, P. Hazra, T. Dasgupta, and M.G. Som. 1999. Genetic variability for three biological nitrogen fixation components in cowpea (*Vigna unguiculata* [L.] Walp.) cultivars. *Crop Research (Hisar)* 18: 222–225.
- Menéndez, C.M., A.E. Hall, and P. Gepts. 1997. A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. *Theoretical and Applied Genetics* 95: 1210–1217.
- Mehta, D.R. and P.P. Zaveri. 1997. Single seed versus single plant selection in cowpea. *Legume Research* 20(2): 130–132.
- Nakawuka, C.K. and E. Adipala. 1997. Identification of sources and inheritance of resistance to Sphaceloma scab in cowpea. *Plant Disease* 81(12): 1395–1399.
- Nkansah, P.K. and C.J. Hodgson. 1995. Interaction between aphid resistant cowpea cultivars and three clones of cowpea aphid and the effect of two light intensity regimes in this interaction. *International Journal of Pest Management* 41: 161–165.
- Nout, M.J.R. 1996. Suitability of high yielding cowpea cultivars for koose, a traditional fried paste of Ghana. *Tropical Science (UK)* 36(4): 229–236.
- Odeigah, P.G.C., A.O. Osanyinpeji, and G.O. Myers. 1996. Induced male sterility in cowpea (*Vigna unguiculata* L. Walp.). *Journal of Genetics and Breeding* 50(2): 171–175.
- Okosun, L.A., M.E. Aken'ova, and B.B. Singh. 1998a. Screening for drought tolerance at seedling stage in cowpea (*Vigna unguiculata* [L.] Walp. I.) The significance of the trait permanent wilting percentage. *Journal of Arid Agriculture* 8: 1–10.

- Okosun, L.A., M.E. Aken'ova, and B.B. Singh. 1998b. Screening for drought tolerance at seedling stage in cowpea (*Vigna unguiculata* [L.] Walp. II.) Selecting for root length and recovery ability traits. *Journal of Arid Agriculture* 8: 11–20.
- Paiva, J.B., E.M. Teófilo, J.H.R. Santos, Lima J.A.A. dos, M.F.B. Gonçalves, e, L. de F. S. Silveira. 1988. “Setentão”: nova cultivar de feijão-de-corda para o estado do Ceará. Fortaleza: UFC, Folder.
- Pandey, K.C., N. Hasan, R.B. Bhaskar, S.T. Ahmed, and K.S. Kohli. 1995. Genetic evaluation of cowpea (*Vigna unguiculata* [L.] Walp.) lines for multiple pest resistance. *Indian Journal of Genetics and Plant Breeding* 55(2): 198–203.
- Ponmariammal, J. and V.L.D. Das. 1996. Diallel analysis for fodder yield and its components in cowpea. *Madras Agricultural Journal* 83(11): 699–701.
- Rios, G.P. e B.P. das Neves. 1982. Resistência de linhagens e cultivares de caupi (*Vigna unguiculata* [L.] Walp) ao vírus do mosaico severo (VMSC). *Fitopatologia Brasileira* 7: 175–184.
- Rios, G.P. E.E. Watt, J.P.P. de Araújo, e, B.P. das Neves. 1982. Cultivar CNC 0434 imune ao mosaico severo do caupi. Pages 113–115 in Reunião nacional de pesquisa de caupi, 1. Goiânia, Resumos. Goiânia: EMBRAPA-CNPAP.
- Roberts, P.A., W.C. Matthews, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to the *Rk* locus in cowpea. *Crop Science* 36: 889–894.
- Roberts, P.A., J.D. Ehlers, A.E. Hall, and W.C. Matthews. 1997. Characterization of new resistance to root-knot nematodes in cowpea. Pages 207–214 in *Advances in cowpea research*, edited by B.B. Singh D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan. Nigeria.
- Rodriguez, I., M.G. Rodriguez, L. Sanchez, and A. Iglesias. 1996. Expression of resistance to *Meloidogyne incognita* in cowpea cultivars (*Vigna unguiculata*). *Revista de Protection Vegetal* 11(1): 63–65.
- Rodriguez, V.J.L.B., M. Menezes, R.S.B. Coelho, and P. Miranda. 1997. Identification of resistance sources on genotypes of cowpea (*Vigna unguiculata* [L.] Walpers) a *Macrophomina phaseolina* (Tass.) Goid., em condicoes de casa-de-vegetacao. *Summa Phytopathologica* 23(2): 170–172.
- Rosario, R.R. del, Y. Lozano, S. Pamorasamit, and M.G. Noel. 1980. The trypsin inhibitor activity of legume seeds. *Philippine Agriculturist* 6(4): 339–334.
- Rocha, M.M., J.A.A Lima, F.R. Freire Filho, C.J.S. Rosal, e, V.C.V. Lima. 1996. Resistência de caupi de tegumento branco a algumas estirpes de comovírus, potyvírus e cucumovírus. Pages 100–101 in Reunião nacional de pesquisa de caupi, 4. Teresina. Resumos. Teresina: EMBRAPA-CPAMN. (EMBRAPA-CPAMN, Documentos 18).
- Santos, A.A. dos, e F.R. Freire Filho. 1986. Genótipos de caupi (*Vigna unguiculata* [L.] Walp.) com resistência de campo ao vírus do mosaico dourado do caupi. Pages 191–203 in *Seminário de pesquisa agropecuária do piauí*, 4. Teresina, Anais. Teresina: EMBRAPA-Uepae de Teresina.
- Santos, A.A. dos, F.R. Freire Filho, e, M.J. Cardoso. 1987. “BR-10 Piauí”, cultivar de feijão macassar (*Vigna unguiculata* [L.] Walp.) com resistência múltipla a vírus. *Fitopatologia Brasileira* 12(4): 402.
- Saber, M.M. and M.H. Hussein. 1998. Induced mutations for resistance to rust disease in cowpea (*Vigna sinensis*). *Bulletin of Faculty of Agriculture, University of Cairo* 49(1): 47–48.
- Sanginga, N., O. Lyasse, and B.B. Singh. 2000. Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant and Soil* 220: 119–128.
- Santos, A.A., M.A.W. Quindere, and M.B. Melo. 1997. Evaluation of cowpea genotypes for resistance to cowpea smut (*Entyloma vignae*). *Fitopatologia Brasileira* 22(1): 77–78.
- Shade, R.E., L.L. Murdock, and L.W. Kitch. 1999. Interactions between cowpea weevil (Coleoptera: Bruchidae) populations and *Vigna* (Leguminosae) species. *Journal of Economic Entomology* 92(3): 740–745.

- Singh, B.B., S.K. Asante, L.E.N. Jackai, and J.d'Hughes. 1996. Screening for resistance to parasitic plants, virus, aphid and bruchid. IITA Annual Report 1996 project 11. Page 24.
- Singh, B.B., S.K. Asante, D. Florini, L.E.N. Jackai, C. Fatokun, and K. Wydra. 1997. Breeding for multiple disease and insect resistance. IITA Annual Report. 1997. Project 11. Page 22.
- Singh, B.B. and H.K. Adu-Dapaah. 1998. A partial male sterile mutant in cowpea. *African Crop Science Journal* 6: 97–101.
- Singh, B.B. 1998. Sources of resistance to septoria, scab, bacterial blight and *Cercospora* leaf shot. IITA Annual Report 1998. Project 11. Pages 24–27.
- Singh, B.B. and J. d' Hughes. 1998. Sources of multiple virus resistance. IITA Annual Report 1998. Project 11. Pages 24–27.
- Singh, B.B. and H. Ajeigbe. 1998. Evaluation of improved cowpea varieties in the Sudan savanna. IITA Annual Report 1998. Project 11. Pages 14–15.
- Singh, B.B. and J. d' Hughes 1999. Sources of multiples virus resistance. IITA Annual Report 1999. Project 11. Page 30.
- Singh, B.B. 1999a. Evaluation of new improved breeding lines without insecticide sprays. IITA Annual Report 1999. Project 11. Page 26.
- Singh, B.B. 1999b. Screening for heat tolerance. IITA Annual Report 1999. Project 11. Page 40.
- Singh, B.B. 1999c. Improved breeding lines with resistance to bruchid. IITA Annual Report 1999. Project 11. Pages 29–30.
- Singh, B.B. 1999d. Breeding for improved quality. IITA Annual Report 1999. Project 11. Pages 31–32.
- Singh, B.B., Y. Mai-Kodomi, and T. Terao. 1999a. A simple screening method for drought tolerance in cowpea. *Indian Journal of Genetics* 59: 211–220.
- Singh, B.B., Y. Mai-Kodomi, and T. Terao. 1999b. Relative drought tolerance of major rainfed crops of the semi-arid tropics. *Indian Journal of Genetics* 59: 1–8.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan. Nigeria.
- Singh, B.B. 2000. Breeding cowpea varieties for wide adaptation by minimizing genotype x environment interactions. Pages 173–181 in *Genotype × environment interactions analysis of IITA mandate crops in sub-Saharan Africa*, edited by I.J. Ekanayake and R. Ortiz. International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Singh, W.M., H. Adams, R. Beveney, and T. Motiram. 1994. Evaluation of the agronomic performance of cowpea (*Vigna unguiculata* [L.] Walp.) varieties in the intermediate savannahs of Guyana. Pages 118–121 in *Annual review conference proceedings, 20–23 October 1992*, National Agricultural Research Institute, Caribbean Agricultural Research and Development Institute, Guyana.
- Sreekumar, S.G., V.G. Nair, and R.B. Asan. 1993. Gujarath cowpea 2 (Chharodi 1): an ideal cowpea variety for intercropping in coconut garden. *Journal of Tropical Agriculture* 31(1): 9–12.
- Tyagi, D.K. and H.S. Chawla. 1999. Effects of seasons and hormones on crossability barriers and in vitro hybrid development between *Vigna radiata* and *Vigna unguiculata*. *Acta Agronomica Hungarica* 47: 147–154.
- Tumwegamire, S., P.R. Rubaihayo, and E. Adipala. 1998. Genetics of resistance to Sphaceloma scab of cowpea. *African Crop Science Journal* 6(3): 227–240.
- Uday, B. and S. Lodha. 1996. *Macrophomina phaseolina* induced changes in plant water relations of resistant and susceptible cowpea genotypes. *Indian Phytopathology* 49(3): 254–259.
- United States Department of Agriculture (USDA). 2000. Notice of release of Green Dixie Blackeye, a green cotyledon blackeye-type southern pea. USDA, ARS, Washington, DC 20250, USA, 28 April 2000.

- Veeranna, R. and M.A. Hussain. 1997. Trichomes as physical barriers for cowpea pod borer *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). Insect Environment 3(1): 15.
- Veerappa, R. 1998. Phenol and tannin reduce the damage of cowpea pod borer *Maruca testulalis*. Insect environment 4: 5–6.
- Van Boxtel, J., B.B. Singh, G. Thottappilly, and A.J. Maule. 2000. Resistance of (*Vigna unguiculata* [L.] Walp.) breeding lines to blackeye cowpea mosaic and cowpea aphid borne mosaic potyvirus isolates under experimental conditions. Journal of Plant Disease and Protection 107: 197–204.
- Vale, C.C. do, e J.A. de A. Lima. 1995. Herança da imunidade da cultivar Macaibo de *Vigna unguiculata* ao vírus do mosaico severo de caupi. Fitopatologia Brasileira, 20(1): 30–32.
- Wydra, K. and B.B. Singh 1998. Breeding for resistance to multiple strains of cowpea bacterial blight. IITA Annual Report 1998. Project 11. Page 27.
- Viswanathan, P.L. and N. Nadarajan. 1996. Genotype  $\times$  environment interaction for grain yield in cowpea. Madras Agricultural Journal. 83(11): 707–708.
- Viswanathan, P.L., S. Murugesan, N. Ramamoorthy, P. Veerabadran, K.S. Jehangir, N. Natarajan, C.V. Dhanakodi, and P.P.R.C. Vamban. 1997. Vamban 1, a new cowpea variety for Tamil Nadu. Madras Agricultural Journal 84(5): 271–272.
- Wang, P.Z., Q.P. Feng, J.L. Yan, and F.X. Han. 1995. Integrated evaluation of elite cowpea germplasm resources. Crop Genetic Resources 3: 14–16.
- Wydra, K. and B.B. Singh 1998. Breeding for resistance to multiple strains of cowpea bacterial blight. IITA Annual Report 1998. Project 11: Pages 25–27.
- Yunes, A.A., M.T. de Sales, M.P. Andrade, R.A. Morais, K.V.S. Fernandes, V.M. Gomes, J. Xavier-Filho, and M.T. de Andrade. 1998. Legume seed cecilins (7S storage proteins) interfere with the development of the cowpea weevil (*Callosobruchus maculatus* [F]). Journal of the Science of Food and Agriculture 76(1): 111–116.
- Zohri, A.A. 1993. Studies on some cowpea cultivars. II. Suitability for *Aspergillus flavus* growth and aflatoxin production. Qatar University Science Journal 13(1): 57–62.

## 1.4

# Breeding and evaluation of cowpeas with high levels of broad-based resistance to root-knot nematodes

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### Abstract

Host-plant resistance to root-knot nematodes (*Meloidogyne* spp.) is often the most practical solution for control of this pest in cowpea. Resistance in current cultivars is conferred by gene *Rk*. This gene has been used extensively by breeders and it provides protection to many isolates of *M. incognita* but only moderate resistance to *M. javanica*. Recently, isolates of *M. incognita* have been identified at multiple sites in California that are virulent to gene *Rk*. Development of cultivars with broad-based resistance would increase the effectiveness of host-plant resistance to root-knot nematodes and simplify nematode management. California Blackeye No. 27 was released in 1999 and has broad-based resistance expressed at a high level due to the additive effect of genes *Rk* plus *rk3*. Improved cultivars are being developed that carry the broad-based resistance gene *Rk*<sup>2</sup>. Twelve sources of additional resistance to root-knot nematodes have been identified recently. Genetic studies on the uniqueness of each source and the potential to pyramid resistance genes are being pursued as part of a comprehensive breeding effort.

### Introduction

Root-knot nematodes (*Meloidogyne* spp.) are distributed widely in warm temperate, subtropical, and tropical regions around the world (Sasser 1980). Nearly all major agronomic, vegetable, and fruit crops, including cowpea, cotton, and tomato, are suitable hosts for one or more root-knot nematode species. At very low population levels, they do little damage to crops. Intensification of cropping with susceptible varieties, particularly on sandy soils, can lead to rapid increases of nematode populations and substantial damage to crops. Soil fumigants are effective in controlling root-knot nematodes and are often used prior to planting high-value orchard or horticultural crops. For most crops, however, genetic resistance and cultural practices, such as periodic fallows and rotation to nonhost crops, are the only practical means of managing these pests. The intensification of agriculture that is occurring in many developing countries will exacerbate root-knot nematode problems. Irrigated land in the tropics that is cropped continuously is especially vulnerable to the buildup of root-knot nematodes to devastating levels. In these situations, cultivation of resistant cowpeas may result in a substantial decrease in root-knot nematode populations. For example, in California in a cover-crop study, resistant cowpea breeding line IT84S-2049 (carrying gene *Rk*<sup>2</sup>) and the resistant cowpea cultivar Iron Clay (carrying gene *Rk*) reduced soil

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population densities of *M. incognita* significantly, compared to fallow and susceptible cowpea treatments (Matthews et al. 1998). The nematode-resistant cowpea cover crops also produced significantly more biomass, compared to the susceptible check, and the reduced nematode populations resulted in significantly higher yields of a susceptible tomato cultivar, compared to yields obtained when planted after susceptible cowpea.

All of the known resistance to root-knot nematodes in cowpea is due to a single gene or locus designated *Rk* by Fery and Dukes (1980), with alleles *rk*, *rk<sup>1</sup>*, *Rk*, and *Rk<sup>2</sup>* (Fery and Dukes 1982; Roberts et al. 1996), except for gene *rk3* whose effectiveness and inheritance are discussed below. Breeders in the USA and elsewhere have incorporated gene *Rk* into many cowpea cultivars developed for dry bean and fresh southernpea production (Table 1). In southeastern USA, gene *Rk* effectively controls *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria* populations (Fery and Dukes 1980). In California, gene *Rk* confers strong resistance to some biotypes of *M. incognita*, but is only partially effective against aggressive isolates of *M. javanica* (Roberts et al. 1997). *Rk*-virulent populations of *M. incognita* were identified at two geographically distinct sites in California in the early 1990s (Roberts and Matthews 1995). Since then, additional sites with *Rk*-virulent field populations of *M. incognita* and *Rk*-aggressive populations of *M. javanica* have been identified (Roberts, personal communication), suggesting that the problem is widespread.

The emergence of root-knot nematode populations that can be damaging to cowpea carrying the *Rk* gene suggests new sources of resistance are needed to ensure the continued effectiveness of resistance as a management tool. In addition, new, broad-based resistance is needed to simplify management decisions, because it is difficult to quantify the virulence profile of root-knot nematodes causing damage in a particular field without conducting expensive and lengthy bioassays. These considerations have prompted efforts to identify new sources of broad-based resistance, to understand its genetic basis, and to incorporate this resistance into cultivars.

## Root-knot nematode resistance classes in cowpea

It is convenient to classify the known resistance to root-knot nematodes in cowpea into three types: (1) resistance conferred by gene *Rk*, (2) broad-based resistance conferred by gene *Rk<sup>2</sup>* and other alleles at the *Rk* locus distinguishable from the resistance conferred by gene *Rk*, and (3) broad-based resistance found in blackeye cultivar CB27 and breeding line H8-8R, which has been shown to be the result of an additive effect of genes *Rk* and *rk3*. Due to the limitations and potential problems in relying on the *Rk* gene as the sole resistance factor in cowpea, we initiated a search for new resistance sources in the early 1990s. Through extensive screening of more than 600 cowpea accessions from the germplasm collection maintained at the University of California (UC) Riverside, we found that IITA breeding lines IT84S-2049 and IT84S-2246 had strong resistance to at least several root-knot nematodes, including isolates of *Rk*-virulent *M. incognita* and *Rk*-aggressive *M. javanica* (Table 1) (Roberts et al. 1992, 1994). In subsequent tests, IT84S-2049 had slightly greater resistance to the marker isolate of *Rk*-virulent *M. incognita* than did IT84S-2246. Therefore, IT84S-2049 was used in a comprehensive genetic study that showed that the resistance is conferred by a dominant allele at the *Rk* locus, or by another tightly linked gene within 0.17 map units of gene *Rk* (Roberts et al. 1996). This allele was designated *Rk<sup>2</sup>*. Accessions PI 441917, PI 441920, and PI 468104 also represent new sources and possess higher levels of resistance to *M. incognita* than *Rk*-cultivar Mississippi Silver (Fery et al.

**Table 1. Summary of level of cowpea resistance to three *Meloidogyne* isolates, genotype (hypothesized genotype in parenthesis), and reference for selected cowpea varieties and accessions.**

| Level of resistance to: <sup>1</sup><br><i>M. incognita</i> |              |           |           |                    |  |   |
|---|--------------|-----------|-----------|--------------------|--|---|
| Cultivar/Accession  | Source       | Avirulent | Virulent  | <i>M. javanica</i> | Genotype                                     | Reference                                 |
| Calif. Blackeye 27  | UC Riverside | Res       | Res       | Res                | <i>RkRk rk3rk3</i>                           | Ehlers et al. 2000a                       |
| IT84S-2049  | IITA         | Res       | Res       | Res                | <i>Rk<sup>2</sup>Rk<sup>2</sup> Rk3Rk3</i>   | Roberts et al. 1996                       |
| IT84S-2246  | IITA         | Res       | Res       | Res                | <i>(Rk<sup>2</sup>Rk<sup>2</sup> Rk3Rk3)</i> | Roberts, unpublished data                 |
| PI441917  | Brazil       | Res(Res)  | Res (Sus) | Res (Sus)          | –  | Fery et al. 1994; (Roberts, unpubl. data) |
| PI 441920   | Brazil       | Res(Res)  | Res (Res) | Res (Res)          | –  | Fery et al. 1994; (Roberts, unpubl. data) |
| Calif. Blackeye 5   | UC Davis     | Res       | Sus       | Mod                | <i>RkRk Rk3Rk3</i>                           | Roberts et al. 1997                       |
| Calif. Blackeye 46  | UC Davis     | Res       | Sus       | Mod                | <i>RkRk Rk3Rk3</i>                           | Roberts et al. 1997                       |
| Iron/Clay   | USA          | Res       | NT        | Mod                | <i>RkRk Rk3Rk3</i>                           | Roberts, unpublished data                 |
| Iron  | USA          | Res       | NT        | Mod                | <i>(RkRk Rk3Rk3)</i>                         | Mackie 1946                               |
| Mississippi Silver  | U Miss       | Res       | Sus       | Mod                | <i>RkRk Rk3Rk3</i>                           | Roberts et al. 1995                       |
| Holstein  | Australia    | Res       | NT        | Mod                | <i>RkRk Rk3Rk3</i>                           | Roberts, unpublished data                 |
| Aloomba   | Australia    | Res       | NT        | NT                 | –  | Roberts, unpublished data                 |
| Christando  | Australia    | Res       | NT        | NT                 | –  | Roberts, unpublished data                 |
| Victor  | USA          | Res       | NT        | Mod                | –  | Roberts, unpublished data                 |
| Carolina Crowder  | USDA         | Res       | NT        | NT                 | <i>RkRk Rk3Rk3</i>                           | Fery and Dukes 1992                       |
| Bettergro Blackeye  | USDA         | Res       | NT        | NT                 | <i>RkRk Rk3Rk3</i>                           | Fery and Dukes 1993                       |
| Bettersnap  | USDA         | Res       | NT        | NT                 | <i>RkRk Rk3Rk3</i>                           | Fery and Dukes 1995a                      |
| TenderCream   | USDA         | Res       | NT        | NT                 | <i>RkRk Rk3Rk3</i>                           | Fery and Dukes 1996                       |
| Chinese Red-WR  | USA          | Res       | NT        | Mod                | –  | Roberts, unpublished data                 |
| N'dout  | Senegal      | Res       | NT        | NT                 | –  | Roberts, unpublished data                 |
| Chinese Red   | USA          | Sus       | Sus       | Sus                | <i>rkrk Rk3Rk3</i>                           | Roberts, unpublished data                 |
| TVu 4552  | Nigeria      | Mod       | Sus       | Sus                | <i>rkrk rk3rk3</i>                           | Ehlers et al. 2000b                       |
| Calif. Blackeye 3   | UC Davis     | Sus       | Sus       | Sus                | <i>rkrk Rk3Rk3</i>                           | Roberts et al. 1997                       |
| Charleston Greenpack  | USDA         | Sus       | NT        | Sus                | <i>rkrk Rk3Rk3</i>                           | Roberts, unpublished data                 |
| Melakh  | Senegal      | Sus       | NT        | Sus                | <i>(rkrk Rk3Rk3)</i>                         | Roberts, unpublished data                 |
| Mouride   | Senegal      | Sus       | NT        | Sus                | <i>(rkrk Rk3Rk3)</i>                         | Roberts, unpublished data                 |
| Cabbage Pea   | USA          | Sus       | NT        | NT                 | <i>(rkrk Rk3Rk3)</i>                         | Roberts, unpublished data                 |
| Red Ripper  | USA          | Sus       | NT        | NT                 | <i>(rkrk Rk3Rk3)</i>                         | Roberts, unpublished data                 |
| Tiny Lady   | USA          | Sus       | NT        | NT                 | <i>(rkrk Rk3Rk3)</i>                         | Roberts, unpublished data                 |

<sup>1</sup>Res = resistant; Mod = moderately resistant; Sus = susceptible to California isolates for work of Roberts or Ehlers et al. and to southeastern US isolates for work of Fery and Dukes.

NT = not tested.

1994). Genetic analysis of these PI accessions indicated that this heightened resistance also is conferred by a single dominant allele at the *Rk* locus. Though more effective than gene *Rk*, these new allelic sources of broad-based resistance are poorly adapted to commercial production in the United States. They have low yields, nonmarketable seed types, and other undesirable traits; therefore, a substantial breeding effort was required to utilize their resistance traits. The resistance in IT84S-2049 has been transferred to large-seeded blackeye cowpea breeding lines adapted to California (Ehlers et al. 1999). While these alleles may be useful for broadening the genetic base of resistance to root-knot nematodes, additional nonallelic resistance is desirable to enhance the durability and perhaps the level of nematode resistance in cowpea.

University of California Riverside blackeye cowpea breeding line H8-8R, originally selected for heat tolerance, was discovered to also have greater levels of resistance to root-knot nematodes than cultivars possessing gene *Rk*, such as California Blackeye 5 (CB5), California Blackeye 46 (CB46), and California Blackeye 88 (CB88) (Roberts et al. 1994). Reproduction and galling on H8-8R caused by *M. javanica* (*Rk*-aggressive) and *Rk*-virulent *M. incognita* were about half that observed on CB5 and CB46 (Ehlers et al. 1996; Roberts et al. 1997). In two years of field testing on ground infested with an *Rk*-virulent population of *M. incognita* and races 3 and 4 of *Fusarium oxysporum* f. sp. *tracheiphilum* (Fusarium wilt), H8-8-27 (a subline of H8-8R) exhibited low galling and had higher yields than entries possessing the *Rk* gene (Ehlers et al. 1995, 1996). H8-8-27 was released as California Blackeye No. 27 (CB27) in 1999 by the California Crop Improvement Association (Ehlers et al. 2000a).

Some of the yield difference observed in these field tests could be attributed to the fact that H8-8-27 also carries a gene that confers resistance to an additional race of Fusarium wilt. The broad-based nematode resistance in H8-8-27 does appear to confer some yield benefit, because it yielded higher than *Rk*-genotypes that also carried the dual resistance to Fusarium wilt. The presence of strong broad-based nematode resistance in H8-8-27 was also indicated by the comparatively low galling scores (in relation to entries carrying gene *Rk*) and low numbers of second-stage root-knot juveniles (extracted from soil at harvest) in a field trial conducted on ground infested with *M. javanica* (Ehlers et al. 1996).

The broad-based nematode resistance found in H8-8R is due to the additive effects of the dominant gene *Rk* and recessive gene *rk3* (Ehlers et al. 2000b). These conclusions were drawn from an allelism test to determine the presence of gene *Rk* in H8-8R and genetic analysis of  $F_1$ ,  $F_2$ , and  $F_2$  – derived  $F_3$  ( $F_{2,3}$ ) generations of crosses between H8-8R and genotypes with gene *Rk* (CB88 and CB46). Resistance assays were conducted with either greenhouse-grown potted plants or a modified growth-pouch technique described by Omwega et al. (1988) in a controlled environment chamber.

The growth-pouch technique employed a commercial seed germination test pouch (16 × 17 cm) that consisted of a paper wick between two sealed sheets of clear plastic. This allowed full view of the developing root system in two dimensions. Ten- to fourteen-day-old plants in pouches were inoculated with second-stage, root-knot juveniles. The nematodes were allowed to develop and complete one generation (indicated when egg masses were observed on the root surface). This took approximately 30 days at 26.7 °C. A nematode egg mass selective dye was used to stain the egg masses, which were then counted under a 10 × magnifying lens to quantify nematode reproduction. Root systems from some pouch tests were processed by extracting nematode eggs with NaOCl

(Hussey and Barker 1973), which were then counted and expressed as eggs per root system and eggs per gram of root. This permitted further discrimination of resistance reactions in the pouch tests.

Resistance in greenhouse tests was determined either by a direct assessment of nematode reproduction on the cowpea plants by counting nematode eggs following their extraction from tomato root systems (Hussey and Barker 1973) or indirectly by using a tomato bioassay technique that consisted of growing susceptible tomatoes in the same soil that hosted the cowpea, and visually rating the tomato root systems for extent of galling.

A *Rk*-avirulent *M. incognita* isolate was used in the allelism test (conducted in growth pouches) to detect the presence of susceptible recombinants in a large  $F_2$  population of a cross between H8-8R and CB88 (a cultivar with gene *Rk*). An *M. javanica* isolate and a *Rk*-virulent *M. incognita* isolate were used to distinguish the heightened resistance phenotype from the phenotype conferred by gene *Rk* in other plant populations ( $F_1$ s,  $F_2$ s, and  $F_3$ s) developed through crosses between H8-8R and genotypes with gene *Rk*.

In the allelism test, a lack of susceptible recombinants indicated that H8-8R, like CB88, is homozygous for gene *Rk* or a similar allele that confers resistance equivalent to that of *Rk* (Ehlers et al. 2000b). This result suggested that H8-8R possesses a unique resistance factor responsible for the enhanced resistance. CB5 is predominant in the pedigree of both CB88 and H8-8R and it is the likely donor of the *Rk* allele presumed present in both lines.

Several tests were conducted with  $F_1$ s obtained from crosses between H8-8-R and CB88 or CB46. In each test, the  $F_1$ s were not distinguishable from the *Rk* parents, indicating that the additional resistance in H8-8R to both *M. javanica* and *Rk*-virulent *M. incognita* is recessive. No differences were found among reciprocal  $F_1$ s, indicating an absence of maternal effects (Ehlers et al. 2000b).

A bimodal  $F_2$  distribution of egg masses per plant was obtained from a preliminary pouch test of a population from the cross CB88  $\times$  H8-8R evaluated for resistance to the *Rk*-virulent *M. incognita* isolate (Ehlers et al. 2000b). This indicated probable segregation of a single gene conferring a higher level of resistance to this isolate. Determination of inheritance was constrained by the difficulty of designating the class of individual plants that fell between the range of the two parents. It was concluded that  $F_3$  family data would provide clearer genotypic separation than data from single, pouch-grown  $F_2$  plants. Therefore, random  $F_{2,3}$  consisting of ten plants per family were evaluated for resistance to the *Rk*-virulent *M. incognita* isolate in growth pouches. The results of three separate tests with  $F_{2,3}$  families were consistent with the segregation of a single recessive gene for a higher level of resistance to this isolate (Table 2) (Ehlers et al. 2000b).

A similar conclusion was reached for the inheritance of resistance to the *M. javanica* isolate using an  $F_2$  population of 100 plants derived from the cross CB88  $\times$  H8-8R. This  $F_2$  pot test indicated the resistance in H8-8R to *M. javanica* is also controlled by a single recessive gene (Table 2). A greenhouse pot test of  $F_3$  families derived from 20 random plants from the  $F_2$  was conducted to further test the hypothesis. The  $F_3$  families could be placed into three classes: (1) five nonsegregating families with all individuals per family expressing a phenotype equivalent to CB88, (2) five nonsegregating families with all individuals per family expressing a phenotype equivalent to H8-8R, and (3) ten families with individual plants within families segregating for the two phenotypes

**Table 2.** Reaction of parental lines and F<sub>2</sub> plants (CB88 × H8-8R) to *Meloidogyne javanica* (Rk-aggressive) in a greenhouse pot test and reaction of parental lines and randomly selected F<sub>2:3</sub> families (CB88 × H8-8R) to *M. incognita* (Rk-virulent) in growth pouches.

| Generation   | Eggs per g of root | Number of plants or families |                       | Chi-square <sup>‡</sup> | <i>P</i> value |
|--|--------------------|------------------------------|-----------------------|-------------------------|----------------|
|  |                    | Observed                     | Expected <sup>†</sup> |                         |                |
| Tested with <i>Meloidogyne javanica</i> (pot test): Parents    |                    |                              |                       |                         |                |
| CB88   | 36714              | 10                           | —                     | —                       | —              |
| H8-8R  | 20547              | 10                           | —                     | —                       | —              |
| F <sub>2</sub> (CB88 × H8-8R)                                  |                    |                              |                       |                         |                |
| Equivalent to CB88 <sup>§</sup>                                | 41395              | 76                           | 74                    |                         |                |
| Equivalent to H8-8R <sup>§</sup>                               | 18464              | 22                           | 24                    | 0.217                   | 0.50–0.75      |
| Unclassified <sup>‡</sup>                                      | 25381              | 2                            | —                     | —                       | —              |
| Tested with <i>Meloidogyne incognita</i> (pouch test): Parents |                    |                              |                       |                         |                |
| CB88   | 28803              | 24                           | —                     | —                       | —              |
| H8-8R  | 14868              | 30                           | —                     | —                       | —              |
| F <sub>2:3</sub> (CB88 × H8-8R)                                |                    |                              |                       |                         |                |
| Equivalent to CB88 <sup>§</sup>                                | 37449              | 63                           | 64                    | —                       | —              |
| Equivalent to H8-8R <sup>§</sup>                               | 20338              | 23                           | 22                    | 0.062                   | 0.75–0.90      |

<sup>†</sup>Expected numbers of plants in F<sub>2</sub> and numbers of F<sub>2:3</sub> families for a single recessive gene model (1:3).

<sup>‡</sup>Determined using Yates correction for continuity.

<sup>§</sup>Classification of F<sub>2</sub> plants and F<sub>2:3</sub> families based on log<sub>10</sub> transformed data.

<sup>‡</sup>Plants not in the range (mean ± SD) of the parental classes.

Source: Ehlers et al. (2000b).

(Ehlers et al. 2000b). The 20 F<sub>3</sub> families segregated 1:2:1, thereby confirming that the additional resistance to *M. javanica* in H8-8R is controlled by a single recessive gene unlinked to gene *Rk*.

To test whether the single recessive gene identified by the *Rk*-virulent *M. incognita* isolate was the same gene conferring resistance to the *M. javanica* isolate, an identical set of 28 F<sub>3</sub> families was evaluated for resistance to both nematodes. Five of the seven F<sub>3</sub> families that were identified as homozygous for resistance to *M. incognita* (resistance equivalent to H8-8R) were also identified as being homozygous for resistance to *M. javanica* (Ehlers et al. 2000b). Although this was not an absolute confirmation, it was strong evidence that the same gene operates to confer the additional resistance to both nematodes.

The single recessive gene identified in this study could be viewed as a modifier gene that operates either as a recessive enhancer of gene *Rk* or as a dominant suppressor of *Rk* (Roberts et al. 1997). An alternative hypothesis is that this recessive gene confers resistance that is independent of the resistance controlled by the *Rk* gene, and that the two genes combine to give the higher level of resistance observed in H8-8R. To test the second hypothesis, examination of the pedigree of H8-8R (UCR breeding line 336 × UCR breeding line 1393) suggests that UCR 1393, and not UCR 336, donated the recessive resistance gene. UCR 336 was the result of a cross between a susceptible parent (CB3) and a parent known to carry only the *Rk* gene (CB5). Among the parental lines used to develop UCR 1393, the most likely donor(s) of a resistance gene unlinked to the *Rk* gene would be Prima and TVu4552. Therefore, we hypothesized that the *Rk* allele most likely came from UCR 336, and the recessive resistance allele came from UCR 1393.

An assessment of TVu4552 and Prima in growth pouches and inoculated with an *Rk*-avirulent *M. incognita* isolate was conducted to determine the probable source of the recessive allele and the nature of its resistance. In this test, the susceptible check (CB3) had a mean egg mass count of 89 (range 37 to 152), and the nematode was controlled by the resistant (gene *Rk*) check (CB46) (mean of 1; range 0 to 3). While Prima was clearly susceptible (mean of 86; range 27 to 155), TVu4552 had a mean of 35 (range 14 to 50) egg masses per root system. Analysis of variance of these data showed that TVu4552 was significantly more resistant than CB3 but significantly more susceptible than CB46. Prima was not different from CB3 (Ehlers et al. 2000b). These results suggest that TVu4552 carries some resistance, possibly the recessive gene, but not *Rk*.

TVu4552 was crossed reciprocally to susceptible CB3 to test whether the resistance of TVu4552 is recessive and thus the probable donor of the gene identified in H8-8R. The parents, reciprocal F<sub>1</sub>s, and a known *Rk* genotype, CB46, were screened in growth pouches using the same *Rk*-avirulent *M. incognita* isolate as before. The results indicated the moderate resistance observed in TVu4552 is recessive (Ehlers et al. 2000b). The symbol *rk3* was proposed for this recessive resistance gene and the probable genotype of TVu4552 is *rkrlrk3rk3* (Table 1).

The high level of resistance observed in H8-8R appears to be the result of an additive effect of the moderate resistance in TVu4552 conferred by a single recessive gene, *rk3*, and dominant gene *Rk*. The occurrence of a root-knot nematode resistance gene in cowpea not linked to the *Rk* locus is an important finding in that when it is combined with *Rk*, strong, broadened resistance results that is effective against *Meloidogyne* spp. isolates that have become virulent to the *Rk* gene. Identification of an independent resistance locus opens up the possibility for new gene combinations that may provide resistance that is

more effective than resistance based on *Rk*. For example, it is possible that combining *rk3* with the *Rk<sup>2</sup>* resistance discovered by Roberts et al. (1996), which confers a higher level of resistance than *Rk* to *Rk*-virulent *M. incognita* and *M. javanica*, could lead to an even higher level of resistance that approaches immunity to virulent isolates.

### Breeding *Rk<sup>2</sup>* resistance into large-seeded blackeyes

The broad-based resistance (*Rk<sup>2</sup>*) present in IITA breeding line IT84S-2049 has been transferred to large-seeded blackeye breeding lines that are well adapted to California. Two crossing cycles back to adapted large-seeded blackeye cowpea cultivars was sufficient to obtain elite large-seeded lines with nematode resistance equivalent to IT84S-2049. In the crossing program, blackeyes fully susceptible to root-knot nematode were used in crosses to IT84S-2049. In this way, resistant *Rk<sup>2</sup>* segregants could be identified easily without the potential confounding effects due to the presence of both *Rk* and *Rk<sup>2</sup>* phenotypes in the populations. Several of the lines with *Rk<sup>2</sup>* resistance are expected to be included in multilocation yield trials in California in 2001.

### New gene combinations and sources of resistance

The existing array of root-knot nematode resistance genes consists of two alleles at one locus (*Rk*, *Rk<sup>2</sup>*), and *rk3*. The genotype of CB27 (derived from H8-8R) is *RkRkrk3rk3*, while IT84S-2049 and the derived advanced breeding lines are probably *Rk<sup>2</sup>Rk<sup>2</sup>Rk3Rk3* (Table 1). It is possible that genotype *Rk<sup>2</sup>Rk<sup>2</sup>rk3rk3* would have even greater resistance due to the additive effect of *rk3* and *Rk<sup>2</sup>*. To determine this possibility, *F<sub>2</sub>* progeny of crosses between H8-8R and IT84S-2049 are being screened in growth-pouch tests with *M. javanica* to detect any individual plants expressing the heightened resistance expected from combining *rk3* and *Rk<sup>2</sup>*.

Through recent extensive screening in both field and growth pouch tests, 11 new sources of resistance to root-knot nematodes have been identified in breeding lines from IITA and in accessions from Australia, Botswana, Kenya, and Niger (Table 3). In addition, breeding line 96-11-27, developed at UC Riverside, is a potentially unique source of resistance because it has wild cowpea parentage (from *Vigna unguiculata* ssp. *pubescens*). Genetic studies are underway to characterize the inheritance of these resistance sources and their relationship to *Rk* and *Rk<sup>2</sup>*, and the potential for combining resistance factors to obtain even more effective resistance.

Some of these new sources of resistance have unique phenotypes (Table 3) or *F<sub>1</sub>* dominance relationships (data not shown) that distinguish them from other known resistance sources, suggesting that they may have unique resistance genes. Highly resistant breeding line IT84S-2049 and most of the other accessions that have equivalent effective resistance to *M. javanica* also express very high resistance to *Rk*-avirulent *M. incognita*. An exception is TVu4765. While highly resistant to *M. javanica*, both in terms of suppressing root galling and nematode reproduction, TVu4765 is more susceptible to *M. incognita* (*Rk*-avirulent) than any of the other accessions when compared to the susceptible check. TVu4765 supported nematode reproduction (as measured by number of egg masses) at a level approximately 40% of that seen with the susceptible check, compared to less than 5% for the other accessions tested (Table 3). This difference may be due to either a new gene that is highly resistant to *M. javanica* but less effective against avirulent isolates of *M. incognita* than gene *Rk*, or perhaps an allele on the *Rk* locus with a root-knot isolate specificity clearly discernable from that of the known alleles (*rk*, *Rk*, *Rk<sup>2</sup>*). Allelism tests of crosses between TVu4765 and genotypes with *Rk* and *Rk<sup>2</sup>* should help to elucidate the genetic basis for this interesting and potentially useful resistance source.

**Table 3.** Egg mass production in growth pouches and root galling reaction in a field test to *Meloidogyne javanica* (*Rk*-aggressive) and egg mass production in growth pouches to an *Rk*-avirulent isolate of *M. incognita* on cowpea genotypes possessing different resistance factors.

| Genotype               | Origin <sup>†</sup>   | Resistance factor <sup>‡</sup> | <i>M. javanica</i>                      |                      | <i>M. incognita</i>                     |
|------------------------|-----------------------|--------------------------------|---|----------------------|---|
|                        |                       |                                | Egg masses per root system <sup>§</sup> | Galling <sup>†</sup> | Egg masses per root system <sup>§</sup> |
| CB3                    | UCD                   | <i>rk</i>                      | 384 b                                   | (2.28)               | 69.1                                    |
| CB46                   | UCD                   | <i>Rk</i>                      | 295 c                                   | 2.13                 | 0.8                                     |
| IT93K-503-1            | IITA (Nigeria)        | ??                             | 53 e                                    | —                    | 0.1                                     |
| IT84S-2049             | IITA (Nigeria)        | <i>Rk</i> <sup>2</sup>         | 54 e                                    | 1.10                 | —                                       |
| IT89KD-288             | IITA (Nigeria)        | ??                             | 66 de                                   | 0.50                 | 0.1                                     |
| TVu 4765               | Niger                 | ??                             | 73 de                                   | 0.96                 | 27.5                                    |
| IT82E-18               | IITA (Nigeria)        | ??                             | 79 de                                   | 1.40                 | 0.5                                     |
| IT92KD-370             | IITA (Nigeria)        | ??                             | 80 de                                   | 0.09                 | —                                       |
| Bots 249C              | Botswana              | ??                             | 06 de                                   | 0.67                 | 0.0                                     |
| Bots 514B              | Botswana              | ??                             | 106 de                                  | 0.07                 | 0.0                                     |
| 96-11-27               | UCR wild × cultivated | ??                             | 116 de                                  | (1.90)               | —                                       |
| KVu 515                | Kenya                 | ??                             | 118 de                                  | 1.00                 | 2.0                                     |
| Bots 444               | Botswana              | ??                             | 122 de                                  | 0.07                 | 0.5                                     |
| Bots B1                | Botswana              | ??                             | 126 d                                   | 0.00                 | 0.0                                     |
| TVu 1015               | Nigeria               | ??                             | 130 d                                   | 0.00                 | 0.0                                     |
| IC2899                 | India                 | ??                             | 211 —                                   | 0.51                 | 0.0                                     |
| TVu 8016               | Nigeria               | ??                             | 456 a                                   | 1.20                 | —                                       |
| H8-9                   | UCR                   | <i>rk</i>                      | —                                       | 3.45                 | —                                       |
| LSD ( <i>P</i> = 0.05) |                       |                                | 59                                      |                      |   |

<sup>†</sup>UCD—University of California, Davis; UCR—University of California, Riverside; IITA—International Institute of Tropical Agriculture.

<sup>‡</sup>*rk* = susceptible allele and *Rk*<sup>2</sup> = broad-based resistance allele found in IT84S-2049.

<sup>§</sup>Means of five replicates. Values in column followed by the same letter are not significantly different according to Duncan's multiple range test (*P* < 0.05).

<sup>†</sup>Galling reaction based on a 0 to 4 scale with 0 = clean and 4 = heavily galled; values in parenthesis are from same field site in a different year.

??Not known.



In response to inoculation by *M. javanica*, accessions Bots 444, Bots B1, and TVu1015 supported nematode reproduction that was more than twice as high as in IT84S-2049, but they exhibited virtually no root galling (Table 3). Again, these differences in resistance phenotypes could be accounted for by either the existence of new resistance alleles or loci, or genetic background effects. Further genetic studies are in progress to clarify the nature and effectiveness of these genotypes.

The phenotypes displayed by accessions IC2899 and TVu8016 are of particular interest (Table 3). IC2899 supports virtually no reproduction of *M. incognita*, but supports high levels of reproduction with very low root galling in response to inoculation with *M. javanica*. TVu8016 supports extremely high reproduction of *M. javanica* and yet its galling score is in the range of most of the accessions with relatively high levels of resistance to this nematode, including IT84S-2049. Although they are not likely candidates as strong resistance sources, genetic investigations of IC2899 and TVu8016 may shed light on the relationship between nematode reproduction and galling in cowpea. These lines may possess a gene(s) for controlling the galling reaction, but lack any genes resisting nematode reproduction. Separate genetic control of reproduction and galling in response to root-knot nematodes has been observed in lima bean (*Phaseolus lunatus*), where analyses of F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations of a cross between a resistance source (PI 256874) and a susceptible commercial lima bean identified two independently inherited genes (Matthews et al. 2000). One of the genes was found to confer resistance to nematode reproduction. The second gene was found to confer resistance to the root galling reaction, but had no effect on reproduction. It is reasonable to expect this also may occur in cowpea.

Observations of some phenotypes in cowpea lines and cultivars that were inoculated with an *Rk*-avirulent isolate of *M. incognita* support the possibility of independent genetic control of these responses to root-knot nematodes. For example, we have observed that cultivar CB3 supports high levels of reproduction with little or no galling, whereas cultivar Chinese Red supports high levels of both reproduction and galling. It may be that while both of these cultivars lack genes resisting reproduction, CB3 may carry one or more genes that control the galling reaction to this nematode. Crosses between CB3 and Chinese Red have been made and will be analyzed to investigate this phenomenon.

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## References

- Ehlers, J.D., A.E. Hall, P.A. Roberts, W.C. Matthews, A.M. Ismail, and A.N. Eckard. 1995. Blackeye varietal improvement. Pages 41–55 in University of California Dry Bean Research: 1995 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, USA.
- Ehlers, J.D., A.E. Hall, P.A. Roberts, W.C. Matthews, A.M. Ismail, B.L. Sanden, C.A. Frate, and A.N. Eckard. 1996. Blackeye varietal improvement. Pages 51–54 in University of California Dry Bean Research: 1996 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, USA.
- Ehlers, J.D., A.E. Hall, A.M. Ismail, P.A. Roberts, W.C. Matthews, B.L. Sanden, C.A. Frate, and S. Mueller. 1999. Blackeye varietal improvement. Pages 47–61 in University of California Dry Bean Research: 1996 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, USA.
- Ehlers, J.D., A.E. Hall, P.N. Patel, P.A. Roberts, and W.C. Matthews. 2000a. Registration of California Blackeye 27 Cowpea. Crop Science 40: 854–855.

- Ehlers, J.D., W.C. Matthews, A.E. Hall, and P.A. Roberts. 2000b. Inheritance of a broad-based form of root-knot nematode resistance in cowpea. *Crop Science* 40: 611–618.
- Fery, R.L. and P.D. Dukes. 1980. Inheritance of root-knot nematode resistance in the cowpea (*Vigna unguiculata* [L.] Walp.). *Journal of American Society of Horticultural Science* 105: 671–674.
- Fery, R.L. and P.D. Dukes. 1982. Inheritance and assessment of a second root-knot resistance factor in southernpea (*Vigna unguiculata* [L.] Walp.). *HortScience* 17: 152 (Abstract).
- Fery, R.L. and P.D. Dukes. 1992. Carolina Crowder southernpea. *HortScience* 27: 1335–1337.
- Fery, R.L. and P.D. Dukes. 1993. Bettergro Blackeye southernpea. *HortScience* 28: 62–63.
- Fery, R.L. and P.D. Dukes. 1995a. Registration of US-566, US-567, and US-568 root-knot nematode resistant cowpea germplasm lines. *Crop Science* 35: 1722.
- Fery, R.L. and P.D. Dukes. 1995b. Bettersnap southernpea. *HortScience* 30: 1318–1319.
- Fery, R.L. and P.D. Dukes. 1996. Tender Cream southernpea. *HortScience* 31: 1250–1251.
- Fery, R.L., P.D. Dukes, and J.D. Thies. 1994. Characterization of new sources of resistance in cowpea to the southern root-knot nematode. *HortScience* 29: 678–679.
- Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. *Plant Disease Reports* 57: 1025–1028.
- Mackie, W.W. 1946. Blackeye beans in California. University of California Agricultural Experimental Bulletin 696.
- Matthews, W.C., J.D. Ehlers, W. Graves, P.A. Roberts, and J.V. Samons. 1998. Use of resistant cover-crop cowpeas in crop rotations to reduce levels of root-knot nematodes. Page 114 in 1998 Annual Meeting Abstracts, American Society of Agronomy, 90th Annual Meeting, 18–22 October 1998, Baltimore, Maryland, USA. ASA, CSSA, SSSA, Madison, Wisconsin, USA.
- Matthews, W.C., D.M. Helms, and P.A. Roberts. 2000. Evidence for independent genetic control of reproduction and galling to *Meloidogyne javanica* in lima bean. Page 49 in Program and Abstracts of the 37th Annual Meeting, Society of Nematologists, 24–28 June 2000, Quebec, Canada. SON, Lawrence, Kansas, USA.
- Omwega, C.O., I.J. Thomason, and P.A. Roberts. 1988. A nondestructive technique for screening bean germplasm for resistance to *Meloidogyne incognita*. *Plant Disease* 72: 970–972.
- Roberts, P.A., W.C. Matthews, A.E. Hall, J.D. Ehlers, S.R. Temple, and D.M. Helms. 1992. Black-eye bean tolerance and resistance to root-knot nematodes. Pages 43–49 in University of California Dry Bean Research: 1992 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, USA.
- Roberts, P.A., W.C. Matthews, A.E. Hall, J.D. Ehlers, S.R. Temple, and D.M. Helms. 1994. Black-eye bean tolerance and resistance to root-knot nematodes. Pages 57–59 in University of California Dry Bean Research: 1994 Progress Report. California Dry Bean Advisory Board, Dinuba, CA, USA.
- Roberts, P.A. and W.C. Matthews. 1995. Virulence in *Meloidogyne* spp. to resistance in cowpea. *Nematologica* 41(3): 336 (abstract)
- Roberts, P.A., C.A. Frate, W.C. Matthews, and P.P. Osterli. 1995. Interactions of virulent *Meloidogyne incognita* and *Fusarium* wilt on resistant cowpea genotypes. *Phytopathology* 85: 1288–1295.
- Roberts, P.A., W.C. Matthews, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to the *Rk* locus of cowpea. *Crop Science* 36: 889–894.
- Roberts, P.A., J.D. Ehlers, A.E. Hall, and W.C. Matthews. 1997. Characterization of new resistance to root-knot nematodes in cowpea. Pages 207–214 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Sasser, J.N. 1980. Root-knot nematodes: a global menace to agriculture. *Plant Disease* 64: 36–41.

## 1.5

# Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *Vigna vexillata*

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### Abstract

Cowpea is grown mainly for its protein-rich grains, which is consumed in various forms in sub-Saharan Africa. Average grain yield in farmers' fields is generally low due to a number of biotic and abiotic stresses. The most important of the biotic stress factors causing extensive grain yield losses in cowpea are postflowering insect pests such as the legume pod borer and pod sucking bugs. Availability of varieties with resistance to these pests will be attractive to cowpea farmers as the crop could then be grown with less dependence on expensive, often adulterated chemicals that are not particularly environmentally friendly. To be able to develop such varieties, it is necessary that genes conferring resistance to these pests are available in the cowpea genome. Genes conferring resistance to these pests were found to exist in the genomes of some wild *Vigna* species such as *V. vexillata* and *V. oblongifolia* and efforts were made to transfer these genes from the wild *Vigna* sp. to cowpea. Pods were retained for up to seven days after pollination when *V. vexillata* lines served as female parents with cowpea. Embryos in pods resulting from these crosses did not develop beyond the globular stage. Several procedures aimed at overcoming this incompatibility were adopted without success. Among the techniques used to overcome incompatibility were in vitro culture of interspecific hybrid embryos, hormonal treatments of flower buds prior to pollination, and polyploidization. No interspecific hybrids were obtained following the several attempts made, thus suggesting that very strong cross-incompatibility exists between cowpea and *V. vexillata*.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is grown mainly for its grain, which contains between 22 and 32% protein on a dry weight basis. The grain is one of the cheapest sources of protein in the diets of peoples of West and Central Africa where cowpea is also an important crop. The dried grain is consumed after being processed into different food forms while the haulms from dried and shelled pods as well as fodder, are a good source of quality feed for livestock. Farmers in the dry savanna areas of West and Central Africa derive some income from selling cowpea fodder to livestock owners, particularly during the dry season.

Every stage in the life cycle of cowpea has at least one major insect pest that could cause serious damage and impact yield negatively. When postflowering insect pests infest

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cowpea fields and cause heavy damage to grain yield, farmers, especially those in the dry savanna area, resort to harvesting the fodder in order to get some income. There is, however, no doubt that farmers get more financial benefit from cowpea grain than from fodder. In order for farmers to obtain high grain yield from their cowpea fields it is necessary for them to spray the cowpea plants with insecticides a number of times. Until recently, cowpea required being sprayed with insecticides up to five times or even more if high grain yield was to be obtained. Relatively high grain yield can now be obtained with two or three insecticide sprays. The high grain yield which can now be obtained with fewer insecticide spray regimes can be attributed to progress that has been made through genetic improvement whereby genes for resistance to some diseases and preflowering insect pests such as aphids have been incorporated into new cowpea varieties. Also, there are cowpea lines that combine these resistance genes with low levels of resistance to the flower bud thrips (*Megalurothrips sjostedti*). Even some traditional farmer varieties have also been improved by introgressing into them these generally simply inherited resistance genes. Furthermore, the population dynamics for most of the insects have been studied and information obtained has been found useful to target the time of intervention by farmers. No cowpea line has been identified as possessing the desired levels of resistance to the legume pod borer (*Maruca vitrata*) and pod sucking bugs (*Clavigralla tomentosicollis*, *Anoplocnemis curvipes*, and *Riptortus dentipes*) all of which are postflowering pests. The legume pod borer and pod sucking bugs can cause tremendous grain-yield losses in cowpea if appropriate control measures are not taken.

The most economical and environmentally friendly way of controlling these insect pests would be through host-plant resistance. Introgressing genes for resistance to the insect pests into cowpea should result in the availability of varieties which can be grown by farmers in sub-Saharan Africa with minimal use of chemicals. This will lead to a reduction in the cost of cowpea production, thereby increasing the profit margin for farmers. Essentially cowpea production will become more attractive to the generally resource-poor farmers in the savanna zones of Africa. In addition, the farmers would be healthier as they no longer need to handle toxic chemicals while at the same time pollution of their environment would be immensely reduced. Because of the potentially immense benefits of growing insect-resistant cowpea varieties, no efforts should be spared in the search for and transfer of the desired genes from landraces and wild *Vigna* species to cultivated cowpea. To this end, a wide range of accessions from the cowpea germplasm collection as well as those of its wild and weedy cross-compatible relatives was screened in order to identify those with genes for resistance to the pests that wreak havoc on cowpea production. None of the tested cultivated cowpea lines and their cross-compatible wild relatives showed the desired high level of resistance to these pests. Several accessions of some *Vigna* species, such as those belonging to *V. vexillata*, *V. davyi*, *V. oblongifolia*, and *V. luteola*, were also screened, among others, for resistance to insect pests of cowpea. The results showed that some accessions of *V. vexillata* and *V. oblongifolia* have good levels of resistance to the insect pests that devastate cowpea. The *Vigna* species whose accessions showed resistance to the major postflowering insect pests of cowpea do not belong to same primary or secondary gene pool as cowpea and this could constitute a major constraint to moving the desirable genes into cultivated cowpea varieties.

A phylogenetic study that was carried out involving various *Vigna* species and based on RFLP markers indicated that among species showing high levels of resistance to the

insect pests, *V. vexillata* is the closest to cowpea (*V. unguiculata*) (Fatokun et al. 1993). In this same study, a wild and cross-compatible relative of cowpea *V. unguiculata* ssp. *dekintiana* var. *pubescens* was linked to *V. vexillata* when the various accessions of *Vigna* species tested were displayed on a minimum spanning tree.

The various accessions of *V. vexillata* showed high levels of resistance to pod sucking bugs, flower thrips, *Maruca vitrata*, bruchid, and *Striga gesnerioides* among others. Possession of these traits makes interspecies crosses between it and cowpea very attractive and worth pursuing. Hence, crosses were initiated between cowpea and *V. vexillata* with the aim of transferring the genes conferring resistance to insect pests from the latter to cowpea. While making these crosses (*V. vexillata* × cowpea) it was observed that some pods were retained for up to seven days or even more when cowpea is the pollen parent. However, in the reciprocal crosses pods were not retained as emasculated flowers drop within one day following cross pollination. On the other hand, the pods that were retained by pollinating *V. vexillata* with cowpea developed slowly as the seeds contained therein. By the time these pods attained their maximum size, they only approximated the size attained by four-day-old pods resulting from selfing. In all the crosses, flowers were emasculated and pollinated a day before anthesis. This was to ensure that pollen tubes reached the ovule in order to release the male (sperm) nuclei in time for fertilization to take place. No viable interspecific hybrid seed was obtained from any of the several hundreds of crosses made, thus suggesting a strong cross incompatibility between the two species, *V. vexillata* and *V. unguiculata*.

## Overcoming interspecies incompatibility

There are a number of procedures that have been used by breeders to overcome barriers that prevent gene exchange between distantly related plant species. These have been used to successfully effect interspecies hybridization in several crops. Among the procedures commonly used are making reciprocal crosses (Thomas and Waines 1982), crossing between different accessions of both species (Harlan and de Wet 1977), polyploidization followed by crossing, polyploidization of the  $F_1$  interspecies hybrid (where the  $F_1$  is sterile), embryo rescue (Przywara et al. 1989), bridging crosses (Hermesen and Ramanna 1973), and hormonal treatment of flower buds prior to or after pollination (Larter and Chaubey 1965; Sitch and Snape 1987), among others. Some of the methods used to overcome constraints to gene exchange through wide crossing in several crops were tried in the attempted cross between cowpea and *V. vexillata* and these are reported in the following sections.

**Crossing several accessions of both species:** Reports of previous wide crossing activities in some crops have shown that hybrids between certain accessions of a species are more productive than others. This is because certain accessions of a species are better combiners with some other individuals of another species. In tobacco (*Nicotiana tabacum*) Pitarelli and Stavely (1975) observed that when three different cultivars were crossed to *N. repanda* only one combination gave  $F_1$  hybrid plants. Harlan and de Wet (1977) also tested a number of *Tripsacum dactyloides* in combination with corn and found that only one of the *T. dactyloides* accessions was effective in transferring genetic material to maize. In the attempted cross between cowpea and *V. vexillata*, some pods are retained on *V. vexillata* when emasculated flowers are pollinated with cowpea but none were retained in reciprocal crosses. It is conceivable that not all lines of *V. vexillata* will respond in the same way as, for example, in the frequency of pod retention when flowers are pollinated with different

cowpea accessions. It is also possible that in some specific combinations the embryos may develop beyond the globular stage. Perhaps some *V. vexillata* lines might even support the development of pods with well-formed seeds to maturity while others do not. Hence several accessions of *V. vexillata* were selected for crossing with cowpea.

Pollen from four wild cowpea relatives belonging to *V. unguiculata* ssp. *dekindtiana* and ten cultivated cowpea (*V. unguiculata*) lines were used to pollinate emasculated flowers of 64 different accessions of *V. vexillata*. There were differences among the accessions of *V. vexillata* used in making these crosses in the frequency of pods they retained following pollination with cowpea lines or wild cowpea relatives. While accession TVNu 73 retained up to 70% pods following the interspecies pollination, only a few pods were retained by some other accessions such as TVNu 1359 (Table 1). It should be noted that the retained pods were on the plants for no longer than eight to ten days after pollination. They shriveled and fell off the plant prematurely. Pods resulting from selfing on *V. vexillata* remain on the parent plants until they dry and are harvested. No appreciable differences were observed in the frequency of pod retention on the basis of which cowpea or *dekindtiana* line was used as pollen parent. Also, there were no observed differences in embryo development when random samples of ovules in retained pods were dissected. Essentially none of the ovules from the interspecific hybridization had an embryo that developed beyond the globular stage.

**Use of mixed pollen:** Cowpea pollen grains do produce tubes albeit at low frequencies when placed on the stigma of *V. vexillata*. Also, some of the developed pollen tubes are malformed and are therefore unable to penetrate the style fast enough to reach the ovule in order to effect fertilization (Barone and Ng 1990). A few pollen grains of the female (*V. vexillata*) plants were deliberately placed on the stigma along with some of cowpea. Pods developed on the *vexillata* plants when the mixed pollen grains were used. The number of normal sized seeds in each pod was few but none of the seeds resulted from interspecific hybridization. Payan and Martin (1975) used the mixed pollen technique to successfully effect interspecies cross in the genus *Passiflora* (passion fruit).

**Application of growth hormones:** Growth promoting hormones have been used to facilitate interspecies crosses in many crops. Generally, hormones are known to prolong the period during which fruits are retained on plants. In *Phaseolus*, Al-Yasiri and Coyne (1964) used growth hormones to prolong the period of pod retention following wide crossing to 30 days as against 15 for untreated pods. Gibberellic acid and NAA are two commonly

**Table 1. Frequency of pod retention by some accessions of *Vigna vexillata* following pollination with cowpea.**

| Accession | Percentage pod retained |
|-----------|-------------------------|
| TVNu 73   | 42                      |
| TVNu 1616 | 41                      |
| TVNu 719  | 36                      |
| TVNu 1344 | 33                      |
| TVNu 64   | 33                      |
| TVNu 72   | 23                      |
| TVNu 1544 | 8                       |
| TVNu 180  | 3                       |
| TVNu 1359 | 1                       |

used hormones to treat flowers in order to enhance interspecies crosses. Two auxins (2,4-D & NAA) and one cytokinin (kinetin) were applied as sprays at low concentrations (approximately 1.0 mg/l) and in various combinations on flowers of *V. vexillata* before or after pollination with cowpea. In particular, 2,4-D was effective in promoting the retention of *V. vexillata* flowers pollinated with cowpea and subsequently the pods resulting from the cross-pollination. Pods that formed from *V. vexillata* flowers sprayed with 2,4-D and pollinated with cowpea developed on the plants and at maturity were bigger in size than those resulting from selfing with no 2,4-D sprayed (Fig. 1). These pods remained on the peduncles until they dried as for normal pods resulting from self-pollination. When pods resulting from flowers sprayed with 2,4-D had matured and were opened, the ovules contained in them did not develop beyond the size of three-day-old ovules of selfed pods. In addition, there was a mass of white colored loose callus-like structures, which filled the spaces between adjacent ovules (Fig. 2). When emasculated flowers were sprayed with 2,4-D but not pollinated, the flowers remained attached to the peduncle for up to six days before falling off. Pods were, however, not initiated from such nonpollinated flowers even with the hormonal treatment. The retention and development of pods on *V. vexillata* following pollination with cowpea and 2,4-D spray is further evidence that fertilization does occur, leading to embryo initiation. According to Barone and Ng (1990), between 15 and 20% of ovules are fertilized when *V. vexillata* flowers are pollinated with cowpea. However, the embryos in ovules could not go through the normal stages of development for some reasons. These observations show that prolonging the retention and development of pods resulting from the *V. vexillata* by cowpea crosses on the female parent did not lead to further embryo development. Deakin et al. (1971) made similar observations in interspecies crosses in cucumber.

The application of NAA as spray to flower buds was not as effective as 2,4-D in promoting retention of pods on *V. vexillata* following pollination with cowpea. The pods resulting from flowers sprayed with NAA increased in size and were only slightly bigger than those that were not sprayed. Also NAA did not increase the frequency of pod retention as compared to when cross-pollinated flowers were not treated with a hormone.

**Embryo rescue:** Developments and improvements in tissue and cell culture techniques have contributed immensely to progress made in the exchange of genes between species in many crops. In vitro culture methods have been used to rescue young interspecific hybrid embryos prior to their abortion. This is particularly important in situations where the cause of incompatibility occurs postfertilization such as endosperm abortion and eventual starvation of the embryo. Fatokun and Singh (1987) needed to rescue embryos of the cross between cowpea and a wild relative, *V. unguiculata* ssp. *pubescens*, otherwise the embryos resulting from the cross collapsed before attaining full development. Barone et al. (1992) reported that the embryo and endosperm resulting from the cross between *V. vexillata* and cowpea collapsed within five and eight days following pollination. The development of an embryo especially during the early stages depends on the existence of a well-formed endosperm, which is the primary source of nourishment for the embryo. Also, it is essential that a harmonious relationship should exist between the embryo and endosperm tissue if the former is to go through the process of development.

When excised, the embryos in ovules resulting from pollinating *V. vexillata* with cowpea attained the globular stage of development (Fatokun 1991). The successful rescue of interspecific hybrid embryos that are this young (i.e., at the globular stage) has been

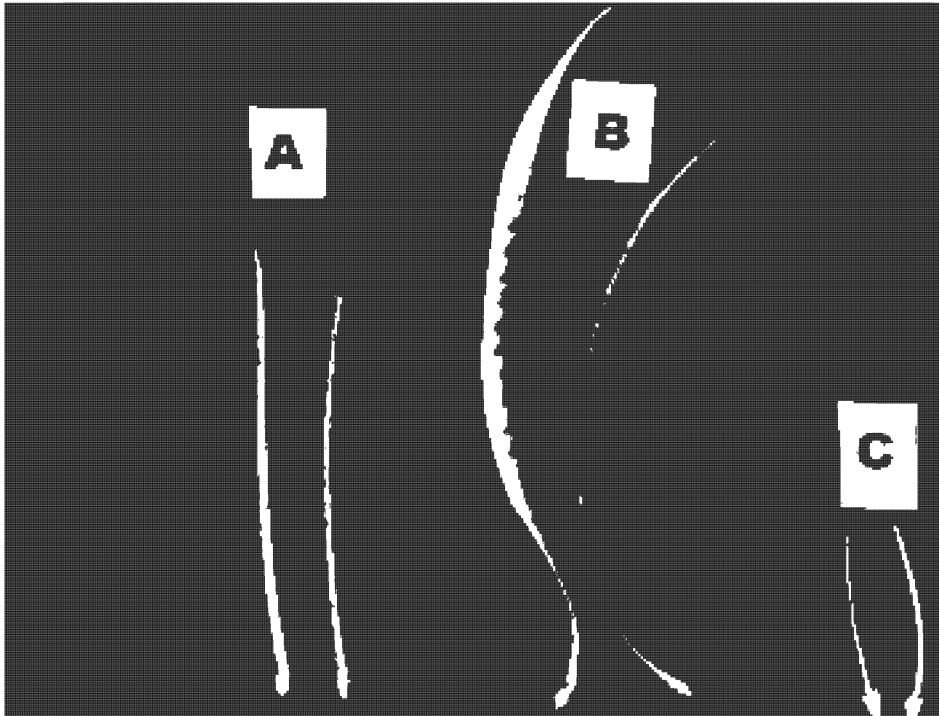


Figure 1. Pods of *Vigna vexillata*; (A) mature selfed pods, (B) mature pods from flowers pollinated with cowpea and sprayed with 2,4-D, and (C) seven-day-old pods from flowers pollinated with cowpea but not treated with 2,4-D.

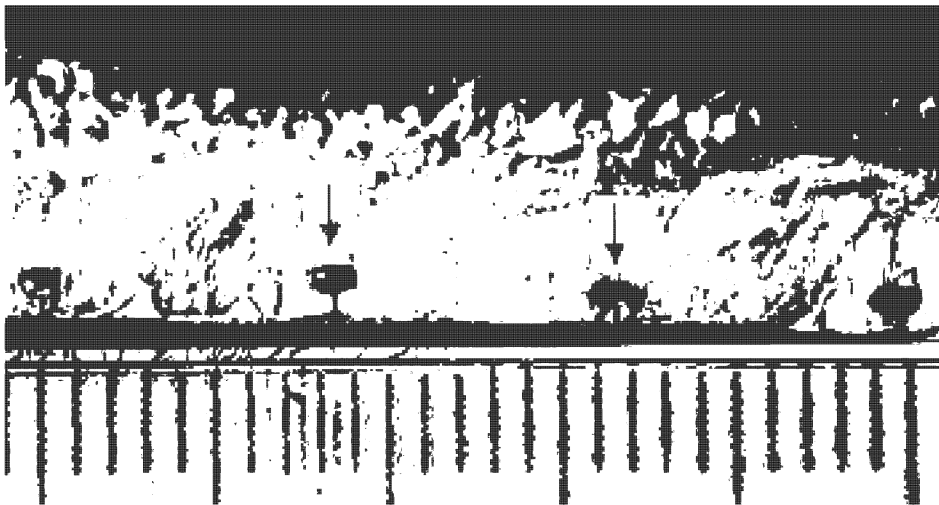


Figure 2. Opened pod of *Vigna vexillata* from flowers pollinated with cowpea and sprayed with 2,4-D showing ovules (arrows) which did not develop into seeds.



difficult to achieve in many plants. Embryo rescue is more successful as the embryo gets older. Usually the development of embryos into plants occurs more readily once they have passed the globular stage of development and beyond. It is obvious from the observations and reports mentioned above that fertilization does take place when pollen of cowpea are placed on the stigma of *V. vexillata*.

Entire ovules resulting from pollinating *V. vexillata* with cowpea were cultured in MS media containing 10% coconut water, 1% casein hydrolysate, and varying levels and combinations of sucrose and benzyl adenine. In ovulo culture was used because the embryos are small and difficult to dissect and excise. The presence of low levels (1–3%) of sucrose along with the other organic components added to the media encouraged the development of young selfed embryos (as young as four days) of both species to develop into plants in the culture tube. However, none of the hybrid embryos developed into plants following placement in the culture media. A few of the ovules (both selfed and hybrid) formed calluses especially in the media containing growth hormone, but when subcultured, no plants could be regenerated from them.

**Polyploidization:** Increasing chromosome number of one or both species can enhance crossability between two species. This is particularly so in cases where the two species being crossed differ in their genome number. However, both cowpea and *V. vexillata* have the same number of chromosomes ( $2N = 22$ ) as all other members of the genus *Vigna* with the exception of *V. glabrescens* which has  $2N = 2X = 44$ . *Vigna glabrescens* is the only naturally occurring polyploid in the subtribe *Phaseolinae* (Marechal et al. 1978). Polyploids were induced in cowpea following treatment of shoot tips of young seedlings with a weak solution of colchicine. Different accessions responded differently to colchicine such that a higher frequency of polyploids were induced in some than in others following similar treatments. The induced polyploids are fertile but produce fewer seeds per pod compared to their diploid counterparts. In addition the plants produced only a few pods each. The polyploid cowpea plants were characterized by thick leaves with large guard cells around the stomates, larger flowers, and pollen grains that were mostly rectangular in shape. Root tip cells obtained from the plants had  $2N = 2X = 44$  chromosomes. Young seedlings of *V. vexillata*, treated with colchicine were more sensitive to the chemical than cowpea. Further growth and development of seedlings were arrested at the shoot tips following application of 0.1% colchicine for a period of 12 hours. Application of colchicine at 0.1% to seedling shoot tips for 24 hours was found to be most effective in cowpea. In *V. vexillata* seedlings treated with colchicine new shoots developed from the roots rather than from the shoot tips. In an attempt to promote development of new shoots from treated buds of *V. vexillata*, young shoots were grafted on cowpea plants as stock. Shoot tips and axillary buds of the scion were treated with 0.1% colchicine for 12 hours. No shoots developed from any of the axillary buds or shoot tips of the scion treated with colchicine. Hence no polyploids could be induced in *V. vexillata* using the same concentration of colchicine that was effective on cowpea. Pollen of *V. vexillata* was placed on the stigma of polyploid cowpea flowers but all such flowers dropped within 24 hours of pollination hence no pods developed on the polyploid cowpea plants.

**Bridge crossing:** Successful interspecific crosses have been made in plants through bridging of crosses. Where direct crosses are not feasible between two species their genomes can be brought together by indirect means. For example *Nicotiana tabacum* does not readily cross with *N. repanda*. However, both species can cross successfully with *N. sylvestris*.

The desirable gene for disease resistance present in *N. repanda* could be transferred to tobacco by first crossing *N. repanda* to *N. sylvestris* and the progeny of this cross was then crossed to tobacco (Burk 1967). In order to effect gene transfer from *V. vexillata* to cowpea, crosses were made between the former and a close relative *V. davyi* on the one hand and between cowpea and *V. unguiculata* ssp. *dekindtiana* on the other. Both *V. vexillata* and *V. davyi* belong to the same section *Plectotropis* in the genus *Vigna* and this was the first reported successful cross between *V. vexillata* and any other *Vigna* species. However, *V. davyi* and *V. unguiculata* ssp. *dekindtiana* could not be crossed successfully. Cowpea and *V. unguiculata* ssp. *dekindtiana* belong to the same section *Catjang* of the genus. The hybrid resulting from the cross between *V. vexillata* and *V. davyi* was partially fertile (Table 2) as that between cowpea and its wild relative. The hybrids (*V. vexillata* × *V. davyi* and *V. unguiculata* × *V. unguiculata* ssp. *dekindtiana*) were crossed to each other and to the four parents but the efforts did not yield the desired products as no seeds were set in the crosses between members from different sections.

**Use of a parthenocarpic cowpea line:** A cowpea line (RI 36) showing parthenocarpy was identified among the progeny of a cross between IT84s-2049 and IT88s-524-B at the University of California, Riverside (J. Ehlers, personal communication). This cowpea line has the capacity to form and retain pods to maturity from emasculated flowers even when not pollinated. This cowpea line therefore served as the female parent and pollinated using a number of *V. vexillata* accessions. Seeds formed in the pods and these appeared to develop normally for the first ten days after which they started to shrivel. There was a mass of cells connecting each seed to the pod wall. Seeds were excised from pods on different days after pollination for placement in the culture media. Embryos could not be readily distinguished in the seed hence all seeds (ovules) were excised and placed in culture media. The only development observed on the cultured seeds was root initiation (Fig. 3) but no shoots were formed.

**Table 2. Morphological attributes of F<sub>1</sub> interspecific hybrid between *Vigna vexillata* and *V. davyi* and their parents.**

| Character               | <sup>†</sup> TVNu 1335 | F <sub>1</sub> | <sup>‡</sup> TVNu 72 |
|-------------------------|------------------------|----------------|----------------------|
| Leaf length (cm)        | 8.3                    | 12.7           | 15.5                 |
| Petiole length (cm)     | 3.8                    | 5.6            | 8.8                  |
| Pod length (cm)         | 9.6                    | 9.0            | 11.4                 |
| Seeds per pod           | 10.8                   | 6.3            | 15.9                 |
| Pollen stainability (%) | 96.2                   | 59.2           | 97.9                 |
| Peduncle length (cm)    | 24.6                   | 25.6           | 17.4                 |

<sup>†</sup>*V. davyi*.

<sup>‡</sup>*V. vexillata*.

## Conclusion

Attempts have been made to cross cowpea with *V. vexillata* using various techniques that have successfully been used to effect wide crosses in some other crops. These efforts did not yield the expected results, thus suggesting the existence of a strong cross incompatibility barrier between cowpea and *V. vexillata*. The causes of incompatibility between the two species are both pre- and postfertilization. In the first place, only a few



**Figure 3.** Root developing from cultured seed of cowpea line RI 36 following pollination with *Vigna vexillata*.

cowpea pollen tubes are able to penetrate the styles of *V. vexillata* and reach the ovule in order to effect fertilization. There is sufficient evidence that fertilization does occur, albeit at relatively low frequency. The fertilization probably gives rise to diploid zygotes, which develop to the globular stage embryo. The nondevelopment of the hybrid embryos beyond the globular stage may be an indication of incomplete fertilization in which the second male nucleus does not fuse with the diploid endosperm nucleus to give the triploid tissue that normally feeds the embryo. Consequently there is no triploid endosperm tissue formed following the cross between cowpea and *V. vexillata*. The absence of the endosperm leads to starvation and subsequent collapse of the embryos.

There is in the genome of *V. vexillata* a repertoire of genes that could confer resistance to several of the pests and diseases to which cowpea succumbs. The attempts made so far, using sexual means, to move desirable genes from *V. vexillata* to cowpea have not yielded the desired results. Perhaps other avenues by which these genes could be accessed should be explored. An approach would be the identification and cloning of these genes which eventually could be used to transform cowpea. This is a much longer route to take but it might be worth the efforts because of the potential benefit.

## References

- Al-Yasiri, A. and D.P. Coyne. 1964. Effects of growth regulators in delaying pod abscission and embryo abortion in the interspecific cross *Phaseolus vulgaris*  $\times$  *P. acutifolius*. Crop Science 4: 433–435.

- Barone, A., A. Del Guidice, and N.Q. Ng. 1992. Barriers to interspecific hybridisation between *Vigna unguiculata* and *V. vexillata*. Sexual Plant Reproduction 5: 195–200.
- Barone, A. and N.Q. Ng. 1990. Embryological study of crosses between *Vigna unguiculata* and *V. vexillata*. Pages 151–160 in Cowpea genetic resources, edited by N.Q. Ng and L.M. Monti. International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Burk, L.G. 1967. An interspecific bridge-cross—*Nicotiana repanda* through *N. sylvestris* to *N. tabacum*. Journal of Heredity 58: 215–218.
- Deakin, J.R., G.W. Bohn, and T.W. Whitaker. 1971. Interspecific hybridisation in *Cucumis*. Economics of Botany 25: 195–210.
- Fatokun, C.A. 1991. Wide hybridisation in cowpea: problems and prospects. Euphytica 54: 137–140.
- Fatokun, C.A., D. Danesh, N.D. Young, and E.L. Stewart. 1993. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theoretical and Applied Genetics 86: 97–104.
- Fatokun, C.A. and B.B. Singh. 1987. Interspecific hybridisation between *Vigna pubescens* and *V. unguiculata* (L.) Walp. through embryo rescue. Plant Cell, Tissue, and Organ Culture 9: 229–233.
- Harlan, J.R. and J.M.J. de Wet. 1977. Pathways of genetic transfer from *Tripsacum* to *Zea mays*. Proceedings of the National Academy of Science, USA 74: 3494–3497.
- Hermesen, J.G. and M.S. Ramanna. 1973. Double bridge hybrids of *Solanum bulbocastanum* and cultivar *S. tuberosum*. Euphytica 22: 457–466.
- Larter, E. and C. Chaubey. 1965. Use of exogenous growth substances in promoting pollen tube growth and fertilisation in barley-rye cross. Canadian Journal of Genetic Cytology 7: 511–518.
- Marechal, R., J.M. Mascherpa, and F. Stainer. 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques, et polliniques traitées par l'analyse informatique. Boissiera 28: 1–273.
- Payan, F.R. and F.W. Martin. 1975. Barriers to the hybridisation of *Passiflora* species. Euphytica 24: 709–716.
- Pittarelli, G.W. and J.R. Stavely. 1975. Direct hybridisation of *Nicotiana rependa* × *N. tabacum*. Journal of Heredity 66: 281–284.
- Przywara, L., D.W.R. White, P.M. Sanders, and D. Maher. 1989. Interspecific hybridisation of *Trifolium repens* with *T. hybridum* using in ovulo embryo culture. Annals of Botany 64: 613–624.
- Sitch, L.A. and J.W. Snape. 1987. Factors affecting haploid production in wheat using the *Hordeum bulbosum* system. 1. Genotype and environmental effects on pollen grain germination, pollen tube growth and the frequency of fertilisation. Euphytica 36: 483–496.
- Thomas, C.V. and J.G. Waines. 1982. Interspecific hybrids between *Phaseolus vulgaris* L. and *P. acutifolius*: Field trials. Annual Report of Bean Improvement Cooperative 25: 58–59.

## 1.6

# Cowpea breeding in the USA: new varieties and improved germplasm

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### Abstract

Cowpea is utilized in the USA as both a vegetable crop and a dry bean, and breeding efforts are focused on development of cultivars for specific end uses. Blackeye cultivars are developed for production of dry beans for national and international markets. California Blackeye No. 27 (CB27), a cultivar with a combination of high-value traits, was released in 1999. CB27 has high yield potential, superior seed quality, heat tolerance, and broad-based resistance to root-knot nematodes and *Fusarium* wilt. Breeding programs in southeastern US have traditionally been directed towards the development of various classes of horticultural-type cultivars for the canning, freezing, fresh market, and home garden market sectors. The most interesting recent development in the horticultural arena is the acceptance of green-seeded cultivars by the freezing industry. Charleston Greenpack is a leading source of raw products for the freezing industry.

### Introduction

Cowpea is an important soil-building crop in the rotation of cotton and vegetable crops in the southern half of the USA. Most cowpea is consumed by people in southeastern US, where it has been a traditional crop since the early 1800s. Cowpea is grown as a vegetable crop in all of the southern states, and it is a popular home garden item throughout the region. Canning or freezing companies process much of the commercial crop in this region, but a significant amount is sold as fresh-shell “peas”. In the southwest, primarily California and Texas, about 45 000 t of dry blackeye type cowpea (“blackeyes”) is produced annually on about 20 000 ha. It has been estimated that 20–30% of the production is exported internationally, mostly to southern Europe, the Middle East, and Asia. Most of the blackeyes are sold through the dry-package trade. Perhaps 5–10% of the blackeye crop is canned.

Immature whole pods are also consumed in the southeast and by Asian communities throughout the US, and specialized cultivars, similar to snap beans (*Phaseolus vulgaris*), have been developed for this purpose.

Prior to the Second World War, cowpea was a major forage crop for horses and cattle (hence the name cowpea). Currently, the acreage of cowpea being used as a soil-building cover crop, particularly in organic agriculture, is increasing rapidly.

### US genetic resources and improvement of cowpea

Reasonably comprehensive germplasm collections have been assembled (about 8000 accessions are held by the United States Department of Agriculture [USDA], and 5500

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accessions by the University of California [UC] Riverside). Passport and characterization data for accessions in the USDA collection are available from the Germplasm Resources Information Network (GRIN) of USDA through the World Wide Web (address: <http://www.ars-grin.gov>). Requests for small quantities of seed of these accessions may be made to USDA or to the UC, Riverside.

Lack of research funding in the US has hindered unlocking the full potential of these collections. Nevertheless, important and unique traits have been identified in cowpea, such as heat and chilling tolerance (Hall 1992; Ismail et al. 1999), and resistance to pests such as root-knot nematodes, cowpea curculio, and *Fusarium* wilt (Ehlers and Hall 1997; Hall et al. 1997).

Cowpea breeding programs are being conducted in the US by USDA at their vegetable laboratory in South Carolina, by Louisiana State University, the University of California, at their Riverside and Davis campuses, at Texas A&M University, and at the University of Arkansas.

The UC breeding programs are developing improved dry-grain blackeye cowpea varieties and complementary management systems that increase profitability through increased yield and grain quality, and decreased production costs. Specific objectives of these programs include development of blackeye varieties with high yield, erect plant type, large grain with low seed coat cracking that cans well, heat tolerance, and resistance to *Fusarium* wilt (races 3 and 4), “early cut-out” disease, root-knot nematodes, cowpea aphid, and lygus bug. New objectives include the development of cover-crop cowpea varieties, and cowpea cultivars with unique grain types such as the persistent green, “sweet,” or large, white grained types.

The cowpea genetics and breeding program at the US Vegetable Laboratory in Charleston, South Carolina, has been in progress for well over three decades. This program has been successful in identifying unique value-added traits and new sources of needed resistance to root-knot nematodes, diseases, and insects (Cuthbert et al. 1974; Cuthbert and Fery 1975, 1979; Fery and Cuthbert 1979; Fery and Dukes 1995a; Fery et al. 1975, 1977, 1994; Schalk and Fery 1982, 1986); in determining the mode of inheritance of major economically important traits (Fery and Cuthbert 1975, 1978; Fery and Dukes 1977, 1980; Fery et al. 1976); and in the development of many cultivars with multiple resistance to pests and diseases. USDA has released 13 cowpea cultivars (recent releases in Table 1) and 10 germplasm/breeding lines in the past 25 years. The green cotyledon gene in cowpea was discovered by this program, and this new gene is the basis of the first commercially successful cowpea cultivars with a “persistent green” seed phenotype (Fery 1998, 1999, 2000; Fery and Dukes 1994; Fery et al. 1993; USDA 2000).

## **New dry grain blackeye variety released**

California Blackeye No. 27 (CB27), developed by the UC Riverside breeding program, was released as a new variety by the California Crop Improvement Association in 1999 (Ehlers et al. 2000a). This is the first new blackeye variety available to California growers in about ten years. Plant Variety Protection (PVP) is being sought. Small quantities of seed are available from UC Riverside for research purposes.

CB27 is an erect, compact blackeye-type cowpea with heat tolerance and high yields and a number of other desirable features, including brighter white seed coat and broader based resistance to *Fusarium* wilt and root-knot nematodes than the currently available

**Table 1. Recently released cowpea cultivars, specific trait improvement, and reference.**

| Cultivar               | Improved trait(s)   | Reference              |
|------------------------|---|------------------------|
| Santee Early Pinkeye   | Early maturing processing cultivar for use to extend season or replace lost/delayed plantings | Fery and Dukes 1990    |
| Carolina Crowder       | Resistant to curculio, blackeye cowpea mosaic virus (BICMV), root-knot nematodes              | Fery and Dukes 1992    |
| Bettergro Blackeye     | Resistant to curculio, root-knot nematodes, rust, and powdery mildew                          | Fery and Dukes 1993    |
| Bettergreen            | First cowpea cultivar with green cotyledon phenotype (cream-type)                             | Fery et al. 1993       |
| Bettersnap             | Snap-type resistant to root-knot nematodes, BICMV, and southern bean mosaic virus             | Fery and Dukes 1995(b) |
| Tender Cream           | Excellent culinary quality and multiple insect, nematode, and disease resistance              | Fery and Dukes 1996    |
| Charleston Greenpack   | First pinkeye-type with green cotyledon trait; no hard seed; resistant to BICMV               | Fery 1998              |
| Petite-N-Green         | Small-seeded, full-season, green cotyledon, pinkeye-type developed for home gardeners         | Fery 1999              |
| California Blackeye 27 | Heat tolerant and broad-based resistance to nematodes, <i>Fusarium</i> wilt                   | Ehlers et al. 2000     |
| Green Pixie            | Green cotyledon, cream-type with seed size and shape characteristics preferred by industry    | Fery 2000              |
| Green Dixie Blackeye   | First blackeye-type cultivar with the green cotyledon phenotype to be released                | USDA 2000              |

varieties, California Blackeye No. 46 (CB46) and California Blackeye No. 5 (CB5) (Table 2). CB27 begins flowering at about 52 days and matures its first flush of pods about 95 days from sowing under typical conditions in California. The average individual seed weight has been 224 mg in California and the grain has excellent canning quality.

CB46 and CB5 carry the nematode resistance gene *Rk* that confers strong resistance to common strains of *Meloidogyne incognita* root-knot nematode. CB27 carries gene *Rk* and another recessive gene, *rk3* (Ehlers et al. 2000b) that act together in an additive fashion to provide greater protection against *Rk*-virulent forms of *M. incognita* and *M. javanica* root-knot nematodes. Reproduction and root galling on CB27 caused by *Rk*-virulent *M. incognita* and *M. javanica* are about half those observed on CB46 and CB5 (Roberts et al. 1997). Several new fields with root-knot nematodes causing galling on CB46 were identified in 1999, indicating the *Rk*-virulent strains of root-knot nematodes may be widespread in California (Ehlers et al. 1999).

CB27 has resistance to both race 3 and race 4 of *Fusarium* wilt, while CB46 only has resistance to race 3 of this disease organism and CB5 is susceptible to both races. Race 3 is the predominant race of *Fusarium* wilt in California, but additional fields with race 4 were identified in 1997, 1998, and 1999, suggesting that this race may be widespread (Ehlers et al. 1999).

## **New horticultural cowpea varieties for southeastern US**

### ***Persistent-green cowpeas***

The development of cowpea cultivars with a persistent-green seed color has been the subject of much interest among both food processors, especially freezers, and plant breeders because seeds of such cultivars can potentially be harvested at the near-dry or dry seed stage of maturity without loss of their green color. The retention of the green color is important because the choice of harvesting method is often a compromise between cost and product quality. Harvesting dry cowpeas can be done efficiently and with minimal losses compared to mechanically harvesting mature-green cowpeas. Also, the crop does not need to be processed immediately and may be stored until it is convenient to freeze. Several hours prior to freezing, the precise amount of grain needed would be soaked in water. This product would have low production and storage costs similar to other dry-grain crops yet the product would be used in high-priced, vegetable-type applications. Compared to other frozen vegetable products that are harvested fresh and have high harvesting and storage costs, the relatively low costs of dehydrated cowpeas and relatively high potential profit margin should encourage processors to use this ingredient in frozen vegetable mixes and other applications, so the market potential may be significant and help increase demand for cowpeas in the USA.

Chambliss (1974) reported that the green testa gene (*gt*) conditions a green seed coat that persists in the dry seed, and this trait results in a processed product with improved consumer appeal. Although a cultivar homozygous for the *gt* gene was released (Chambliss 1979), the green testa trait was never accepted by the processing industry. Fery et al. (1993) discovered a green cotyledon mutant in the cream-type cultivar Carolina Cream, and released a green cotyledon selection as Bettergreen. The new trait is similar to the green cotyledon trait reported in lima bean by Magruder and Wester (1941). The green cotyledon trait revolutionized the lima bean industry, and the quick acceptance of the green cotyledon



**Table 2.** Comparative features of California Blackeye No. 27 (CB27), California Blackeye No. 5 (CB5), and California Blackeye No. 46 (CB46) blackeye cowpeas.

| Entry | <i>Fusarium</i> wilt |        | Resistance to root-knot nematodes <i>M. incognita</i> |          |                    |      | Plant habit        | Seed weight<br>(g/100 seeds) |
|-------|----------------------|--------|---|----------|--------------------|------|--------------------|------------------------------|
|       | Race 3               | Race 4 | avirul.   | virulent | <i>M. javanica</i> | Heat |                    |                              |
| CB27  | Yes                  | Yes    | Yes   | Yes      | Yes                | Yes  | Compact            | 22                           |
| CB46  | Yes                  | No     | Yes   | No       | Partial            | No   | Moderately compact | 22                           |
| CB5   | No                   | No     | Yes   | No       | Partial            | No   | Vine               | 25                           |

avirul. = avirulent, effectively controlled by gene *Rk*; virulent = not effectively controlled by gene *Rk* alone.

cultivars Bettergreen and Charleston Greenpack by the freezing industry indicates that the trait will have a similar impact on the cowpea industry as most processing horticultural cultivars are now harvested at the near-dry or dry seed stage of maturity. Fery and Dukes (1994) concluded that the green cotyledon trait in southernpea is conditioned by a single recessive gene, symbolized *gc*, and that this gene is neither allelic to nor linked with the *gt* gene. Apparently, these genes prevent the normal breakdown of chlorophyll that occurs as seeds reach maturity. Dry persistent-green grain stored in sacks retains its green color for many months, however, the green color of the grain can be bleached by exposure to sunlight for several weeks, giving the grain its background color, e.g., white. Apparently there are no negative pleiotropic effects on yield or other agronomic characters (Freire Filho, unpublished report).

Several important new persistent-green cowpea varieties have recently been developed, including Charleston Greenpack (Fery 1998), Petite-N-Green (Fery 1999), Green Pixie (Fery 2000), and Green Dixie (USDA 2000).

Bettergreen was the first southernpea cultivar to be developed that exhibits the green cotyledon trait (Fery et al. 1993). It was derived from a single mutant plant harvested from a 1986 field planting of Carolina Cream. The mutant plant was homozygous for a newly discovered gene (*gc*) conditioning a unique green cotyledon trait. Bettergreen has a medium, bushy plant habit. A typical pod is slightly curved, 15 cm long, and contains 12 to 14 peas. Pods are green when immature, green with a distinct purple shading at green-shell maturity, and pale tan or straw when dry. The fresh peas are small and ovate-reniform in shape. The dry peas have a smooth seed coat and can be harvested at dry seed maturity without loss of the seeds fresh green color. Bettergreen has resistance to the cowpea curculio, *Cercospora* leaf spot, southern blight, rust, and powdery mildew. The cultivar exhibits tolerance to seedling diseases. Bettergreen is used in the US as a commercial cultivar by the frozen food industry.

Charleston Greenpack was the first pinkeye-type southernpea to be developed that exhibits the green cotyledon trait (Fery 1998). Charleston Greenpack originated as a bulk of an  $F_8$  [Kiawah  $\times$  (Kiawah  $\times$  Bettergreen)] population grown in 1994. Except for the green seed color and a tendency for a slightly smaller pea size, the phenotype of Charleston Greenpack is quite similar to those of the leading US pinkeye-type processing cultivars Coronet and Pinkeye Purple Hull-BVR. The plant habit is low, bushy, and somewhat more compact than that of either Coronet or Pinkeye Purple Hull-BVR. A typical Charleston Greenpack pod is moderately curved, 17 cm long, and contains 14 peas. Pod color is green when immature and dark purple when ready for mature-green or dry harvest. The fresh peas are kidney-shaped and have a pink eye. The dry peas have a smooth seed coat, and are slightly smaller than those of Coronet and Pinkeye Purple Hull-BVR. Charleston Greenpack has excellent field resistance to blackeye cowpea mosaic virus (BICMV), a major pathogen of southernpea in the USA. In the brief period since it was first released in 1997, Charleston Greenpack has already become a leading pinkeye-type cultivar for the US frozen food industry. Charleston Greenpack peas produce an attractive frozen pack. Protection for Charleston Greenpack is being sought under the US Plant Variety Protection Act.

Petite-N-Green is a small-seeded, full-season, green cotyledon, pinkeye-type southernpea that was released in 1998 (Fery 1999). Petite-N-Green originated as a bulk of an  $F_9$  (Coronet  $\times$  Bettergreen) population grown in 1994. Petite-N-Green has a low, bushy

plant habit similar to that of Coronet. It has a more procumbent vine than does Charleston Greenpack. Petite-N-Green produces dry pods at Charleston, South Carolina, in 70 to 76 days, 4 to 7 days later than Charleston Greenpack and 2 to 9 days later than Coronet and Pinkeye Purple Hull-BVR. A typical Petite-N-Green pod is moderately curved, 14 cm long, and contains 14 peas. Pod color is green when immature, and dark purple when ready for mature-green harvest or when dry. Fresh peas are ovate to kidney-shaped and have a pink eye that is quite similar to fresh Charleston Greenpack, Coronet, and Pinkeye Purple Hull-BVR peas. Dry peas are small and have a smooth seed coat. Petite-N-Green peas are 12–20% smaller than Charleston Greenpack peas, 11–25% smaller than Coronet peas, and 12–24% smaller than Pinkeye Purple Hull-BVR peas. Petite-N-Green yields are comparable to those of Charleston Greenpack, Coronet, and Pinkeye Purple Hull-BVR. Petite-N-Green is recommended particularly for use as a home-garden cultivar in southeastern USA. The peas can be harvested not only fresh for immediate consumption or storage in home freezers, but also when fully dry for storage as an attractive dry pack. The dry peas can be removed from storage and soaked to restore a near-fresh green color. Protection for Petite-N-Green is being sought under the US Plant Variety Protection Act.

Green Pixie is a small-seeded, green cotyledon, cream-type southernpea that was released in 1999 (Fery 2000). Green Pixie originated as a bulk of an  $F_2$  (Bettergreen  $\times$  White Acre) population grown in 1994. Green Pixie has a high, bushy plant habit similar to that of White Acre. Green Pixie produces dry pods at Charleston, South Carolina, in about 76 days, 5 days later than Bettergreen, and 5 days earlier than White Acre. A typical Green Pixie pod is slightly curved, about 15 cm long, and contains about 16 peas. Pod color is light green when immature, purple when ready for mature-green harvest, and light straw color when dry. Green Pixie peas are rhomboid-kidney in shape, similar to the shape of fresh White Acre peas, but very different from the ovate to reniform shape characteristic of fresh Bettergreen peas. Dry peas are small and have a smooth seed coat. Green Pixie peas are similar in size to White Acre peas, but much smaller than Bettergreen peas. Green Pixie was developed for use by the frozen food industry, either as a replacement for the popular White Acre or as a substitute for Bettergreen when grown to produce the raw product for a blended pack of Bettergreen and White Acre.

Green Dixie Blackeye is the first blackeye-type southernpea to be released that exhibits the green cotyledon phenotype (USDA 2000). Green Dixie Blackeye originated as a bulk of an  $F_2$  (Bettergreen  $\times$  Bettergro Blackeye) population grown in 1994. Green Dixie Blackeye has a high bushy plant habit. A typical Green Dixie Blackeye pod is slightly curved, 21 cm long, and contains 14 peas. Pod color is light green when immature, light green with a tendency for slight pigmentation (purple) on the tip when ready for mature-green harvest, and light straw color when dry. Green Dixie Blackeye peas have an oblong shape. The dry peas have a smooth seed coat and small, black-colored, hilar eyes. Results of replicated tests conducted at Charleston, South Carolina, indicate that Green Dixie Blackeye has a much greater yield potential than Bettergro Blackeye. Green Dixie Blackeye is recommended for use by home gardeners and the dry-pack bean industry. The peas can be harvested not only fresh for immediate consumption or storage in home freezers, but also when fully dry for storage or sale as an attractive dry pack. The dry peas can be soaked to restore a near-fresh green color.

The UC Riverside program is also developing persistent-green California blackeye-type varieties for potential use in frozen products. Like Green Dixie Blackeye, the grain

of these varieties resembles fresh-shell blackeyes after being soaked in water for several hours. Because the green color bleaches to white after prolonged exposure to sunlight in the field, “double-flush” production practices, wherein growers accumulate two flushes of pods over 120–140 days, will not be possible. In other aspects, however, production, harvesting, and storage practices would be as for traditional blackeye cowpeas that are now produced in California.

### ***Fresh green pods***

Certain southernpea cultivars are grown for their immature fresh pods or snaps, and some processors have traditionally included a small portion of snaps in the processed product (Fery 1990). Lorz and Halsey (1964) noted that “the snap ingredient has always consisted of immature pods of standard shell-pea varieties.” The cultivar Snapea was developed specifically for its attractive, long, low fiber pods (Lorz and Halsey 1964). Patel and Hall (1986) evaluated five vegetable cowpea (snap-type) breeding lines and a snap bean cultivar in a summer field test at Riverside, California. They concluded that vegetable cowpea lines have a potential for producing large yields of pods in environments in which snap bean produces only small yields due to hot weather. Fery (1981) observed earlier that southernpeas are tolerant to drought and hot weather, and can be grown quite successfully under conditions that are totally unsuitable for table legumes such as the common bean and the lima bean. Fery and Dukes (1995b) released the edible podded cultivar Bettersnap in January 1994. Bettersnap, which is resistant to root-knot nematodes and blackeye cowpea mosaic and southern bean mosaic viruses, quickly replaced Snapea as the snap-type cultivar of choice for commercial food processors.

### **Development of new specialty cowpea grain types**

One major problem facing the cowpea industry in the USA is stagnant demand. Per capita consumption of dry cowpeas has steadily decreased in the USA, in part because this product takes more time to prepare than other foods, and little time is available for meal preparation with most families having both spouses working full-time. Also, more and more meals are eaten outside the home, and cowpeas have not been frequently offered on restaurant menus or used in convenience foods. Increased awareness of the health benefits of consuming grain legumes has helped increase demand in some markets, but new types of cowpeas and quick-to-prepare cowpea food products are needed to stimulate cowpea consumption in the USA.

### **Development of all-white cowpeas for value-added products**

Cowpea is processed into many traditional West African foods, such as *akara*, that are delicious yet virtually unknown outside West Africa. Such foods could find wide acceptance in US markets as processed convenience foods or “fast-foods”.

*Akara* is traditionally prepared from cowpeas that are soaked, dehulled, and milled wet. If the milled product is not used immediately, expensive or laborious drying or refrigeration is necessary for its preservation. Dry milling of whole grain cowpea would be much more efficient than wet milling and produce an easily storable product. This would make possible the development of “ready to cook” cowpea flour mixes for *akara* production or for use in other products. Unlike pigmented cowpea cultivars, an all-white cowpea would produce an all-white flour that would be preferred for most products. Cowpea flour can

be substituted for wheat flour up to 30% in the preparation of yeast breads without loss in quality (K.H. McWatters, personal communication).

High yielding breeding lines have been developed at UC Riverside with large all-white grains that are adapted to the US by crossing California blackeye varieties with the all-white cultivars Bambey 21 from Senegal and Montiero from Brazil. In crosses between Bambey 21 and California blackeyes, a ratio of 3 blackeye:1 all-white type seed coat was observed in the  $F_2$  generation (J.D. Ehlers, unpublished data), indicating a single recessive gene confers this trait. Plants having all-white seed may be recognized in the vegetative stage because they lack any red pigmentation on the stem or branch nodes or on other plant parts. Presumably, Bambey 21 carries a gene-blocking formation of pigments.

Complex segregation is observed in the  $F_2$  generation of crosses between blackeyes and Montiero. In Montiero, the capacity for pigment to be produced is retained but pigment is restricted to a barely visible ring around the hilum.

One all-white line from the UC Riverside breeding program, 97-15-33, developed using Bambey 21 as a source of the all-white character, was compared to four other cowpea lines for use in *akara* production and found to be as good as the control blackeye variety (McWatters et al. 2000).

### **“Sweet” cowpea**

Breeding line 24-125B is a sweet-tasting cowpea developed by the breeding program of the Research Institute for Agricultural Development (IRAD)/Purdue University Bean–Cowpea Collaborative Research Program (CRSP) that is based in Maroua, Cameroon. Line 24-125B was developed from a single cross of two IITA lines, IT86D-364 and IT81D-1138 (L.W. Kitch, personal communication, 1999) neither of which is considered “sweet”. In 1993, a single plant selection was made from an  $F_4$  family at Maroua. The following year, seed of the resulting  $F_5$  family was bulked and used for yield trials. Cameroonian farmers who had been brought to the IRAD/Purdue University CRSP project plots as part of a farmer-assisted selection process noted that this line tasted “sweet” or “good”. For over three years, Cameroonian farmers consistently chose this line as one of their favorites (Kitch et al. 1998). Subsequent analysis of the sugar content of dry seeds of this line by Purdue University researchers revealed that it has a sugar content of about 6% compared to sugar content of about 2% for “normal” cowpea varieties (L. Murdock, unpublished data). Purdue researchers also conducted a triangular taste panel test comparing cooked samples of 24-125B with its nonsweet sister line 24-125A. In this test, two samples of the nonsweet line 24-125A and one sample of the sweet line were placed before a panel. The tasters, who were generally unfamiliar with cowpeas, were able to correctly differentiate the sweet cowpea from its nonsweet sister line about 83% of the time.

The discovery of the sweet trait opens up the possibility of developing new products and markets for cowpea in the US and elsewhere. One possibility is the development of “sweet” versions of existing market classes. Another possibility is the development of new market classes. One type might resemble garden peas (*Pisum sativum*) having grain that are sweet, round-shaped, and persistent-green in color.

The sweet trait is being rapidly bred into cultivars targeted to the US, Senegal, and Ghana. Line 24-125B has been crossed to CB27, to CB46, to the Senegal variety Melakh, and to the Ghanaian variety Sul-518 for development of locally adapted “sweet” varieties and for genetic analysis of the trait.  $F_1$  data from several crosses indicate that sweetness is

completely recessive.  $F_2$  seed of selected crosses was sent to Ghana and Senegal in June 2000, and is being grown in California.  $F_3$  seed from  $F_2$  plants of these crosses will be analyzed for sugar content and inheritance of "sweetness" trait determined. If the trait is simply inherited, a backcross procedure would be appropriate to introduce the trait into adapted cultivars suited to many regions.

## **Progress in breeding for pest resistance**

### ***Improved nematode resistance in blackeye cowpeas***

Resistance to root-knot nematodes in US cultivars and probably most other cultivars in the world is based on the *Rk* allele. *Rk* provides very strong protection from most isolates of *Meloidogyne incognita* but only moderate resistance to *M. javanica* (Roberts et al. 1997). Also, gene *Rk*-virulent strains of *M. incognita* have been identified at several locations in California. Therefore, cowpea cultivars with effective broad-based resistance to root-knot nematodes are needed. At the last world cowpea conference, Roberts et al. (1997) reported that IITA breeding line IT84S-2049 had much more effective resistance to root-knot nematodes (*M. incognita* and *M. javanica*) than cultivars possessing the *Rk* resistance gene, and that this resistance is due to an allele at the *Rk* locus, designated *Rk*<sup>2</sup> (Roberts et al. 1996). Unfortunately, IT84S-2049 is poorly adapted to the US and has poor quality seeds from the standpoint of small size (about 0.13 g/seed), and high frequency of seed coat splitting. Therefore, a limited backcrossing program was used to develop high yielding and large-seeded blackeye breeding lines that possess the IT84S-2049 resistance (Ehlers et al. 1999).

### ***Identification and improvement of insect resistance***

Insect-resistant cowpea varieties may become very important in the near future to maintain high bean quality and yield levels in the USA. Restrictions on the use of currently available pesticides are likely to increase, while their effectiveness in some cases is decreasing due to the development of insecticide-resistant insect biotypes. Also, due to the high cost of pesticide registration, few new insecticides for minor crops such as cowpea may be available. A major goal of the breeding programs at the USDA vegetable laboratory and the UC Riverside is the development of pest-resistant cultivars that require minimal applications of pesticides.

Lygus bug is the most devastating pest of cowpea in California. Early season infestations of lygus bugs reduce the yield of cowpea by feeding on reproductive buds causing them to abort. The extent of yield loss depends on the timing of the infestation, the phenological stage of the crop, and the intensity and duration of the attack, and a cultivar's ability to recover. Late infestations of lygus bugs damage pods and developing seeds, causing seed or pod abortion, seed pitting or malformation, and superficial scarring to the seed coat. Even superficial scarring of the seed coat is important because it lowers the value of the grain.

Hundreds of cowpea accessions have been screened for resistance to lygus at UC Riverside and several promising lines have been identified. In 1999, the grain yields and lygus-induced seed damage of CB46, three exotic African cowpea lines, and three lines developed at UC Riverside from crosses between wild and cultivated cowpeas were evaluated under both lygus bug-protected and unprotected conditions at Riverside. The six lines were chosen based on their performance in similar trials or from unprotected lygus

screening nurseries conducted in 1998. Lygus bug-induced grain yield losses in CB46 were 29% and 14% of the seed of this variety had lygus damage (Table 3). Five of the six entries had significantly less grain yield loss due to lygus than CB46, and all six entries had lower seed damage (Table 3). These data indicate that progress is being made in identifying lygus-resistant germplasm. IT92KD-370 and IT96-11-27 also had significantly less lygus-induced seed damage than CB46 in similar trials conducted in 1998.

Wild cowpeas are a potential source of insect-resistance genes, but are themselves difficult to evaluate for resistance to lygus because of their photoperiod sensitivity, slow early growth and development, and morphological and grain characteristics that differ substantially from cultivated cowpeas. UC Riverside has developed many breeding lines derived from three-way crosses between cultivated and wild cowpeas [(wild cowpea  $\times$  cowpea)  $\times$  cowpea] that are similar to cultivated cowpeas. Lines 96-11-27 and 96-11-38, developed from crosses to wild cowpea accession TVNu 597 (ssp. *pubescens*), have exhibited lygus resistance in terms of less grain yield and seed quality reductions than the standard cultivar CB46 (Table 3).

Over the last five years, more than 1000 accessions and wild cowpea  $\times$  cultivated and exotic  $\times$  adapted breeding lines have been screened in the field for resistance to lygus by the UC Riverside program. From this work, IITA breeding lines IT93K-2046, IT93K-273-2-1, IT92KD-370, and IT86D-716 appear to have moderate resistance to lygus bud blasting or lygus-induced seed damage. This resistance is being bred into lines adapted to California. The resistance appears to have high heritability since it was possible to visually identify resistant  $F_3$  families developed from crosses between California blackeyes and IITA lines IT93K-2046, IT93K-273-2-1, IT92KD-370, and IT86D-716 (Ehlers et al. 1999). Observations in the field suggest that IT93K-2046 also has resistance to the California biotype(s) of cowpea aphid and this resistance has been transferred to breeding lines after crosses with susceptible California cultivars.

Strong resistance to cowpea aphid in the US has been difficult to identify. Unfortunately, the aphid resistance that is effective in Africa is not effective against biotypes of this pest in the US. In 1999, IITA line IT93K-2046 and several breeding lines (99CV-564-2, 99CV-564-4, 99CV-565-3, and 99CV576-4) developed from crosses of this line and California blackeye cultivars, were initially attacked, but rapidly recovered and produced pods in both replicates of a screening nursery in which all other lines were completely destroyed by aphids. Several selections were made in each of the resistant  $F_6$  lines and are being further tested for resistance to aphids.

## Cowpea cover crops—progress in breeding

Cowpea is increasingly finding favor as a warm-season, nitrogen-fixing cover crop, particularly in organic vegetable production systems in the US. These systems need low-cost sources of organic nitrogen, and cowpea can have high levels of biological nitrogen fixation.

Cowpea cover-crop varieties in the US are needed that produce abundant biomass, have strong nematode resistance, photoperiod sensitivity, resistance to *Fusarium* wilt, reduced pod shattering, vigorous plant growth, and high yields of small seeds. Photoperiod sensitive cowpea cover-crop varieties will fix much more nitrogen and produce much more biomass than present-day, neutral cowpea cultivars grown in the US because, under long day lengths, photoperiod sensitivity prevents the early transition to reproductive growth

**Table 3. Grain yield of protected and unprotected, yield loss due to lygus, and percentage damaged seed under unprotected conditions of check cultivar CB46 and exotic (E) or wild × cultivated (W × C) cowpea lines at Riverside in 1999.**

| Line      | Type  | Grain yield |             | Yield loss (%) | Lygus damaged seed (%) | Seed        |                            |
|-----------|-------|-------------|-------------|----------------|------------------------|-------------|----------------------------|
|           |       | Protected   | Unprotected |                |                        | Size        | Color/pattern <sup>†</sup> |
|           |       | kg/ha       |             |                |                        | g/100 seeds |                            |
| 96-11-38  | W × C | 2014        | 1826        | 9              | 5                      | 18.0        | B-W/holstein               |
| IT93K-452 | E     | 1661        | 1500        | 10             | 6                      | 15.6        | Blackeye                   |
| 96-11-27  | W × C | 2017        | 1723        | 14             | 6                      | 13.6        | B-W/holstein               |
| IT93K-370 | E     | 1766        | 1475        | 15             | 8                      | 16.7        | Blackeye                   |
| 96-11-29  | W × C | 1719        | 1463        | 15             | 3                      | 12.8        | B-W/holstein               |
| 96-11-111 | W × C | 1710        | 1355        | 21             | 7                      | 11.6        | Solid black                |
| CB46      | Check | 2520        | 1753        | 29             | 14                     | 20.4        | Blackeye                   |
| Mean      |       | 1996        | 1651        | 16             | 7                      | 15.5        |                            |
| LSD(0.05) |       | 330         | 311         | 9              | 4                      | 1.0         |                            |
| CV (%)    |       | 11          | 13          | 42             | 41                     | 4.1         |                            |

<sup>†</sup>B-W/holstein = black pigment covers about 75% of surface area on white background.



that leads to sharp decreases in biomass production and nitrogen fixation. In the US, seed of photoperiod sensitive varieties can only be produced reliably in warm fall regions such as the low-elevation deserts of California and southern Florida.

At UC Riverside, complementary parental lines have been identified and crossed to develop a variety with the desired traits listed above. UCR 779, a nematode-susceptible landrace from Botswana that has a very aggressive spreading plant habit and high biomass production (in the absence of root-knot nematodes), has been hybridized with IT89KD-288 and IT84S-2049 breeding lines from IITA that have high biomass and very strong resistance to root-knot nematodes (Roberts et al. 1997) and other desirable traits (Aguilar et al. 1998). Nematode-resistant  $F_5$  breeding lines with photoperiod sensitivity, nonshattering pods, small seed size, and high biomass production have been identified from these crosses thus far.

## Future possibilities

Successful genetic transformation of cowpea, which appears imminent at this time, will open up new possibilities for improvement, particularly in the area of insect resistance.

Candidate genes that show insecticidal activities with a number of African cowpea pests have been identified (Machuka 2000). These genes code for *Bacillus thuringiensis* (Bt) endotoxin crystal proteins, plant lectins, protease and alpha-amylase inhibitor proteins (Shade et al. 1994), chitinases and ribosomal inactivating proteins. Little is known, however, about the effectiveness of these genes in controlling major US cowpea pests such as the lygus bug and cowpea curculio.

Recent improvements in the cowpea genetic map (Fatokun et al. 2000; Ogundiwin et al. 2000) could potentially make applied breeding programs more efficient through marker-assisted selection.

Wild cowpea relative *Vigna vexillata* is highly resistant to many insect pests, but until now it has not been possible to hybridize this species with cowpea (Fatokun 2000). The recent report of the successful hybridization of this species with cowpea (Gomathinayagam and Muthiah 2000) offers the possibility of obtaining insect resistance that is not available from the primary gene pool of the species.

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## References

- Aguilar, J., J.D. Ehlers, and W. Graves. 1998. Desert cover crop varieties identified. California Vegetable Journal, December. Pages 5, 6, and 21.
- Chambliss, O.L. 1974. Green seed coat: a mutant in southernpea of value to the processing industry. HortScience 9: 126.
- Chambliss, O.L. 1979. Freezegreen southernpea. HortScience 14: 193.
- Cuthbert, F.P. Jr., R.L. Fery, and O.L. Chambliss. 1974. Breeding for resistance to the cowpea curculio in southern peas. HortScience 9: 69–70.
- Cuthbert, F.P. Jr. and R.L. Fery. 1975. CR 17-1-13, CR 18-13-1, and CR 22-2-21, cowpea curculio resistant southernpea germplasm. HortScience 10: 628.

- Cuthbert, F.P., Jr. and R.L. Fery. 1979. Value of plant resistance for reducing cowpea curculio damage to the southernpea (*Vigna unguiculata* [L.] Walp.). *Journal of the American Society of Horticultural Science* 104: 199–201.
- Ehlers, J.D. and A.E. Hall. 1997. Cowpea (*Vigna unguiculata* [L.] Walp.). 1997. *Field Crops Research* 53: 187–204.
- Ehlers, J.D., A.E. Hall, A.M. Ismail, P.A. Roberts, W.C. Matthews, B.L. Sanden, C.A. Frate, and S. Mueller. 1999. Blackeye varietal improvement. Pages 47–61 in *University of California Dry Bean Research 1999 Progress Report*, California Dry Bean Advisory Board, Dinuba, CA, USA.
- Ehlers, J.D., A.E. Hall, P.N. Patel, P.A. Roberts, and W.C. Matthews. 2000a. Registration of California Blackeye 27 Cowpea. *Crop Science* 40: 854–855.
- Ehlers, J.D., W.C. Matthews, A.E. Hall, and P.A. Roberts. 2000b. Inheritance of a broad-based form of nematode resistance in cowpea. *Crop Science* 40: 611–618.
- Fatokun, C.A. 2000. Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and *Vigna vexillata*. *Proceedings of the Third World Cowpea Conference*, 4–7 September 2000. Ibadan, Nigeria.
- Fatokun, C.A., B. Ubi, T.H.N. Ellis, C. Li, and G. Scoles. 2000. A genetic linkage map of cowpea based on DNA markers. *Proceedings of the Third World Cowpea Conference*, 4–7 September 2000. Ibadan, Nigeria. (Abstract.)
- Fery, R.L. 1981. Cowpea production in the United States. *HortScience* 16: 473–474.
- Fery, R.L. 1990. The cowpea: production, utilization, and research in the United States. *Horticultural Review* 12: 197–222.
- Fery, R.L. 1998. Charleston Greenpack, a pinkeye-type southernpea with a green cotyledon phenotype. *HortScience* 33: 907–908.
- Fery, R.L. 1999. Petite-N-Green, a small-seeded, full-season, green cotyledon, pinkeye-type southernpea. *HortScience* 34: 938–939.
- Fery, R.L. 2000. Green Pixie, a small-seeded, green cotyledon, cream-type southernpea. *HortScience* 35(5): 954–955.
- Fery, R.L. and F.P. Cuthbert, Jr. 1975. Inheritance of pod resistance to the cowpea curculio infestation in southernpeas. *Journal of Heredity* 66: 43–44.
- Fery, R.L. and F.P. Cuthbert, Jr. 1978. Inheritance and selection of nonpreference resistance to the cowpea curculio in the southernpea (*Vigna unguiculata* [L.] Walp.). *Journal of the American Society of Horticultural Science* 103: 370–372.
- Fery, R.L. and F.P. Cuthbert, Jr. 1979. Measurement of pod-wall resistance to the cowpea curculio in the southernpea (*Vigna unguiculata* [L.] Walp.). *HortScience* 14: 29–30.
- Fery, R.L., P.D. Dukes, and F.P. Cuthbert, Jr. 1975. CR 17-1-34, *Cercospora* leaf spot-resistant southernpea germplasm. *HortScience* 10: 627.
- Fery, R.L. and B.B. Singh. 1997. Cowpea genetics: a review of recent literature. Pages 13–29 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan Nigeria.
- Fery, R.L., P.D. Dukes, and F.P. Cuthbert, Jr. 1976. The inheritance of *Cercospora* leaf spot resistance in the southernpea (*Vigna unguiculata* [L.] Walp.). *Journal of the American Society of Horticultural Science* 101: 148–149.
- Fery, R.L. and P.D. Dukes. 1977. An assessment of two genes for *Cercospora* leaf spot resistance in the southernpea (*Vigna unguiculata* [L.] Walp.). *HortScience* 12: 454–456.
- Fery, R.L., P.D. Dukes, and F.P. Cuthbert, Jr. 1977. Yield-loss of southernpea (*Vigna unguiculata*) caused by *Cercospora* leaf spot. *Plant Disease Report* 61: 741–743.
- Fery, R.L. and P.D. Dukes. 1980. Inheritance of root-knot nematode resistance in the cowpea (*Vigna unguiculata* [L.] Walp.). *Journal of the American Society of Horticultural Science* 105: 671–674.
- Fery, R.L. and P.D. Dukes. 1990. Santee Early Pinkeye southernpea. *HortScience* 25: 990–991.
- Fery, R.L. and P.D. Dukes. 1992. Carolina Crowder southernpea. *HortScience* 27: 1335–1337.
- Fery, R.L. and P.D. Dukes. 1993. Bettergro Blackeye southernpea. *HortScience* 28: 62–63.

- Fery, R.L. and P.D. Dukes. 1994. Genetic analysis of the green cotyledon trait in southernpea (*Vigna unguiculata* [L.] Walp.). *Journal of the American Society of Horticultural Science* 119: 1054–1056.
- Fery, R.L. and P.D. Dukes. 1995a. Registration of US-566, US-567, and US-568 root-knot nematode resistant cowpea germplasm lines. *Crop Science* 35: 1722.
- Fery, R.L. and P.D. Dukes. 1995b. Bettersnap southernpea. *HortScience* 30: 1318–1319.
- Fery, R.L. and P.D. Dukes. 1996. Tender Cream southernpea. *HortScience* 31: 1250–1251.
- Fery, R.L. P.D. Dukes, and F.P. Maguire. 1993. Bettergreen southernpea. *HortScience* 28: 856.
- Fery, R.L., P.D. Dukes, and J.A. Thies. 1994. Characterization of new sources of resistance in cowpea to the southern root-knot nematode. *HortScience* 29: 678–779.
- Freire Filho, F.R., O.L. Chambliss, and A.G. Hunter. 1996. Genetic analysis of crosses to produce persistent green seeds in southernpeas using gt and gc genes. Auburn University Research Report, USA.
- Gomathinayagam, P. and A.R. Muthiah. 2000. Study on backcrossed embryo rescued crossed regenerants (*Vigna vexillata* [L.] A. Rich × *Vigna unguiculata* [L.] Walp.) with *Vigna unguiculata* [L.] Walp.) Proceedings of the Third World Cowpea Conference, 4–7 September 2000. Ibadan, Nigeria.
- Hall, A.E. 1992. Breeding for heat tolerance. *Plant Breeding Reviews* 10: 129–168.
- Hall, A.E., B.B. Singh, and J.D. Ehlers. 1997. Cowpea breeding. *Plant Breeding Reviews* 15: 215–274.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1997. Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. *Crop Science* 37: 1270–1277.
- Ismail, A.M., A.E. Hall, and T.J. Close. 1999. Allelic variation of a dehydrin gene co-segregates with chilling tolerance during seedling emergence. *Proceedings of the Natural Academy of Science* 96: 13566–13570.
- Kitch, L.W., O. Boukar, C. Endondo, and L.L. Murdock. 1998. Farmer acceptability criteria in breeding cowpea. *Experimental Agriculture* 34: 475–486.
- Lorz, A.P. and L.H. Halsey. 1964. Snapea, a new cream type southern pea variety for snap pods use. University of Florida Agricultural Experimental Station Circular S-160, Florida, USA.
- Machuka, J. 2000. Potential role of transgenic approaches in the control of cowpea insect pests. Proceedings of the Third World Cowpea Conference, 4–7 September 2000. Ibadan, Nigeria.
- Magruder, R. and R.E. Wester. 1941. Green cotyledon, a new character in the mature lima bean (*Phaseolus lunatus* L.). *Proceedings of the American Society of Horticultural Science* 398: 581–584.
- McWatters, K.H., C.-Y.T. Hung, Y.-C. Hung, M.S. Chinnan, and R.D. Phillips. 2001. Akara making characteristics of five US varieties of cowpeas (*Vigna unguiculata*). *Journal of Food Quality* 24(1): 53–66.
- Ogundiwin, E.A., C.A. Fatokun, G. Thottappilly, M.E. Aken'Ova, and M. Pillay. 2000. Genetic linkage map of *Vigna vexillata* based on DNA markers and its potential usefulness in cowpea improvement. Proceedings of the Third World Cowpea Conference, 4–7 September 2000. Ibadan, Nigeria. (Abstract).
- Patel, P.N. and A.E. Hall. 1986. Registration of snap-cowpea germplasm. *Crop Science* 26: 207–208.
- Roberts, P.A., W.C. Matthews, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to the *Rk* locus in cowpea. *Crop Science* 36: 889–894.
- Roberts, P.A., J.D. Ehlers, A.E. Hall, and W.C. Matthews. 1997. Characterization of new resistance to root-knot nematodes in cowpea. Pages 207–214 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan Nigeria.

- Schalk, J.M. and R.L. Fery. 1982. Southern green stink bug and leaffooted bug: Effect on cowpea production. *Journal of Economic Entomology* 75: 72–75.
- Schalk, J.M. and R.L. Fery. 1986. Resistance in cowpea to the southern green stink bug. *Hort-Science* 21: 1189–1190.
- Shade, R.E., H.E. Schroeder, J.J. Pueyo, L.M. Tabe, L.L. Murdock, T.J.V. Higgins, and M.J. Crispceels. 1994. Transgenic pea seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. *Biocontrol Science and Technology* 12: 793–796.
- US Department of Agriculture. 2000. Notice of release of Green Dixie Blackeye, a green cotyledon, blackeye-type southernpea. USDA, ARS, Washington, DC 20250. 28 April 2000.



## **Section II**

# **Cowpea integrated pest management**



## 2.1

# The importance of alternative host plants for the biological control of two key cowpea insect pests, the pod borer *Maruca vitrata* (Fabricius) and the flower thrips *Megalurothrips sjostedti* (Trybom)

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### Abstract

The interactions between naturally occurring and cultivated host plants, and biological control, are first evaluated for the major lepidopteran pest attacking cowpea in West Africa, the pod borer *Maruca vitrata*. Significantly higher larval mortality due to parasitism by the ovolarval parasitoid *Phanerotoma leucobasis* was observed on wild alternative host plants in perennial habitats (e.g., *Pterocarpus santalinoides*) than in agroecosystems such as the cowpea field. Experimental assessment of the impact of the only egg parasitoid recorded from *M. vitrata*, *Trichogrammatoidea ?eldanae*, indicated that it is present in a variety of agroecosystems. A second important legume pest, the flower thrips *Megalurothrips sjostedti*, is attacked by the larval parasitoid *Ceranisus menes*, but overall parasitism rates are low, depending on host plant and season. However, another parasitoid of the same genus, *C. femoratus*, recently discovered in Cameroon, has showed higher efficiency in parasitizing *M. sjostedti* on most of the important host plants, including cowpea. The potential of this new parasitoid as a biocontrol candidate in West Africa is being assessed through experimental releases in Benin and Ghana.

### Introduction

In West Africa, cowpea (*Vigna unguiculata* Walp.) is cultivated mainly as a rainfed crop from April to November, depending on the location. In the moist savanna with a bimodal rainfall pattern, where cowpea can produce two crops, the first rainy season lasts from April to July, and the second from mid-September to November, with a short dry spell from August to early September. In the regions of monomodal rainfall, the beginning and length of the rainy season usually depend on the latitude. In the areas considered in this review (see below), the monomodal rainy season normally lasts from May to November. During the long dry season from December to March, cowpea is cultivated on residual moisture in small isolated areas only (e.g., the Ouémé valley in southern Benin, or the fadamas in northern Nigeria) (Arodokoun 1996; Bottenberg et al. 1997). As a consequence, insect pests attacking cowpea would need either to find alternative hosts to survive during this

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period or to diapause. For the two pests considered in this paper, the pod borer *Maruca vitrata* Fabricius (Lep., Pyralidae) and the flower thrips *Megalurothrips sjostedti* Trybom (Thys., Thripidae), Arodokoun et al. (2001) and Tamò et al. (1993b) have demonstrated that neither species goes through diapause during the dry season, both of them being capable of feeding and reproducing on a wide range of alternative host plants in the absence of cowpea. Parallel studies (Arodokoun 1996; Tamò et al. 1997; Zenz 1999) have indicated that natural enemies of both *M. vitrata* and *M. sjostedti*, and particularly parasitic Hymenoptera, also survive on the same alternative host plant habitat.

This paper summarizes the status of knowledge on the interactions between these two cowpea pests, their most important natural enemies, and the alternative wild, host plant habitat.

## Case study I: the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera, Pyralidae)

### *The host plants*

The host range of *M. vitrata* was studied in Nigeria and Benin by Taylor (1978), Atachi and Djihou (1994), Zenz (1999), and Arodokoun et al. (2001). However, only the latter two studies provide information concerning year-long monitoring of host plants across ecological regions (from the coast to the southern Guinea savanna). The larvae of other Pyralidae occurring in West Africa (e.g., *Mussidia nigrivenella* Ragonot [Lep., Pyralidae], see Sétamou et al. 2000), particularly their early instars, are difficult to distinguish from *M. vitrata*, and could be mistaken for the latter. For this reason, the larvae sampled by Arodokoun et al. (2001) and suspected to be *M. vitrata*, were reared until the emergence of the adult moth.

The studies by Zenz (1999) and Arodokoun et al. (2001) used quantitative sampling procedures which permitted an assessment of the seasonal abundance of *M. vitrata* on the different host plants. This information was used to establish a list of the most important noncrop host plants organized by seasons and habitats (Table 1). Apart from cowpea, other cultivated plants such as pigeonpea (*Cajanus cajan*) and common bean (*Phaseolus vulgaris*) are also attacked by larvae of *M. vitrata*, but were not considered in this study. Without exception, all plants listed in Table 1, and the less important plants cited by Zenz (1999) and Arodokoun et al. (2001), belong to the family of the Fabaceae, which lets us conclude that *M. vitrata* is a stenophagous insect. Although all these host plants occur naturally in the wild, some of them, e.g., the herbaceous legumes *Centrosema pubescens* and *Pueraria phaseoloides*, have been introduced as cover crops during the first half of last century, mainly from tropical America and Asia, but are now part of the spontaneous vegetation. Another interesting feature concerning the association of *M. vitrata* with these host plants is its feeding habit. None of the larval instars feed on growing pods, as is the case for cultivated legumes (cowpea and common beans). Instead, they either feed inside single flowers or spin a web around the whole inflorescence, feeding on several flowers.

The most important outcome of these studies is the certainty that, in the area under study, *M. vitrata* does not need cowpea, nor any other cultivated legume, as an obligate host plant in order to complete its annual cycle. This is particularly important during the main dry season, when cowpea cultivation is restricted to moister areas, and cowpea possibly offers a less favorable microhabitat for *M. vitrata* larval development than trees such as

*Pterocarpus santalinoides* (Leumann 1994). During the intermediate period from August to November (short dry spell and subsequent short rainy season), cowpea is planted with the return of the rain in mid-September, and thus becomes available for *M. vitrata* larvae approximately one month later, depending on the variety planted. Since the cowpea crop from the first rainy season would have been harvested by late July/early August, this leaves a two-month gap without cowpea. This gap is filled by several *Tephrosia* and *Sesbania* species; the most important of them are given in Table 1.

### The interactions with its natural enemies

Earlier studies of the natural enemies complex of *M. vitrata* (e.g., Usua and Singh 1978) focused on organisms associated with *M. vitrata* larvae on cowpea. Away from that crop-centered approach for studying natural enemies of a pest, Arodokoun (1996) was the first to look for *M. vitrata* natural enemies, particularly parasitic Hymenoptera, on alternative wild host plants, and to compare their occurrence and parasitism levels with those found on cowpea. Percentage parasitism for each parasitoid was calculated after Bellows et al. (1992) and van Driesche et al. (1991), and is summarized in Figure 1 for each of the important host plants (Arodokoun 1996).

**Table 1. Flowering season and habitat of major alternative host plants for *Maruca vitrata* in southern and central Benin (all belonging to the family Fabaceae) (adapted from Zenz [1999] and Arodokoun et al. [2001]).**

| Host plant  | Habitat                        |
|---|--------------------------------|
| Flowering during the main dry season<br>(December–March)      |                                |
| <i>Centrosema pubescens</i>                                   | Ubiquitous                     |
| <i>Lonchocarpus sericeus</i>                                  | Wetland, river banks (coast)   |
| <i>Milletia thonningii</i>                                    | Firmland (savanna)             |
| <i>Pterocarpus erinaceus</i>                                  | Firmland (savanna)             |
| <i>Pterocarpus santalinoides</i>                              | Wetland, river banks (savanna) |
| <i>Pueraria phaseoloides</i>                                  | Ubiquitous                     |
| Flowering during the main rainy season<br>(April–July)        |                                |
| <i>Afromosia laxiflora</i>                                    | Firmland (savanna)             |
| <i>Andira inernis</i>   | Firmland (savanna)             |
| <i>Canavalia virosa</i>                                       | Firmland (savanna)             |
| <i>Centrosema pubescens</i>                                   | Wetland (savanna)              |
| <i>Dolichos africanus</i>                                     | Firmland (savanna)             |
| <i>Lonchocarpus cyanescens</i>                                | Firmland (coast, savanna)      |
| <i>Lonchocarpus sericeus</i>                                  | Firmland (coast, savanna)      |
| <i>Pterocarpus santalinoides</i>                              | Firmland (savanna)             |
| <i>Pueraria phaseoloides</i>                                  | Wetland (savanna)              |
| Flowering during the intermediate period<br>(August–November) |                                |
| <i>Sesbania pachycarpa</i>                                    | Firmland (savanna)             |
| <i>Tephrosia candida</i>                                      | Firmland (savanna)             |
| <i>Tephrosia humilis</i>                                      | Firmland (savanna)             |
| <i>Tephrosia platycarpa</i>                                   | Firmland (savanna)             |
| <i>Vigna racemosa</i>   | Firmland (savanna)             |

Compared to cowpea, aggregate parasitism levels were generally higher on wild alternative host plants, with the exception of *Lonchocarpus sericeus*. Highest overall rates were observed on *P. santalinoides* during the main dry season, followed by *L. cyanescens*, while on the herbaceous legumes parasitism rates were lower than 15%. The difference between woody plant species (trees and shrubs such as *P. santalinoides*, *L. sericeus*, and *L. cyanescens*) and herbaceous legumes lies not only in the apparently higher parasitism rates, but even more in the composition of the parasitoid community. Although Arodokoun (1996) observed a total of eight different species of larval parasitoids, and one unidentified parasitic nematode, only three of them seemed to be important and are therefore used in our comparison below.

The dominant parasitoid recovered from *M. vitrata* larvae collected in the flowers of the woody plant species (Fig. 1) was *Phanerotoma leucobasis* Kriechbaumer (Hym., Braconidae). Parasitoids of the genus *Phanerotoma* were already observed by Taylor (1967), Usua (1975), and Usua and Singh (1978), but were not identified to species level. *P. leucobasis* was also found on the herbaceous legumes *P. phaseoloides*, *T. platycarpa*, and cowpea (Fig. 1), but with much lower parasitism rates, and in a lower proportion compared with other parasitoid species. Zenz (1999) recovered *P. leucobasis* mainly from *Dolichos africanus* and *Tephrosia* spp., while only one specimen was reared from a total of three larvae found on *L. cyanescens*. The main reason for the discrepancy between the

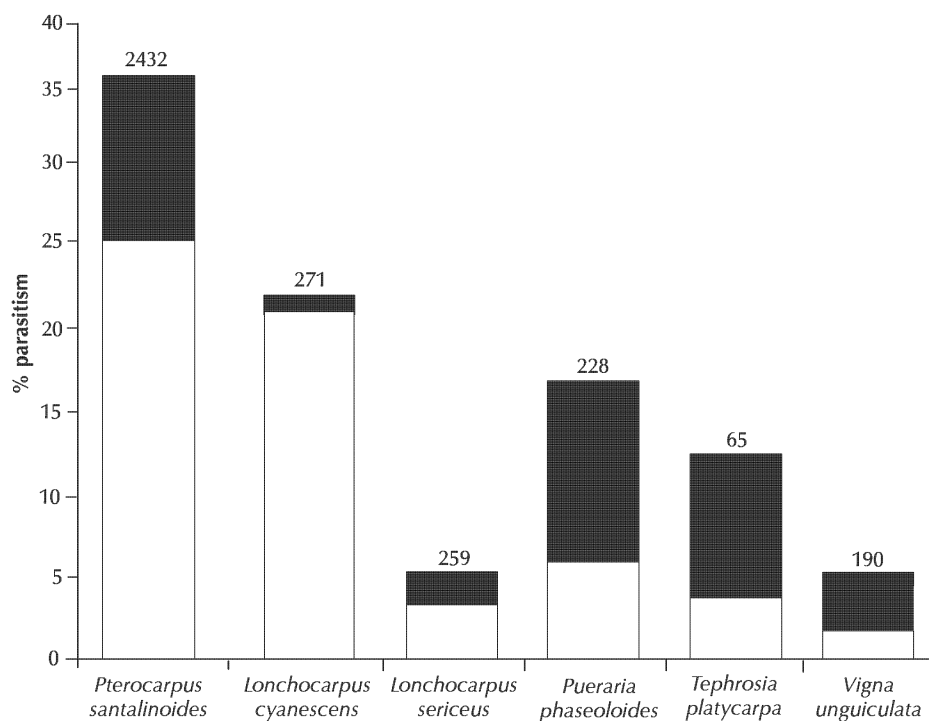


Figure 1. Parasitism levels exhibited by larvae of *M. vitrata* collected from different host plants in southern and central Benin (□ = *Phanerotoma leucobasis*, ■ = *Braunsia kriegeri*, ▒ = *Pristomerus* sp., ■ = others). Numbers on top of the bars are the total numbers of larvae reared during 1993–1995 for each host plant (modified from Arodokoun 1996).

result of Arodokoun (1996) and Zenz (1999) is because the latter examined the vegetation surrounding cowpea fields during the rainy season, while Arodokoun (1996) determined the presence of *M. vitrata* and its natural enemies in areas of high incidence of major alternative host plants throughout the year.

On the herbaceous legumes in Figure 1, the predominant parasitoid was *Braunsia kriegeri* Enderlein (Hym., Braconidae), the most commonly reported parasitoid from *M. vitrata* on cowpea, and thus the one being more often observed in previous studies (see Tamò et al. 1997). However, overall parasitism rates for *B. kriegeri* were much lower than those seen for *P. leucobasis*, averaging mainly between 5 and 10%. This parasitoid was also observed by Zenz (1999) and, although based on fewer samples, his data corroborate the results of Arodokoun (1996).

The third larval parasitoid worth mentioning was *Pristomerus* sp. (Hym., Ichneumonidae), which could not be identified to species level. In the study of Arodokoun (1996), it was recovered from two host plants only, cowpea, with less than 1% parasitism, and *P. santalinoides*, where it was able to parasitize up to 4% of *M. vitrata* larvae.

Among the egg parasitoids attacking *M. vitrata*, *Trichogrammatoidea ?eldanae* Viggiani (Hym., Trichogrammatidae) was the only one discovered in West Africa (Tamò et al. 1997). The minute size of *M. vitrata* eggs, and the way they are scattered on the plant organs used for oviposition (leaves, peduncles, flowers) (Jackai 1981), precluded any direct observation of parasitism in the field. Instead, Arodokoun (1996) exposed sentinel *M. vitrata* eggs, freshly oviposited on cowpea leaves in the laboratory on selected host plants. Because of the polyphagous nature of Trichogrammatids, nonhost crops such as maize and cassava were also included in his investigation, in order to determine the habitat diversity of *T. ?eldanae*. Apparent parasitism rates were calculated using the same approaches used for the larvae, and are reported in Table 2. During the wet season, *M. vitrata* eggs exposed on cowpea developed quite high parasitism rates, reaching over 50% on average. These parasitism rates might actually have been underestimated, because eggs unsuitable for oviposition and egg predation were not taken into account in the calculation of percentage parasitism. On the only host plant available for comparison, the tree *L. sericeus*, the parasitism level was less than a third of that recorded on cowpea. A different picture was encountered during the dry season, where egg parasitism levels close to those found on cowpea during the rainy season were observed on the cover crop *C. ensiformis*, followed by maize with nearly 30% parasitism. Surprisingly, also on the

**Table 2. Parasitism of *Maruca vitrata* eggs exposed on different host plants during different seasons ([a] long rainy season, [b] long dry season) by *Trichogrammatoidea ?eldanae* [Arodokoun 1996]).**

| Plant species                    | Eggs exposed | Eggs parasitized | Parasitism (%) |
|----------------------------------|--------------|------------------|----------------|
| <i>Lonchocarpus sericeus</i>     | 6138         | 1000             | 16.3           |
| <i>Vigna unguiculata</i>         | 4954         | 2671             | 53.9           |
| <i>Canavalia ensiformis</i>      | 1554         | 810              | 52.1           |
| <i>Manihot esculenta</i>         | 223          | 33               | 14.8           |
| <i>Pterocarpus santalinoides</i> | 7648         | 1030             | 13.5           |
| <i>Tephrosia candida</i>         | 1550         | 16               | 1.0            |
| <i>Vigna unguiculata</i>         | 5730         | 934              | 16.3           |
| <i>Zea mays</i>                  | 822          | 245              | 29.8           |

other nonhost for *M. vitrata*, cassava, there was still a substantial proportion (14.8%) of eggs parasitized by *T. ?eldanae*.

Using the same procedure of exposing *M. vitrata* eggs oviposited on cowpea leaves, Zenz (1999) found similar parasitism levels when exposed in cowpea fields.

In addition to eggs of *M. vitrata*, *T. ?eldanae* also attacked eggs of two common polyphagous leaf feeders, *Trichoplusia limbirena* (Guence) (Lep., Noctuidae) and *Syl-epta derogata* (Fabricius) (Lep., Pyralidae) collected from cowpea fields, with parasitism rates averaging over 70% (Arodokoun 1996). In the laboratory, the same author observed *T. ?eldanae* successfully parasitizing eggs of the maize stem- and earborers *Sesamia calamistis* Hampton (Lep., Noctuidae), *Eldana saccharina* Walker (Lep., Pyralidae), and *M. nigrivenella*.

With all this information at hand, we can now attempt to identify main interactions concerning the host plant, the insect pest, and the associated natural enemies. For instance, it was observed that the ovolarval parasitoid *P. leucobasis* could successfully parasitize *M. vitrata* on trees and shrubs, but less so on herbaceous legumes. Exactly the reverse trend was observed for the egg parasitoid *T. ?eldanae*, whose parasitism levels were much higher on herbaceous legumes and lower on the taller plants. Although adult *P. leucobasis* usually emerge from the 2nd instar larvae, the female oviposits inside the *M. vitrata* egg, but the egg of the parasitoid does not hatch and start development until close to the hatching of the 1st instar *M. vitrata* larva. As it was demonstrated in a laboratory experiment (Arodokoun, unpublished data), *M. vitrata* eggs could be simultaneously parasitized by both parasitoids. In this case, however, it was always *T. ?eldanae* which completed development and emergence, evidently because of its shorter developmental time. Although they were not validated with field experiments, the above findings could explain why *T. ?eldanae*, because of its minute size and the hence reduced flight capabilities, would be more successful than *P. leucobasis* in parasitizing *M. vitrata* eggs on plants with prostrate growth habit. In its turn, *P. leucobasis* being a better flyer, can parasitize *M. vitrata* eggs on taller shrubs and trees in the absence of *T. ?eldanae*.

Another type of interaction involves the two other hosts of *T. ?eldanae* encountered on cowpea, the moths *T. limbirena* and *S. derogata*. Both insects are polyphagous, but have been mostly observed feeding on leaves of annual crop plants such as cowpea, cotton, okra, etc. (e.g., Dufay 1982; Silvie 1989). In fact, during the extensive collecting surveys of Arodokoun (1996), they were never recorded on major wild host plants for *M. vitrata*. Given the high parasitism rates by *T. ?eldanae* on eggs of both *T. limbirena* and *S. derogata* recorded on cowpea, their presence in the cropping systems could substantially increase its population level, and subsequently its ability and efficacy in parasitizing *M. vitrata* eggs. On the other hand, the absence of these moths on trees such as *L. sericeus* and *P. santalinoides*, together with the above discussed low dispersal capability of *T. ?eldanae* on taller legume trees, is probably an additional explanation for the lower parasitism rates observed there.

The high levels of parasitism encountered in the maize field are not surprising. *T. ?eldanae* has been reported attacking eggs of the cereal stemborers *Sesamia calamistis* Hampson (Lep., Noctuidae) (Bosque-Pérez et al. 1994) and *Eldana saccharina* (Walker) (Lep., Pyralidae) (Conlong and Hastings 1984). These findings were confirmed by laboratory studies, where *T. ?eldanae* was able to successfully parasitize eggs of *S. calamistis*, *E. saccharina*, and *M. nigrivenella* (Arodokoun 1996). Given the fact that cowpea is very

often intercropped with cereals such as sorghum, millet, and maize, the polyphagous habit of *T. ?eldanae* could increase its persistence and possibly also its efficiency in such a cropping system.

## Case study II: the bean flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera, Thripidae)

### The host plants

The first report of *M. sjostedti* on alternative host plants in West Africa was given by Taylor (1974), who described this insect feeding and reproducing on two legumes, *C. pubescens* and pigeonpea. Subsequent studies by Tamò et al. (1993b) and Zenz (1999) gave more detailed, albeit not exhaustive, lists of alternative host plants for different ecological regions, ranging from the coast of Benin and Ghana, up to the Sudan savanna in Burkina Faso. As already the case for *M. vitrata*, the data collected on the abundance of *M. sjostedti* also allowed the establishment of the same type of list, indicating the most important alternative host plants according to season and habitat (Table 3).

Most of these alternative host plants belong to the Fabaceae and are also host plants for *M. vitrata*, which might be competing with *M. sjostedti* in the case of scarce resources, as already noticed for cowpea (Tamò et al. 1993a). However, some of the important relay hosts, and many of the secondary host plants (Tamò et al. 1993b; Zenz 1999) belong to

**Table 3. Flowering season, habitat, and location of major host plants for *Megalurothrips sjostedti* in West and Central Africa (adapted from Tamò et al. [1993b] and Zenz [1999]).**

| Host plant   | Bot. family    | Habitat                        |
|--|----------------|--------------------------------|
| Flowering during the main dry season (December–March)      |                |                                |
| <i>Berlinia grandiflora</i>                                | Caesalpinaceae | Wetland, river banks (savanna) |
| <i>Centrosema pubescens</i>                                | Fabaceae       | Ubiquitous                     |
| <i>Lonchocarpus sericeus</i>                               | Fabaceae       | Wetland, river banks (coast)   |
| <i>Milletia thonningii</i>                                 | Fabaceae       | Firmland (savanna)             |
| <i>Pterocarpus erinaceus</i>                               | Fabaceae       | Firmland (savanna)             |
| <i>Pterocarpus santalinoides</i>                           | Fabaceae       | Wetland, river banks (savanna) |
| <i>Pueraria phaseoloides</i>                               | Fabaceae       | Ubiquitous                     |
| Flowering during the main rainy season (April–July)        |                |                                |
| <i>Afromosia laxiflora</i>                                 | Fabaceae       | Firmland (savanna)             |
| <i>Centrosema pubescens</i>                                | Fabaceae       | Wetland (savanna)              |
| <i>Dolichos africanus</i>                                  | Fabaceae       | Firmland (savanna)             |
| <i>Lonchocarpus cyanescens</i>                             | Fabaceae       | Firmland (coast, savanna)      |
| <i>Lonchocarpus sericeus</i>                               | Fabaceae       | Firmland (coast, savanna)      |
| <i>Pterocarpus santalinoides</i>                           | Fabaceae       | Firmland (savanna)             |
| <i>Pueraria phaseoloides</i>                               | Fabaceae       | Wetland (savanna)              |
| Flowering during the intermediate period (August–November) |                |                                |
| <i>Cochlospermum planchoni</i>                             | Bixaceae       | Firmland (savanna)             |
| <i>Piliostigma thonningii</i>                              | Caesalpinaceae | Firmland (savanna)             |
| <i>Sesbania pachycarpa</i>                                 | Fabaceae       | Firmland (savanna)             |
| <i>Tephrosia candida</i>                                   | Fabaceae       | Firmland (savanna)             |
| <i>Tephrosia platycarpa</i>                                | Fabaceae       | Firmland (savanna)             |

the other two families of the Leguminosae, Caesalpiniaceae, and Mimosaceae. The only exception is *C. planchoni*, a shrub with conspicuous, bright yellow flowers, which is the only nonleguminous plant that can sustain high populations of *M. sjostedti* (Zenz 1999). Other nonleguminous plants are just occasional hosts, where in most of the cases adult *M. sjostedti* were observed feeding on pollen, but no larval development was observed (Tamò et al. 1993b).

As opposed to the case for *M. vitrata*, adult *M. sjostedti* were present in the dry savanna throughout the year (Bottenberg et al. 1997), indicating a much higher degree of adaptability to difficult conditions, which might be a consequence of their capability to feed and reproduce on more diverse types of plants.

### **Interactions with its natural enemies**

Among the important natural enemies found attacking *M. sjostedti* (Tamò et al. 1997), three have been reported from host plants other than cowpea: the predator *Orius albidipennis* Reuter (Het., Anthocoridae) (Fritzsche and Tamò 2000), the egg parasitoid *Megaphragma* spp. (Hym., Trichogrammatidae) (Tamò et al. 1993b), and the larval parasitoid *Ceranisus menes* Walker (Hym., Eulophidae) (Tamò et al. 1993b, 1997; Zenz 1999). While no quantitative data were available for evaluating the dynamics and impact of *O. albidipennis* on alternative host plants, preliminary observations on *Megaphragma* spp. indicated higher parasitism rates on selected alternative host plants such as *P. phaseoloides*.

Since its first report in Benin in the early 1990s, *C. menes* has been the object of intensified observations. Over 10 000 *M. sjostedti* larvae were collected from the flowers of 12 host-plant species by Zenz (1999) and reared until either the emergence of the adult thrips or the pupa of the parasitoid. Similarly, a recent study by Tamò and coworkers (unpublished data) covered all ecological zones of Benin and was carried out during five consecutive years. Larvae of *M. sjostedti* were collected from 19 host plants, five of which (*P. santalinoides*, *L. sericeus*, *L. cyanescens*, *T. candida*, and cowpea) received particular attention, because they hosted the largest populations of *M. sjostedti*. The results of these two studies are summarized in Table 4.

With exception of two plants, parasitism rates never exceeded 6%, with an overall mean parasitism of 2.6%. The lowest rates were observed on cowpea, with barely 0.1% which confirms previous data by Tamò et al. (1993b), while the highest rates were found on *C. planchoni* (19.1%) and *E. senegalensis* (14.5%). However, these high rates were obtained with a rather low number of samples and over a short period of time (Zenz 1999).

During a survey to assess the presence of insect pests and associated natural enemies on multipurpose legumes along the forest margins of southern Cameroon in February 1998, the first author came across a species of *Ceranisus* with completely dark brown metasoma, which was subsequently identified as *Ceranisus femoratus* Gahan (Triapitsyn, University of California, Riverside, pers. comm.). Adult *C. femoratus* were first encountered in flowers of *C. pubescens* and *Milletia* sp. around the IITA Humid Forest Center at Nkolbison (Yaoundé, Cameroon), and were also obtained in large numbers from parasitized larvae of *M. sjostedti* (Tamò & Tindo, unpublished data). A study was subsequently conducted to assess the presence of this newly discovered parasitoid on cowpea and to measure its possible impact on *M. sjostedti* (Ndam 1998). As it is shown clearly from the results in Table 5, *C. femoratus* was able to parasitize a substantial percentage of *M. sjostedti* larvae collected from cowpea flowers in two locations in Cameroon. Following

**Table 4. Assessment of parasitism inflicted by *Ceranisia menes* on larvae of *Megalurothrips sjostedti* collected from different host plants in Benin. Only plants from where at least 200 larvae were collected are considered in this table (1 = Zenz 1999; 2 = Tamò et al., unpublished).**

| Host plant                       | Viable <i>M. sjostedti</i><br>collected | Larvae<br>parasitized | Parasitism<br>(%) | Source |
|----------------------------------|---|-----------------------|-------------------|--------|
| <i>Cajanus cajan</i>             | 694                                     | 3                     | 0.4               | (1)    |
| <i>Cochlospermum planchonii</i>  | 235                                     | 45                    | 19.1              | (1)    |
| <i>Centrosema pubescens</i>      | 2694                                    | 36                    | 1.3               | (1)    |
| <i>Dolichos africanus</i>        | 374                                     | 30                    | 8.0               | (1)    |
| <i>Erythrina senegalensis</i>    | 558                                     | 81                    | 14.5              | (1)    |
| <i>Lonchocarpus cyanescens</i>   | 231                                     | 8                     | 3.5               | (1)    |
| <i>Lonchocarpus cyanescens</i>   | 5670                                    | 217                   | 3.8               | (2)    |
| <i>Lonchocarpus sericeus</i>     | 8357                                    | 95                    | 1.1               | (2)    |
| <i>Pterocarpus santalinoides</i> | 7590                                    | 30                    | 0.4               | (2)    |
| <i>Tephrosia bracteolata</i>     | 1750                                    | 102                   | 5.8               | (1)    |
| <i>Tephrosia candida</i>         | 2270                                    | 70                    | 3.1               | (1)    |
| <i>Tephrosia candida</i>         | 8220                                    | 361                   | 4.4               | (2)    |
| <i>Tephrosia platycarpa</i>      | 1142                                    | 52                    | 4.6               | (1)    |
| <i>Vigna unguiculata</i>         | 3822                                    | 5                     | 0.1               | (2)    |
| Total                            | 43607                                   | 1135                  | 2.6               |        |

**Table 5. Assessment of parasitism inflicted by *Ceranisia femoratus* on larvae of *Megalurothrips sjostedti* on cowpea in southern Cameroon (Ndam 1998).**

| Host plant              | Viable <i>M. sjostedti</i><br>collected | Larvae<br>parasitized | Parasitism<br>(%) |
|-------------------------|---|-----------------------|-------------------|
| Nkolbison ( 1st season) | 548                                     | 84                    | 15.3              |
| Nkolbison (2nd season)  | 696                                     | 143                   | 20.5              |
| Mbalmayo (1st season)   | 463                                     | 75                    | 16.2              |
| Mbalmayo (2nd season)   | 160                                     | 47                    | 29.4              |
| Total                   | 1867                                    | 349                   | 18.7              |

the delivery of standard quarantine import permits, *C. femoratus* was introduced into the laboratories of the IITA-Benin research station in Agoukamey (near Cotonou, Benin). In collaboration with the national plant protection services, experimental releases were subsequently carried out both in Benin (July 1999) and Ghana (December 1999). In Ghana, the releases were followed by establishment at one site (Pokuase), in spite of the very low thrips population throughout the rainy season. However, because of the untimely onset of the dry season, the second release site at Gomoa Buduatta was hit by a severe bush fire shortly after the release, leaving only few *C. pubescens* plants bearing flowers during the dry season. Unfortunately, these plants were attacked by aphids, and were subsequently colonized by ants which interfered with the activity of *C. femoratus*, as it is often the case with hymenopterous parasitoids (e.g., Cudjoe et al. 1993). Whereas *C. femoratus* could still be found over one year after the release at the first site, repeated attempts to recover the parasitoid from *C. pubescens* flowers at the second site remained unsuccessful.



In Benin, *C. femoratus* was released at two sites around Ouidah and at one site on the IITA-Benin research station. As it had been the case for the second release site in Ghana, both sites around Ouidah were also affected by two consecutive severe, dry seasons characterized by the same type of problems, i.e., bush fires and interference by ants. Again, the consequence was nonestablishment of the released parasitoids on *C. pubescens*. Fortunately, a totally different scenario was found after the experimental release at the IITA-Benin station. Here, *C. femoratus* was able to establish a large population first on *T. candida*, from where it subsequently spread to adjacent patches of *Dioclea guianensis*, *C. pubescens*, and cowpea fields. Although the population density and the related parasitism were subjected to seasonal variations (with peaks over 60% on *D. guianensis* and *T. candida*), depending on the flowering pattern of the host plants, *C. femoratus* is now firmly established and has started spreading outside the fence of the station. All these data from the experimental releases are still being compiled and analyzed, and will be published soon.

## General discussion and conclusion

The interactions between insect pests, their natural enemies, and the natural vegetation have led quite often to more efficient biological control, because of the increased availability of refugia and alternative prey for natural enemies during off-seasons, but also to their higher diversity in the natural vegetation (e.g., Altieri et al. 1993; Waage and Hawksworth 1991).

Our first case study involving *M. vitrata* also suggests that the availability of alternative host plants positively affects parasitism rates, and should consequently reduce overall pest densities. However, given the high pest damage observed in the field (e.g., Bottenberg et al. 1998), one might suspect that the permanent availability of alternative host plants flowering at different periods of the year is rather the cause of much higher population levels of *M. vitrata* than might be expected if cowpea was the only host plant. One of the possible explanations for this occurrence is the fact that *M. vitrata* slowly migrates from the coastal savanna in the south to the dry savanna in the north during the main rainy season, following the Intertropical Convergence Front, as suggested by Arodokoun (1996) and Bottenberg et al. (1997). During this semimigratory movement, *M. vitrata* can produce several generations on various host plants, thus building up large populations, which subsequently migrate into cowpea fields. It could be hypothesized that, during this host switch, some natural enemies might not be able to follow *M. vitrata* on some of the important host plants. The data available so far, however, stemming from the southern and central part of Benin, do not allow us to draw a final conclusion on this issue. On the other hand, *M. vitrata* might still be a problem because it is not indigenous in West Africa, thus lacking efficient natural enemies which may be available elsewhere (Tamò et al. 1997).

Because of the semimigratory habit of *M. vitrata*, possible biological control interventions have to be considered at two different levels. The first option, at the cowpea field level, would be the inundative release of locally available, mass-reared trichogrammatids (*T. ?eldanae*), preferably in conjunction with the use of pheromone trap-derived thresholds (Downham et al. this volume). This approach would be particularly suitable in areas where *M. vitrata* does not have suitable alternative host plants during the dry season, but it rather invades the cowpea fields like a migrant pest (e.g., coming from the South, as it is the case for the Kano region, see Bottenberg et al. 1997). The second option would be more appropriate where alternative host plants are abundant and constitute a major factor influencing the dynamics of *M. vitrata* populations. In this case, the objective would be to reduce overall population pressure, which could be obtained

by the introduction of presumed more efficient natural enemies such as *Phanerotoma philippiniensis* and *Bassus javanicus* (Hym., Braconidae) observed in southeast Asia (Tamò et al. 1997).

In our second case study dealing with the flower thrips *M. sjostedti*, the interactions between natural enemies and wild vegetation are characterized by very low occurrence of the endemic larval parasitoid *C. menes*, irrespective of host plant, ecological zone, and season. The inability of *C. menes* to control *M. sjostedti* is thought to be primarily caused by a high degree of physiological incompatibility (Diop 2000), which supports the hypothesis put forward by Tamò et al. (1993b) that *M. sjostedti* might not be of West African origin. This hypothesis is further reinforced by the fact that the newly introduced exotic parasitoid *C. femoratus* is showing higher parasitism rates than the local *C. menes*.

To render the understanding of these interactions more complicated, not only the two main pests *M. vitrata* and *M. sjostedti* might not be indigenous, but also some of their important host plants are exotic. This is particularly interesting for *T. candida* (introduced from Asia) and *D. guianensis* (from tropical South America) (Hutchison and Dalziel 1958), two of the host plants that have shown the best response to *C. femoratus* so far. While hymenopterous parasitoids can be more habitat specific rather than host specific (Vinson 1976), this hypothesis needs to be verified for our case study involving *M. sjostedti* and its parasitoid *C. femoratus*, and compared to observations on flower thrips and associated parasitoids in the area of origin of the two above exotic host plants. At the same time, it would be interesting to conduct, at the experimental release sites in West Africa, some studies on the possible role of semiochemicals involved in host habitat location, as they are known from the literature (e.g., Elzen et al. 1983). We hope that the outcome of all these studies can be used to optimize further releases of *C. femoratus* in conjunction with habitat management interventions.

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## References

- Altieri, M.A., J.R. Cure, and M.A. Garcia. 1993. The role and enhancement of parasitic Hymenoptera biodiversity in agroecosystems. Pages 257–275 in *Hymenoptera and biodiversity*, edited by J. LaSalle and I.D. Gauld. CAB International, Wallingford, UK.
- Arodokoun, D. 1996. Importance des plantes-hôtes alternatives et des ennemis naturels indigènes dans le contrôle biologique de *Maruca testulalis* Geyer (Lepidoptera: Pyralidae), ravageur de *Vigna unguiculata* Walp. PhD thesis, University of Laval, Québec, Canada. 181 pp.
- Arodokoun, D.Y., M. Tamò, C. Cloutier, and R. Adeoti. 2001. The importance of alternative host plants for the annual cycle of the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae). *Insect Science and its Application* (submitted).
- Atachi, P. and Z.C. Djihou. 1994. Record of the host plants of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) in the Republic of Benin. *Annales de la Société Entomologique de France* 30(2): 169–174.
- Bellows, T.S., R.G. van Driesche, and J.S. Elkinton. 1992. Life-table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology* 37: 587–614.
- Bosque-Pérez, N.A., J.A. Ubeku, and A. Polaszek. 1994. Survey for parasites of *Sesamia calamitis* (Lep.: Noctuidae) and *Eldana saccharina* (Lep.: Pyralidae) in southwestern Nigeria. *Entomophaga* 39: 367–376.

- Bottenberg, H., M. Tamò, and B.B. Singh. 1998. Impact of host plant resistance and cropping system on cowpea pest damage, pest populations, and associated natural enemies. *Agriculture, Ecosystems and Environment* 70: 217–229.
- Bottenberg, H., M. Tamò, D. Arodokoun, L.E.N. Jackai, B.B. Singh, and O. Youm. 1997. Population dynamics and migration of cowpea pests in northern Nigeria: implications for integrated pest management. Pages 271–284 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Conlong, D.E. and H. Hastings. 1984. Evaluation of egg parasitoids in the biological control of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Pages 168–172 in *Proceedings of the 58th Annual Congress*, 25–28 June 1984, Durban and Mount Edgecombe, South Africa.
- Cudjoe, A.R., P. Neuenschwander, and M.J.W. Copland. 1993. Interference by ants in biological control of the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) in Ghana. *Bulletin of Entomological Research* 83: 15–22.
- Diop, K. 2000. The biology of *Ceraninus menes* (Walker) (Hym., Eulophidae), a parasitoid of the bean flower thrips *Megalurothrips sjostedti* (Trybom) (Thys., Thripidae): a comparison between African and Asian populations. PhD thesis, University of Ghana, Legon, Ghana. 165 pp.
- Dufay, C. 1982. The Plusiinae of the Comoro archipelago (Lep. Noctuidae). *Bulletin de la Société Entomologique de France* 87: 5–6, 220–228.
- Elzen, G.W., H.J. Williams, and S.B. Vinson. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. *Environmental Entomology* 12: 1872–1876.
- Fritzsche, M. and M. Tamò. 2000. Influence of thrips prey species on the life-history and behaviour of *Orius albidipennis* (Reuter) (Heteroptera). *Entomologia Experimentalis et Applicata* 96: 111–118.
- Hutchison, L.D. and M.D. Dalziel. 1958. Pages 292–828 in *Flora of West tropical Africa*. Vol I, Part II. The Whitefriars Press, Ltd, London and Tonbridge Wells, UK.
- Jackai, L.E.N. 1981. Use of an oil-soluble dye to determine the oviposition sites of the legume pod-borer *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Insect Science and its Application* 2: 205–207.
- Leumann, C. 1994. Populationsdynamik des Hülsebohrrers *Maruca testulalis* auf der Kuherbse *Vigna unguiculata* und auf *Pterocarpus santalinoides*, einer alternativen Wirtspflanze, während der Trockenzeit im Süden von Bénin. Diplomarbeit, ETH Zürich, Switzerland. 83 pp.
- Ndam, H. 1998. Dynamique de population de deux insectes ravageurs (*Megalurothrips sjostedti* et *Maruca vitrata*) du niébé au Sud Cameroun et leurs ennemis naturels. Mémoire de fin d'étude d'ingénieur agronome Université de Dschang. 39 pp.
- Sétamou, M., F. Schulthess, S. Gounou, H.-M. Poehling, and C. Borgemeister. 2000. Host plants and population dynamics of the ear borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) in Benin. *Environmental Entomology* 29: 516–524.
- Silvie, P. 1989. Chemical control of *Sylepta derogata*, a phyllophagous pest of cotton. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent. 54: 1019–1027.
- Tamò, M., J. Baumgärtner, and A.P. Gutierrez. 1993a. Analysis of the cowpea (*Vigna unguiculata* Walp.) agroecosystem in West Africa: II. Modelling the interactions between cowpea and the bean flower thrips *Megalurothrips sjostedti* (Trybom). *Ecological Modelling* 70: 89–113.
- Tamò, M., J. Baumgärtner, V. Delucchi, and H.R. Herren. 1993b. Assessment of key factors responsible for the pest status of the bean flower thrips *Megalurothrips sjostedti* (Trybom) (Thysanoptera, Thripidae). *Bulletin of Entomological Research* 83: 251–258.
- Tamò, M., H. Bottenberg, D. Arodokoun, and R. Adeoti. 1997. The feasibility of classical biological control of two major cowpea insect pests. Pages 259–270 in *Advances in cowpea research*, edited by

- B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Agriculture (IITA) and Japan International Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Taylor, T.A. 1967. The bionomics of *Maruca testulalis* Gey. (Lepidoptera: Pyralidae), a major pest of cowpea in Nigeria. *Journal of the West Africa Science Association* 12: 111–129.
- Taylor, T.A. 1974. On the population dynamics of *Taeniothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) on cowpea and an alternate host, *Centrosema pubescens* Benth., in Nigeria. *Review of Zoology of Africa* 88: 689–702.
- Taylor, T.A. 1978. *Maruca testulalis*: an important pest of tropical grain legumes. Pages 193–200 in *Pest of grain legumes: ecology and control*, edited by S.R. Singh, H.V. Van Endem, and T.A. Taylor. Academy Press, London, UK.
- Usua, E.J. 1975. Studies in relation to *Maruca testulalis*. Pages 52–54 in *Proceedings of IITA Collaborators Meeting on Grain Legume Improvement*, 9–13 June 1975, Ibadan, Nigeria.
- Usua, E.J. and S.R. Singh. 1978. Parasites and predators of the cowpea pod borer, *Maruca testulalis* (Lepidoptera: Pyralidae). *Nigerian Journal of Entomology* 3: 100–102.
- van Driesche, R.G., T.S. Bellows, J.S. Elkinton, and J.R. Gould. 1991. The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. *Environmental Entomology* 20: 1–7.
- Vinson, S.B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21: 109–133.
- Waage, J.K. and D.L. Hawksworth. 1991. Biodiversity of microorganisms and invertebrates: its role in sustainable agriculture. Pages 149–162 in *Proceedings of the First Workshop on the Ecological Foundations of Sustainable Agriculture (WEFSA 1)*, 26–27 July 1990, London, UK. CAB International, Wallingford, UK.
- Zenz, N. 1999. Effect of mulch application in combination with NPK fertilizer in cowpea (*Vigna unguiculata* [L.] Walp.; Leguminosae) on two key pests, *Maruca vitrata* F. (Lepidoptera: Pyralidae) and *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), and their respective parasitoids. PhD thesis, University of Hohenheim, Germany. 379 pp.

## 2.2

# Recent advances in research on cowpea diseases

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### Abstract

Cowpea diseases induced by various pathogenic groups (fungi, bacteria, viruses, nematodes, and parasitic flowering plants) constitute one of the most important constraints to cowpea production in all agroecological zones where the crop is grown. This paper presents an overview of the major research findings on cowpea diseases since the 1995 World Cowpea Conference. The focal points include consideration of the present state of scientific knowledge of these diseases with special emphasis on new information on etiology, biology, distribution, epidemiology, economic significance, and integrated disease management options. Knowledge gaps that should be bridged through research to minimize losses from these diseases are highlighted wherever necessary.

### Introduction

Cowpea diseases induced by species of pathogens belonging to various pathogenic groups (fungi, bacteria, viruses, nematodes, and parasitic flowering plants) constitute one of the most important constraints to profitable cowpea production in all agroecological zones where the crop is cultivated. At the September 1995 Second World Cowpea Conference, our knowledge about diseases from 1984 (when the first World Cowpea Conference was held) to 1995 was updated by lead papers on shoot and pod diseases (Emechebe and Florini 1997), parasitic plants (Lane et al. 1997; Singh and Emechebe 1997), nematodes and other soilborne diseases (Florini 1997; Roberts et al. 1997), and viruses (Hampton et al. 1997; Huguenot et al. 1997). This paper presents an overview of the major research findings on cowpea diseases (induced by all the different pathogenic groups) since 1995; it highlights information on new disease records and, wherever possible, updates information on the major pathogens, paying special attention to their biology, distribution, epidemiology, economic significance, and integrated management options.

### *Distribution of research publications among geographical regions, pathogen groups, and themes during the past five years*

To prepare this paper, we reviewed over 340 scientific papers on cowpea diseases published during the last five years. We have classified these papers to obtain the relative contributions from the main geographical regions—Africa, Asia, Australia, Europe, South America and the Caribbean, and North America—as well as their relative distribution among the

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principal themes (biology, control, economic importance, etiology, epidemiology, systems, and techniques/methodology) and pathogen groups.

About 25% and 36% of the publications were contributed by scientists located in Africa and Asia, respectively, while those based in North America and in South America produced almost 30% (Table 1). Table 2 shows that among the pathogen groups, the fungi attracted about 36% of the publications, viruses 33%, and nematodes 20% and only about 6% each of the papers were devoted to bacteria and parasitic flowering plants. It is encouraging that about 42% of the papers dwelt on the control of the diseases, with about 50% of these papers being on host-plant resistance (Table 3). Of the remaining 50% of the publications on disease control, three ecologically sustainable options (biological, cultural, and botanical) attracted about 34% and pesticides about 17%. This is considered a fair balance. Similarly, the relative number of publications on other research themes (especially epidemiology, physiology, and techniques) appears to be satisfactory; it is expected that in future the effort presently devoted to etiology and surveys will be channeled to these areas. In the following sections, only publications that provide new information or that clarify hitherto existing controversies are reviewed.

**Table 1. Relative contributions by geographical area to scientific publications on cowpea diseases (1995–2000).**

| Geographical area           | Number of papers contributed | Percentage of papers contributed |
|-----------------------------|------------------------------|----------------------------------|
| Africa                      | 85                           | 24.8                             |
| Asia                        | 123                          | 35.9                             |
| Australia                   | 5                            | 1.4                              |
| Europe                      | 28                           | 8.2                              |
| South America and Caribbean | 27                           | 7.9                              |
| North America               | 75                           | 21.8                             |
| Total                       | 343                          | 100.0                            |

**Table 2. Publications on cowpea diseases according to pathogen groups.**

| Group of pathogens | Number of papers | Percentage of papers |
|--------------------|------------------|----------------------|
| Bacteria           | 21               | 6.1                  |
| Fungi              | 122              | 35.6                 |
| Nematodes          | 69               | 20.1                 |
| Parasitic plants   | 19               | 5.5                  |
| Viruses            | 112              | 32.7                 |
| Total              | 343              | 100.0                |

**Table 3. Publications on cowpea diseases grouped according to themes.**

| Theme            | Number of papers | Percentage of papers |
|------------------|------------------|----------------------|
| Biology          | 40               | 11.3                 |
| Control:         | 147              | 41.7                 |
| Biocontrol       | (17)             | (4.8)                |
| Botanicals       | (11)             | (3.1)                |
| Cultural         | (22)             | (6.1)                |
| HPR              | (72)             | (20.40)              |
| Pesticidal       | (26)             | (7.30)               |
| Cropping systems | 7                | 2.0                  |
| Distribution     | 33               | 9.3                  |
| Epidemiology     | 34               | 9.6                  |
| Etiology         | 38               | 10.8                 |
| Mycotoxins       | 1                | 0.3                  |
| Physiology       | 33               | 9.3                  |
| Techniques       | 20               | 5.7                  |
| Total            | 352              | 100.0                |

## Bacterial diseases

The controversy about the etiology of bacterial blight and bacterial pustule appears to have persisted after 1995 despite the suggestion by Emechebe and Florini (1997) that the pustule pathogen be regarded as a strain of *Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye and not a distinct pathovar of *X. campestris*, namely *X. campestris* pv. *vigna unguiculata* Patel and Jindal as proposed by Patel and Jindal (1982). However, in a related publication, Allen et al. (1998) stated categorically that cowpea blight is induced by *X. campestris* pv. *vignicola* while bacterial pustule is induced by *X. campestris* pv. *vigna unguiculata*, not a strain of pv. *vignicola* from which it is distinct in pathogenicity, the single feature for definition of pathovars. To clarify the situation, Khatri-Chhetri et al. (1998a) studied 55 strains of the bacterium (36 from blight lesions, 13 from pustules, and six reference strains) for their metabolizing pattern of 95 carbon sources using the Biolog GM Microplate System. They reported considerable variation in metabolic fingerprints of the strains, which were generally correlated with their origin and identification, but not with blight or pustule development and pathogenicity. They concluded that the strains isolated from blight and pustules from West Africa belonged to the same pathovar, *vignicola*. In a subsequent study, Verdier et al. (1998) analyzed isolates from cowpea leaves with blight or pustule lesions (collected from 11 countries in various geographical areas) and selected on the basis of pathological and physiological characteristics. The strains were analyzed for genotype markers by ribotyping and by RFLP analysis with a plasmid probe, *pth* B, containing a gene required for pathogenicity from *X. campestris* pv. *manihotis*. Based on polymorphism detected by *pth* B among *X. campestris* pv. *vignicola* strains, nine haplotypes were defined. However, the genetic variation was independent of geographic origin of the strains and of pathogenic variation. Based on these results and those of an earlier study on pathogenic and biochemical characterizations (Khatri-Chhetri et al. 1998b), Verdier et al. (1998) concluded that the strains isolated from leaves with blight symptoms or minute pustules belonged to the same pathovar, *vignicola*. This implies that bacterial pustule and blight are induced by different strains of the same bacterial species.

There have been doubts about the taxonomy of the genus *Xanthomonas* that presently has more than 140 pathovars (Bradbury 1986). To sort out the relationship between the many pathovars and species, a series of studies on the taxonomy of the genus has been undertaken during the last decade to address the species delineation within the genus (Vauterin et al. 2000). These studies used analytical fingerprinting techniques such as electrophoresis of whole-cell proteins and gas-chromatographic analysis of cellular fatty acids. Results showed that *X. campestris* pathovars are phenotypically much more diverse than previously documented. The DNA homology matrix distinguished 20 genomic groups within the genus *Xanthomonas* (Vauterin et al. 1995). Of these, four groups corresponded to four existing species of *Xanthomonas*, while 16 DNA homology groups were new and not consistent with the existing pathovar classification; these latter 16 genomic groups were then described as new species (Vauterin et al. 1995). As a result of the complex arrangements resulting from the DNA homology relationships within the genus, the bacterial blight/pustule pathogen has been placed in the species *X. axonopodis* and is currently designated as *X. axonopodis* pv. *vignicola* (Vauterin et al. 1995, 2000). This nomenclature has received international recognition as evidenced by the fact that authorities in CAB International have since 1996, consistently included the new name in square parenthesis after the former name (*X. campestris* pv. *vignicola*) in reporting abstracts of authors that had not conformed to the new specific name in their monthly publication *Review of Plant Pathology*.

Other areas of cowpea bacterial disease research that received noteworthy attention in the past five years were pathogen survival, field inoculation and screening techniques, and disease control by seed treatment. A study at Minjibir, Kano (Nigeria) showed that planting spreader lines two weeks before the test lines compared to simultaneous planting of spreader and test lines increased disease pressure and screening efficiency (Amusa and Okechukwu 1998). Using this method, only nine of 45 lines previously rated resistant (following simultaneous planting of spreader and test lines) were confirmed to be resistant. Related studies in India have shown that plants spray-inoculated at 10–100 days of age were most susceptible to infection at one month of age (Rakesh et al. 1995). It was also shown that the highest infection occurred when plants were spray-inoculated twice at 24-hour intervals under humid conditions. Earlier, Rakesh et al. (1994a) had found that maximum disease incidence was obtained by a spray inoculum concentration of  $8.6 \times 10^8$  c.f.u./ml, with disease symptoms appearing five days after inoculation.

Survival studies were conducted in Benin Republic and India. In Benin, Sikirou et al. (1998a, b) reported that *X. campestris* pv. *vignicola* in infected tissue did not survive more than two months on the soil surface or when buried in the soil at a depth of 10–20 cm in the field. They also reported that out of 12 legume species leaf-infiltrated with *X. campestris* pv. *vignicola*, only *Sphenostylis stenocarpa* (African yam bean) exhibited clear symptoms of bacterial blight. The results of survival studies done in India contradict those of Sikirou (1998a, b). A study of the survival of the bacterium in infected seed and trash revealed that the bacterium remained viable in infected seed for 390 days at 5–10 °C and 250 days at 10–40 °C (Rakesh et al. 1994b). In the soil, they showed that *X. campestris* pv. *vignicola* survived for 260 days at 10 °C and 100 days at 40 °C.

Good progress towards the control of bacterial blight by seed treatment was reported during the last five years. In India, Thammaiah and Khan (1995a) found that chemical



seed treatment with copper oxychloride for 15–60 min completely suppressed seed-transmitted bacterial blight, as did treatment with root or leaf extract of *Adhatoda zeylanica* for 60 min (Thammaiah et al. 1995). Hot water treatment of infected seeds at 52 °C for 15 min was also effective (Thammaiah and Khan 1995b), higher temperatures being detrimental to seed germination. By contrast, Sikirou (1999) in Benin has reported that seed treatment in hot water at 60 °C for 30 min or 70 °C for 10 min, or treatment in hot air (65 °C for 120–144 hr or 70 °C for 96 hr) eliminated the bacterium from infected seeds without inhibiting seed germination. The same researcher has found that in cowpea grown in alternate rows with maize, the disease severity and incidence were reduced by up to 43% and 41%, respectively while alternate rows of cassava reduced disease severity by up to 42% and disease incidence by 34% (Sikirou 1999). There was only one report on yield loss caused by bacterial blight during the period—64% grain yield loss was reported in Benin by Sikirou (1999) in one of her several field experiments.

An important research contribution during the period under review was the development of a semiselective medium (SSM) for easy isolation of the cowpea bacterial blight pathogen. To accomplish this, Khatri-Chhetri et al. (1998b) evaluated 12 carbon and five nitrogen sources for selectivity towards *X. axonopodis* pv. *vignicola* while 25 antibiotics were screened for inhibitory effects on saprophytic contaminants frequently isolated from cowpea leaves (e.g., *Pseudomonas fluorescens*, *Erwinia herbicola*, and *Bacillus subtilis*). They have designated SSM as cefazoline-cellobiose-methionine medium (CCMM), defined its composition and the characteristic appearance of the colonies of *X. axonopodis* pv. *vignicola*, and how the latter can be distinguished from saprophytes not completely inhibited by the CCMM.

## **Fungal diseases**

### ***New records of fungal diseases and races***

During the last five years there have been reports of new records of localized occurrences either of well-known diseases or races in new areas, or of diseases that had not been reported before on cowpea. These are described below.

### ***Alternaria leaf spot in South Africa***

Grange and Aveling (1998) have shown that the pathogen of what they considered a destructive foliar disease of cowpea in South Africa is *Alternaria cassiae* Juriar and Khan. The disease occurs also in Botswana. The identification of the pathogen to a specific level makes it the first report of the species in cowpea. However, Emechebe and Florini (1997) listed leaf spot induced by *Alternaria* sp. as one of the minor diseases of cowpea, noting its occurrence in Zimbabwe according to Maramba (1983) and Maringa et al. (1985). Foliar symptoms, beginning as semicircular water-soaked lesions at the leaf margin, enlarge towards the center of the leaf and become necrotic. Sporulation occurs at the lower leaf surface as a black velvety mass.

### ***Choanephora pod rot in Colombia***

This widely distributed pod rot induced by *Choanephora cucurbitarum* (Berk. and Rav.) Thaxt was reported for the first time in the province of Cordoba, Colombia (Munoz and Tamayo 1994).

### **Colletotrichum stem disease in South Africa**

This was reported as a new disease in South Africa (Smith et al. 1999a). This is probably the first record of the fungus (*C. dematium*) in cowpea; cowpea was not given as one of its hosts in the comprehensive list by Allen and Lenne (1998). The stem lesions are light brown and appear about 48 hours after inoculation (Smith et al. 1999a).

### **Latent anthracnose**

A latent anthracnose in cowpea leaves was reported recently by Latunde-Dada et al. (1999) who have provided details of the infection process. Anthracnose lesions appear on senescent leaves after a prolonged symptomless period of host colonization lasting more than two weeks. The disease is of no economic importance.

### **Phomopsis pod spot in the USA**

A pod spot disease of cowpea in the State of Mississippi (USA) was observed in 1994 and later shown to be induced by *Phomopsis longicola* (Roy and Ratnayake 1997). The disease, probably a new record on cowpea, appears on mature pods as scattered, irregular black spots. The fungus is seedborne and also infects soybean.

### **Pre- and postemergence damping-off induced by *Pythium ultimum* in South Africa**

This was a major problem in smallholder rural farms under wet soil conditions, causing seed rot as well as pre- and postemergence damping-off (Aveling and Adandonon 2000). Germinated seedlings failing to emerge above the soil level were characterized by water-soaked lesions that girdled the hypocotyl. Emerged seedlings had necrotic taproots and few lateral roots. Infected hypocotyls above soil level had light brown lesions while some seedlings showed symptoms of wilting.

### **A new race of *Fusarium oxysporum* f.sp. *tracheiphilum* in California, USA**

Race 4 of the cowpea *Fusarium* wilt fungus (*F. oxysporum* f. sp. *tracheiphilum*) has appeared in California (USA) and has been designated as Fot Race 4, which has caused severe wilting in cultivars CB46 and CB88, known for their resistance to races 1, 2, and 3 (Smith et al. 1999b).

### **Macrophomina blight**

Among the fungal diseases, *Macrophomina* blight (*Macrophomina phaseolina*) has received relatively high research attention from workers in Africa and Asia because it has remained a serious constraint to cowpea production in the drier savannas and the Sahel, the principal zones for cowpea production. Apart from having a wide host range, the fungus produces sclerotia that remain viable in the soil for many years and it has been difficult to find suitable sources of resistance genes among cowpea genotypes. Consequently most of the effort has been directed towards developing sustainable control options and to a better understanding of the role of soil environment on pathogenesis.

Temperature, being an important environmental factor, was recently studied by Ratnoo et al. (1997), in pot experiments in India. Their results indicated that *Macrophomina* blight is favored by high temperatures. Thus, of the four temperature regimes investigated, highest disease indices were obtained at 25–40 °C and 20–35 °C (disease indices being 100% and 94.5%, respectively) compared to 10–25 °C and 15–30 °C. Ratnoo et al. (1997) also found that disease development was low in flooded soil compared to drier soil. A similar and more recent study was conducted in Niger by Afouda (1999).

He showed that optimum temperature for growth for all eight isolates of *M. phaseolina* was 30–35 °C, with no growth occurring at below 13 °C or above 40 °C. Afouda (1999) also studied different combinations of soil water and temperature regimes. He found that when inoculated seeds are sown under stress conditions (daily temperature cycle of 33 °C for 13 hr and 23 °C for 11 hr and plants watered twice a week) the disease incidence was 92% and seedling mortality was 68%. By contrast, sowing inoculated seeds under apparently normal conditions (daily temperature cycle of 28 °C for 13 hr and 22 °C for 11 hr, with plants watered regularly) resulted in disease incidence of 15% and seedling mortality of 5%.

Attempts to develop sustainable control options have focused mostly on seed treatments with fungicides and biological control agents. For example, Ramadoso (1994) and Arjunan and Raguchander (1996), in two independent studies in India, obtained good control of seedling blight by treating seeds with carbendazim or thiram. Another study (Latha et al. 1997) has shown that addition of ZnSO<sub>4</sub> at 50 kg/ha to Zn deficient soil, significantly reduced *Macrophomina* root rot, compared to untreated soil; apparently Zn had a fungicidal effect on the pathogen. The search for biocontrol agents has focused on *Trichoderma* spp. and *Bacillus* spp. Ushamalini and his associates evaluated *Trichoderma* spp. first in vitro (Ushamalini et al. 1997a) and then in field (Ushamalini et al. 1997b) and obtained effective control in both cases with *T. harzianum* and *T. viride*. In pot experiments in Niger, Afouda (1999) evaluated 13 isolates of *Bacillus subtilis* and found isolate A11 to be most promising. Thus, plants infected with *M. phaseolina* and treated with *B. subtilis* A11 showed the lowest blight incidence of 13% and the highest percentage germination of 72% compared to blight incidence of 70% and germination percentage of 40% for check, inoculated with *M. phaseolina*. Also Ushamalini et al. (1997c) have screened 21 botanicals for their efficacy to inhibit the growth of *M. phaseolina* under in vitro conditions. They showed that extracts of three plant species (*Vitex negundo*, *Adenocalymma alliaceum*, and *Ocimum sanctum*) effectively inhibited mycelia growth and sclerotic germination of *M. phaseolina*. Finally, efforts to find sources of resistance to *M. phaseolina* have continued. For example, Mahabeer et al. (1995) evaluated 30 cowpea varieties in India and found that none was completely resistant while five were moderately resistant. In contrast, Rodrigues et al. (1997) reported that 10 cowpea genotypes in Brazil were resistant to *M. phaseolina*.

To improve the efficiency of detecting *M. phaseolina* infection of cowpea seed—important for plant quarantine and planting seed certification purposes—Afouda (1999) raised antibodies against the cytosol and extracellular components of *M. phaseolina* and used them in a double antigen sandwich ELISA (DAS-ELISA). He found that three of his four antigens detected specifically *M. phaseolina* in protein extracts of the fungus as well as in samples from infected plants. The most effective antigen (designated 848/3) detected 15–30 ng/ml protein extracts of *M. phaseolina* but was less specific than the next most effective antigen (848/4) which has the advantage of not cross-reacting with any of the nine other fungi used in the sensitivity test (Afouda 1999).

### **Diseases induced by *Colletotrichum* species**

Two major diseases of cowpea (anthracnose and brown blotch) are induced by two distinct species of the genus *Colletotrichum*. Emechebe and Florini (1997), on the basis of the information available by September 1995, suggested that the cowpea anthracnose pathogen be regarded as a species that is distinct from *C. lindemuthianum*, the *Phaseolus*

bean anthracnose pathogen. Indeed, Latunde-Dada et al. (1996) have provided strong evidence in favor of considering the cowpea anthracnose pathogen as a form of *C. destructivum* O'Gara. This has been accepted by the authors of a recent major review of cowpea diseases (Allen et al. 1998). Cowpea brown blotch pathogen is *C. capsici* while *C. truncatum* is presently considered to refer to the same fungus (Allen et al. 1998). New information from the relatively little research done on the two diseases in the past five years is now presented.

Using essentially the same methodology, Adebitan et al. (1996) and Adebitan and Ikotun (1996) studied the effect of plant spacing and cropping pattern on brown blotch and anthracnose at Ibadan (Nigeria). They reported a greater reduction of brown blotch in monocropped cowpea (Adebitan et al. 1996). It was shown that wide spacing of cowpea resulted in lower incidence and severity of brown blotch compared to the closer planted crop, both monocrop and intercrop. As expected, similar results were obtained in the anthracnose trial. Anthracnose incidence and severity were lower in the intercrop relative to the sole crop while reduction in both inter- and intrarow spacing resulted in an increase in the incidence and severity of anthracnose (Adebitan and Ikotun 1996). Adebitan (1996) has also evaluated the effects of weed infestation and application of phosphorus fertilizer on anthracnose. The severity of anthracnose was lowest in plots given 80 kg/ha  $P_2O_5$  and highest in plots that did not receive phosphorus fertilizer. Also, plots kept weed-free until harvest had 56.9% lower anthracnose incidence and 43.2% lower severity when compared to the unweeded check plot.

Apart from these studies on effects of cultural practices, some attention was devoted to developing disease control options. Thus, Bankole and Adebajo (1996) working in southwestern Nigeria, have reported that seed treatment or soil drenching with dense ( $1 \times 10^8$  conidia/ml) conidial suspension of *Trichoderma viride* effectively reduced brown blotch infection. In addition, foliar application of spore suspension of *T. viride* once or twice weekly, beginning three days after inoculation of seedlings with the pathogen, reduced brown blotch in the field. The yield of plots sprayed twice weekly with suspension of *T. viride* was not significantly different from that of plots sprayed weekly with benomyl. Another study in Nigeria has shown that water or alcohol extract of *Piper betle*, *Ocimum sanctum*, and *Citrus limon* were effective in checking the incidence and spread of anthracnose in the field. Extracts of *P. betle* were the most effective in both the laboratory and the field (Amadioha 1999).

### **Sphaceloma scab**

Scab of cowpea is induced by a *Sphaceloma* sp. (Emechebe 1980) which is usually regarded as the anamorph of the perfect species, *Elsinoe phaseoli* Jenkins. It is considered as the most important disease of cowpea wherever it occurs in both the northern and southern Guinea savanna zones of West and Central Africa (Emechebe and Shoyinka 1985). Its occurrence in East Africa has been confirmed by reports from Uganda (Iceduna et al. 1994; Nakawuka and Adipala 1997; Edema et al. 1997). In Brazil, Central America, and Suriname, it is one of the most destructive diseases of cowpea (Lin and Rios 1985).

Despite its importance, cowpea scab has received relatively little research attention. In one of these studies, Nakawuka and Adipala (1997) found that 10 out of 75 cowpea genotypes evaluated in Uganda were resistant to the pathogen based on the foliar symptoms of the disease, while 24 were resistant based on pod infection. In a separate experiment, the same workers (Nakawuka and Adipala 1997) studied the nature of inheritance of resistance

to *Sphaceloma* scab. Their data showed that additive genetic variance constituted the major portion of the total genetic variance for resistance to scab in their varieties.

Given the difficulty of isolating the pathogen from infected tissue (Emechébe 1981) and the absolute necessity for artificial cultures for detailed work, Mungo et al. (1998a) recently developed a culture medium for easy isolation of the scab pathogen. The cowpea isolate of *Sphaceloma* sp. appears to be restricted to cowpea (*Vigna unguiculata*); artificial inoculation of many species of legumes (including *Vigna radiata*, *Phaseolus lunatus*, and *P. vulgaris*) did not produce any symptoms with the exception of *Lablab purpureus* (hairy bean) (Emechébe 1981). The cowpea scab fungus, therefore, differs in pathogenicity from the isolate of *Elsinoë phaseoli* reported by Jenkins (1931) which has a much wider host range, including *P. vulgaris*, *V. radiata*, and cowpea. Recently, it was reported that *Sphaceloma* occurred on nine out of 14 major weed species found in cowpea fields in the northern Guinea savanna of Nigeria (Adebitan 1998). Apparently, isolates of *Sphaceloma* sp. were obtained from scab lesions on these weeds and seven of them were pathogenic, to varying degrees, on cowpea. These findings do not imply that the plant species that yielded the isolates are new hosts for the *Sphaceloma* sp. that induces cowpea scab under natural conditions. Rather, the results indicate that cowpea is one of the hosts of the isolates obtained from the seven weed species.

Some work has been done on the epidemiology of the fungus. Mungo et al. (1998b) showed that inoculum for primary infection under field conditions originated either from the infected seed or from infected cowpea debris, with primary lesions appearing on the hypocotyl or epicotyl (but not on the unifoliate primary leaves) about 25 days after sowing. Secondary spread of the fungus was by rain splash and wind-driven moisture. Earlier work (Emechébe 1980) had shown that cowpea scab development is exacerbated by moderate temperatures (23–28 °C), three or more consecutive days of wet weather, and consequent high relative humidity. These requirements probably partly explain the recent report from Uganda by Edema et al. (1997) that the incidence and severity of scab were higher during the second season. They also found that scab was favored by high plant populations (conducive for rain splash dispersal) while growing cowpea in intrarow mixtures with other crops resulted in less disease.

### **Diseases induced by *Thanatephorus cucumeris* (*Rhizoctonia solani*)**

*Thanatephorus cucumeris* (Frank) Donk, usually occurring in nature and artificial culture media in its anamorphic state, *Rhizoctonia solani* Kuhn, is soilborne and ubiquitous. *T. cucumeris* has a broad host range and comprises about 12 anastomosis groups (AG), and it has been suggested that these groups be accorded taxonomic status (Allen et al. 1998). The fungus induces two distinctly different diseases in cowpea—web blight and a root rot/seedling disease complex—separated in time and space. Web blight is induced by aerial types, usually belonging to AG-1, while the strains that induce root rots/seedling diseases are strongly soilborne, in contrast to the aerial strain, which has only a transient association with the soil. The two diseases are important throughout the humid tropical lowlands, and are regarded as major diseases in the forest belt of West Africa (Allen et al. 1998). They can also be severe under localized, waterlogged conditions in both moist and dry savanna regions. Similarly, web blight is destructive in Latin America and in hot humid regions of India (Lin and Rios 1985; Verma and Mishra 1989). The root and seedling phase results in root rot and in damping-off/seedling blight, the latter being due

to collar/foot rot. Both phases of the disease complex are seed-transmitted (Emechebe and McDonald 1979).

Publications of research efforts on diseases induced by *R. solani* during the period under review have originated from only two countries—India and Brazil. In all cases, the root rot/seedling disease complex had been the target. Noronha et al. (1998) evaluated 20 genotypes of cowpea for their reaction to *R. solani* under both screenhouse and field conditions in Brazil. In the screenhouse, seeds were sown in sterilized soil that was artificially inoculated with the pathogen while in the field they were sown in naturally infested field. Their results showed that none of the varieties was resistant in both environments, being either moderately susceptible or susceptible. In India, Sunder et al. (1996) studied the effects of inoculum characteristics on pathogenicity of *R. solani*. They showed that an increase in inoculum dose from 50 to 200 mg/pot resulted in an increase in seedling mortality and that the pathogenicity of the isolate declined as the age of the inoculum increased. Their results also revealed that inoculum grown at 25 °C or 30 °C and pH 6.5–7.5 produced higher seedling mortality compared to inoculum cultivated at 20 °C or 35 °C.

Efforts to develop control options concentrated on efficacy of biological control agents and fungicides, both deployed as seed treatments. In Brazil, Noronha et al. (1995) screened isolates of *Bacillus subtilis* for their efficacy against *R. solani* as seed treatment in the screenhouse and in the field. Initially 40 isolates of *B. subtilis* were tested against one isolate of the pathogen inoculum at a dose of 50 mg (of inoculum grown on rice grain) per kg of potted soil. The best three isolates were then evaluated in pot culture against four isolates of *R. solani*, each at four inoculum levels of 50, 100, 200, and 300 mg/kg of soil. The most efficacious isolate of *B. subtilis* was finally evaluated in the field against four inoculum levels of the pathogen. In all cases, cowpea seeds were inoculated with *B. subtilis* by dipping in bacterial suspension containing  $1 \times 10^9$  cell/ml. The results showed that seed treatment with *B. subtilis* significantly reduced seedling mortality and was superior to seed treatment with quintozone, a fungicide. Instead of *B. subtilis* isolates, those of fluorescent *Pseudomonas* spp. were screened as seed treatment (seeds dipped in bacterial suspension containing  $1 \times 10^8$  cell/ml) against *R. solani* also in Brazil by Barbosa et al. (1995), using a protocol remarkably similar to that of Noronha et al. (1995). The results showed that one of the isolates of *P. fluorescens* significantly reduced seedling mortality induced by *R. solani* at all levels of pathogen inoculum tested, the level of control being superior to that obtained with seed treatment with the fungicide, quintozone. An evaluation of fungicidal seed treatments was conducted in India by Ram et al. (1995) who found that carbendazim and thiophanate-methyl were not only the most effective among seven fungicides but they also significantly reduced seedling mortality, compared to the other fungicides and the check.

### ***Cercospora* and *Pseudocercospora* leaf spots**

*Cercospora* leaf spot is induced by *Cercospora canescens* Ellis and Martin, while *Pseudocercospora* leaf spot is induced by *Mycosphaerella cruenta* Latham in the form of its anamorph, *Pseudocercospora cruenta* (Sacc.) Deighton, formerly *C. cruenta* (Emechebe and Shoyinka 1985). Before *C. cruenta* was redesignated as *P. cruenta*, the diseases induced by what were considered as two species of the genus, *Cercospora* were known as *Cercospora* leaf spots. *Pseudocercospora* leaf spot is characterized by chlorotic or necrotic spots on the upper leaf surface and profuse masses of conidiophores and conidia,

appearing as downy gray to black mats, on the lower leaf surface. *Cercospora* leaf spot is characterized mostly by circular to irregular cherry red to reddish-brown lesions on both leaf surfaces. Both pathogens survive the no-crop period on infected crop residue and in infected seed (Williams 1975; Patel 1985) although Emechebe and McDonald (1979) were unable to demonstrate seed-to-plant transmission of *C. canescens*. Both leaf spots have been reported from all cowpea growing regions of the world. *P. cruenta* induces leaf spot on several legumes and *C. canescens* on an even wider range of legumes—the list of susceptibles of each pathogen is provided by Allen et al. (1998). However *Pseudocercospora* leaf spot is economically more important than *Cercospora* leaf spot.

Emechebe and Florini (1997) noted that very little work on the two cowpea diseases had been done between 1985 and 1995. This situation did not change between 1995 and 2000. The few reports that appeared during this period focused on varietal resistance and fungicidal control. In China, 131 cowpea accessions were evaluated for their reaction to *P. cruenta* in the field subsequent to artificial inoculation (Lin et al. 1995). It was shown that 15 accessions were immune and seven were highly resistant. Although only six cowpea cultivars were evaluated in Singapore by Leina et al. (1996), one variety was found to be highly resistant to *P. cruenta*. In another experiment, Leina et al. (1996) demonstrated a direct correlation between the variation in peroxidase activity in the soluble fraction of inoculated leaves and resistance to infection in cowpea cultivars; they also showed that the soluble fraction of inoculated leaves had higher preoxidase activity than either the mitochondrial or chloroplast extracts.

Evaluation of fungicides for the control of *Pseudocercospora* leaf spot was conducted in Bangladesh and Nigeria. In Nigeria, Amadi (1995) evaluated three fungicides (benomyl, mancozeb, and captafol) for the control *Cercospora* leaf spot in Ilorin. He reported that weekly spraying of benomyl, beginning at three weeks after planting, gave the best control of the diseases and the highest grain yield. A trial by Haque et al. (1994) in Bangladesh tested the efficacy of six fungicides against *Pseudocercospora* leaf spot. Their results showed that the best control of the disease and the highest grain yield were obtained by three to four sprays of benomyl after 12 days.

### **Brown rust**

Brown rust is induced by *Uromyces appendiculatus* (Pers) Unger (synonym: *U. vignae* Barclay). It occurs in all cowpea producing areas of the world, in contrast to the localized occurrence of pink rust, *Phakospora* spp. (*P. pachyrhizi* occurs in Cambodia, China, Ghana, Nigeria, and Sierra Leone while *P. meibomia* occurs in Brazil.) Cowpea brown rust is considered a major disease in the rainforest and southern Guinea savanna zones of West Africa and in midaltitude areas of East Africa (Emechebe and Shoyinka 1985). Konate and Ouedraogo (1988) have reported moderate to high intensities of brown rust in the northern Guinea savanna of Burkina Faso. Also Stofella et al. (1990) have shown that brown rust is one of the most important fungal diseases of cowpea at Fort Pierce, Florida, USA. *U. appendiculatus* survives the period between crops as teliospores in infected crop residue.

Although some authorities (e.g., Allen et al. 1998) question the global economic importance of brown rust, the disease received the highest research attention among the fungal diseases of cowpea during the 1995/2000 period. Several research areas were covered, such as inoculation techniques, disease physiology, host-plant resistance, and mechanism of resistance, mutation breeding, and cultural and fungicidal control.

Obviously, not all the papers on these topics are reviewed here; rather only a few papers on the various themes are considered. Among these themes, rust disease physiology was given a lot of attention. For example, Heath (1998) studied the involvement of reactive oxygen species in hypersensitive response (HR) of cowpea to infection by the rust. His results provided evidence that the rust fungi initially negate nonspecific defensive responses in both resistant and susceptible cells as part of the establishment of biotrophy. His data also suggested that the HR in the cowpea–cowpea rust fungus pathosystem is not triggered by an oxidative burst. In another study on the role of calcium in signal transduction during cowpea HR to brown rust, Xu and Heath (1998) demonstrated that the elevation of  $\text{Ca}^{2+}$  ion level was involved in signal transduction leading to the HR during rust fungal infection. Another study in the same laboratory purified and characterized two novel HR-inducing specific elicitors produced by the cowpea rust fungus and found that the two specific elicitors were products of two avirulence genes corresponding to the two genes for resistance in the resistant cultivar (D'-Silva and Heath 1997). The phenomenon of slow rusting has also been detected in cowpea infected by brown rust fungus in India (Cherian et al. 1996a, b).

The genetics of resistance in a few cultivars have been determined. A study of inheritance of resistance in cultivar Calico Crowder has suggested the presence of dominant and recessive resistance components (Ryerson and Heath 1996). In India, Rangaiah (1997) investigated the inheritance of resistance in a resistant cowpea genotype and showed that a minimum of two genes control resistance. Brown rust control with fungicides was also studied in India by Kale and Anahosur (1996) who found that triadimefon and mancozeb sprays effectively controlled brown rust in cowpea.

Effective methods for artificial inoculation of cowpea with uredospores of *U. appendiculatus* have been described. Zeng et al. (1999) reported that in China the optimum conditions for host infection consisted of spore concentration of  $3.24 \times 10^5/\text{ml}$  and ambient temperature of 23–26 °C. They also found that good germination of uredospores was obtained in sterilized tap or distilled water containing 1% each of glucose and sucrose and leaf extract of cowpea seedling. Earlier, Kale and Anahosur (1994) effectively inoculated cowpea with uredospore suspension by spraying 45-day-old plants four times on alternate days.

## Other fungal diseases

Research findings on other fungal diseases reported during the period under review are presented in Table 4.



**Table 4. Summary of research findings on some fungal diseases reported from 1995 to July 2000.**

| Disease/pathogen  | Major research findings/reference   |
|---|---|
| Black leaf spot or leaf smut ( <i>Protomycopsis phaseoli</i> Ramak. & Subram. or <i>Entyloma vignae</i> Batista)          | Out of 156 genotypes evaluated for resistance to <i>E. vignae</i> in Brazil, only 3 were resistant (Santos et al. 1997).<br>In vitro studies in Nigeria showed <i>Trichoderma harzianum</i> more effective than <i>T. koningii</i> and <i>Trichoderma</i> sp. in reducing radial growth of <i>P. phaseoli</i> while field studies indicated that <i>Trichoderma</i> sp. was better than <i>T. koningii</i> and <i>T. harzianum</i> (Adejumo et al. 1999) in the control of the leaf smut.   |
| <i>Fusarium</i> wilt ( <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> [E.F. Smith] Snyd. & Hans.)                    | Extracts of 3 out of 21 plant species inhibited mycelia growth and spore germination in India (Ushamalini et al. 1997a).<br>Greatest suppression of mycelia growth of wilt pathogen obtained with <i>Bacillus subtilis</i> followed by <i>T. harzianum</i> and <i>Pseudomonas fluorescens</i> (Ushamalini et al. 1997b).<br>Study of interaction between <i>F. oxysporum</i> f.sp. <i>tracheiphilum</i> and <i>Meloidogyne incognita</i> showed that:<br><ol style="list-style-type: none"> <li>(1) Infection by <i>M. incognita</i> did not predispose wilt resistant cowpea to wilt disease.</li> <li>(2) Wilt occurred in nematode-treated plots in wilt susceptible var.</li> <li>(3) Yield of wilt resistant genotypes reduced by about 17% by nematode infection in nontreated plot.</li> <li>(4) In nontreated plots yield of wilt susceptible genotypes was reduced by about 37–65% by combined nematode and wilt infections. (Roberts et al. 1995).</li> </ol> |
| <i>Phytophthora</i> stem rot or red stem canker ( <i>P. vignae</i> Purss and <i>P. cactorum</i> [Leb. & Chon] Schroet)    | Cowpea inoculation with mycorrhizal fungi ( <i>Glomus intraradices</i> ) could provide some degree of reduction of <i>P. vignae</i> stem and root rot disease in cowpea, independent of nutritional or other growth effects in USA (Fernando and Linderman 1997).   |
| <i>Sclerotium</i> basal stem rot or wilt ( <i>Sclerotium rolfsii</i> Sacc. [teliomorph = <i>Corticium rolfsii</i> Curzi]) | Out of 20 cowpea lines evaluated for resistance to <i>S. rolfsii</i> , only one line was moderately resistant (Muqit et al. 1996).<br>Six fungicides evaluated as seed treatment; vitax-200 gave best control of <i>C. rolfsii</i> and highest grain yield (Rahman et al. 1994).  |
| <i>Pythium</i> soft stem rot ( <i>Pythium aphanidermatum</i> [Edson] Fitz)  | Two isolates each of <i>Trichoderma viride</i> and <i>Bacillus cereus</i> and 3 of <i>B. subtilis</i> evaluated in vitro and in vivo for efficacy against <i>P. aphanidermatum</i> . <i>T. viride</i> hyperparasitized pathogen mycelium while bacterial isolates inhibited it, in vitro. Applied as soil treatments, the antagonists reduced seedling infection. Efficacy of antagonists increased with increase in dose (Bankole and Adebanjo 1998).  |

## Viral diseases

### Introduction

Hampton et al. (1997) have listed nine viruses considered most damaging to cowpea, seven of which that are seedborne being the following: blackeye cowpea mosaic potyvirus (BICMV), cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic virus (CMV), cowpea mosaic comovirus (CPMV), cowpea severe mosaic comovirus (CPSMV), southern bean mosaic sobemovirus (SBMV), and cowpea mottle carmovirus (CPMoV). The two nonseedborne viruses considered important by Hampton et al. (1997) are cowpea golden mosaic geminivirus (CGMV) and cowpea chlorotic mottle bromovirus (CCMV). They listed about eight other viruses considered to be of minor or undetermined importance. Major research findings on eight of the nine viruses published during the past five years are summarized below. We found no specific publication on CGMV during the period.

### Blackeye cowpea mosaic potyvirus (BICMV)

BICMV occurs wherever the crop is grown. It is particularly damaging when it occurs in combination with other viruses (Hampton et al. 1997). Based on the number of publications on it, BICMV has received fairly good attention from researchers during the period under review.

Recent reports have confirmed the occurrence of BICMV in northeast Asia (Reeves 1997), Indonesia (Hadiastono 1996), and Sri Lanka (Premala et al. 1996). It appears that there are several sources of genetic resistance among cowpea genotypes. Thus, Bashir and Hampton (1996) evaluated only 51 cultivars and lines for resistance to seven geographically and pathologically diverse isolates of BICMV in Pakistan and found five to be immune to all isolates and three immune to all but one of the isolates. Another study in the USA (Hunter et al. 1996) has shown that it is possible to screen cowpea simultaneously for resistance to the virus and *Meloidogyne incognita*. However, mixed infection of BICMV and cucumber mosaic virus (CMV) resulted in severe cowpea stunt disease symptoms and high concentration of CMV coat protein 20 days after inoculation of all plants without extreme resistance to BICMV (Anderson et al. 1996). It seems that resistance to BICMV determined through symptom observation is not adequate when evaluating germplasm for cowpea stunt resistance and that rapid development of symptoms on dually infected plants may not be due solely to increased CMV concentration. On the other hand, when 45 and 54 seedborne isolates of BICMV and cowpea aphid-borne mosaic virus (CABMV), respectively, were compared using various serological tests and definitive host reactions, isolates of BICMV were clearly distinguished serologically from CABMV isolates (Bashir and Hampton 1996). Although isolate comparison on selected cowpea genotypes partitioned most isolates into two distinct groups, a few isolates from seeds of a particular cowpea variety were definitely BICMV by all serological tests but behaved as CABMV in definitive cowpea genotypes. Recently, Boxtel et al. (2000) found that 10 elite lines of cowpea differed widely in their susceptibility to both BICMV and CABMV and did not always show correlation between field performance and resistance to virus infection under glasshouse conditions. The importance of high seedborne transmission of BICMV suggests that in addition to control through HPR, some control could be obtained by production and planting virus-free seeds as suggested for Taiwan by Chang et al. (1994).

### ***Cowpea aphid-borne mosaic potyvirus (CABMV)***

CABMV is widely distributed in the world and causes severe crop losses either alone or in combination with other viruses (Hampton et al. 1997). Based on the number of publications on it, CABMV has received the greatest attention from researchers. The occurrence of CABMV on cowpea grown commercially in the USA was reported in 1997 (Kline and Anderson 1997). Recent reports have also confirmed its occurrence in Zimbabwe (Gubba 1994) and Nepal (Dahal and Albrechtsen 1996).

To facilitate diagnosis of CABMV infections, especially its distinction from BICMV, Bashir and Hampton have developed a procedure for purifying isolates of the two viruses (Bashir and Hampton 1995) and standardized both the direct antigen coating ELISA (DAC-ELISA) and double antibody sandwich ELISA (DAS-ELISA) (Bashir et al. 1995). However, large-scale surveys for BICMV and CABMV showed that several CABMV isolates from Southern Africa were either poorly or not recognized by monoclonal antibodies prepared for isolates collected in West Africa (Huguenot et al. 1996). Consequently, three new monoclonal antibodies prepared against the Maputo (Mozambique) isolates were included in a revised panel of monoclonal antibodies, resulting in assignment of isolates to appropriate serotypes, including a newly created serotype (Huguenot et al. 1996). Another contribution to improved diagnosis is a rapid technique for detecting CABMV from cowpea seeds developed by Konate and Neya (1996) in Burkina Faso.

As a first step in developing resistant varieties, cowpea genotypes have been screened for their reaction in several countries. In Pakistan, Muhammad et al. (1996) screened 51 lines against seven isolates of CABMV and found three immune. Similarly Kannan and Doraiswamy (1994) detected 30 immune lines out of the 50 evaluated by them.

### ***Cowpea mosaic comovirus (CPMV)***

CPMV is considered to be one of the most important cowpea viruses in Africa (Hampton et al. 1997). Most of the work reported during the last five years focused on the molecular components and their functions. Lekkerkerker et al. (1996) studied the functional domains of the movement protein (MP) and found that it contains at least two distinct domains, one that is involved in tubule formation and a second that is involved in the incorporation of the virus particle into the tubule. Subsequent work of Kasteel et al. (1997) revealed that apart from the MP and the capsid proteins (CP) of CPMV, no other infection-specific proteins existed in the infected tissue. This agrees with recent findings of Verver et al. (1998) that both CP and the MP are absolutely required for cell-to-cell movement of CPMV. A study in India has demonstrated that transmission of CPMV by *Myzus persicae* requires: (1) a 24-hr inoculation feeding period, (2) optimum acquisition period of 10 min, (3) pre-acquisition fasting of one hour, and (4) a minimum of eight aphids per plant (Nagaraju and Murthy 1997).

As noted by Hampton et al. (1997), the best and most practical method of control of CPMV is the use of resistant cultivars. In this regard, Nagaraju and Keshavamurthi (1998) have reported that eight out of 20 lines were resistant to CPMV.

### ***Cucumber mosaic cucumovirus (CMV)***

Despite its common and widespread occurrence (through both seed and aphid transmission) CMV is considered a mild cowpea pathogen, except in infection-sensitive genotypes or when combined with BICMV (Hampton et al. 1997). However, Gillaspie et al. (1998) have reported a new seedborne strain of CMV that induces severe symptoms on many

cowpea genotypes in Georgia (USA). This strain [CMV-Csb] is symptomatic on tobacco but it produces more severe cowpea stunt symptoms when present in combination with BCMV than do the more prevalent CMV isolates.

The detailed structure of CMV has been determined by Wikoff et al. (1997), and it is very similar to that of cowpea chlorotic mosaic virus. Other workers have shown that the RNA2 of CMV is involved in resistance breaking in cowpea (Jang et al. 1996). Most cowpea genotypes are resistant to strain Y of CMV (CMV-Y), the resistance being dependent on existence of a resistance (R) gene in these genotypes. Nasu et al. (1996) have found that inheritance of HR as a component of the resistance to CMV-Y in these a genotypes (typified by Kurodane Sanjaku cultivar of Japan) is controlled by a single dominant gene. Another component of the resistance to CMV in most cowpea genotypes, apart from HR, is the localization of systemic infection. The results of a recent study by Kim et al. (1997) have suggested that an inhibition response in protoplasts, where an HR does not occur, leads to a localization of infection in the whole plant and that different cowpea genes are involved in eliciting the HR and the localization response. It is interesting that induction of resistance to CMV by applying the systemic fungicide, ferimzone, has been attributed to the production of salicylic acid in the treated plants (Nakayama et al. 1996)

### ***Cowpea chlorotic mottle bromovirus (CCMV)***

CCMV can cause heavy crop damage in susceptible cowpea cultivars, alone or in mixed infections (Hampton et al. 1997). Although it has been isolated from two weed species in Nigeria (Thottappilly et al. 1993), CCMV infection of cowpea in nature appears to be confined to North and South America (Hampton et al. 1997). Most of the work reported during the period under review was targeted at understanding the molecular basis for infection and viral movement within the infected plant. Jong et al. (1997) concluded that under normal circumstances, the rate of CCMV cell-to-cell spread in cowpea is limited primarily by factors other than the movement protein. In this regard, Schneider et al. (1997) found that virion formation is not required for systemic infection and that the carboxyl two thirds of the CP is required and sufficient for systemic movement of the viral RNA. They suggested that the CP of CCMV is multifunctional, with a distinct, long-distance movement function and a role in virion formation. Rao (1997) has also concluded that CCMV moves in a nonvirion form. The molecular basis for reduction in virulence in the strain (CCMV-T) that produces intense and extensive chlorosis to that in the strain (CCMV-M) that produces mild symptoms has been provided by Filho et al. (2000). They found that the genetic determinants of symptom expression is located in the third portion of the coat protein gene—specifically, amino acid residue Ala 151 in CMV-T is changed to Val 151 in the CCMV-M.

### ***Cowpea mottle carmovirus (CPMoV)***

CPMoV was first isolated in Nigeria by Shoyinka et al. (1978). Since that time it has been reported from Benin, Côte d'Ivoire, Pakistan, and Togo (Hampton et al. 1997). It appears that the only publication specifically devoted to CPMoV was by Gillaspie et al. (1999). They described sensitive reverse transcription-polymerase chain reaction (RT-PCR) method for detection of CPMoV in newly acquired cowpea germplasm. The RT-PCR method was up to 10<sup>5</sup> times more sensitive than direct action coating ELISA (DAC-ELISA) in detecting CPMoV and gave no false positive reaction as is seen sometimes with ELISA (Gillaspie et al. 1999).

### ***Southern bean mosaic sobemovirus (SBMV)***

The cowpea strain of SBMV (SBMV-C) often occurs in mixtures with other beetle-transmitted viruses, including CCMV and CPSMV (Hampton et al. 1997). The few publications on SBMV-C during the last five years were devoted to its molecular structure and functions. Thus, Hacker (1995) has identified the location of the CP binding site (between nucleotide 1410 and 1436) on the SBMV-C genome. Later, Hacker and Sivakumara (1997) reported on the mapping and expression of SBMV genomic and subgenomic RNAs. The same workers (Sivakumaran et al. 1998) have identified the viral genes needed for cell-to-cell movement of SBMV-C. They found that open reading frames 1 and 3 (ORF1 and ORF3) proteins and the CP are required for SBMV-C cell-to-cell movement.

### ***Cowpea severe mosaic comovirus (CPSMV)***

The symptoms of CPSMV in some cowpea genotypes are similar to those of CPMV but these symptoms, contrary to the term “severe”, may not be more severe than those of CPMV. According to Hampton et al. (1997), CPSMV comprises at least nine serotypes and an unknown number of pathogenic variants. Given this extent of pathogenic variation, it is not surprising that a large proportion of the few, new publications were on HPR-related research. In Brazil, 181 cowpea genotypes were screened for their reaction to CPSMV and five genotypes were found to be immune and nine genotypes resistant (Paz et al. 1999). Umaharan et al. (1997b) reported that four cowpea genotypes were immune, one was tolerant, and three were resistant (out of 160 evaluated) to the Trinidad isolate. With respect to inheritance of resistance, Umaharan (1997a) found that in Trinidad resistance was controlled by three major genes and that resistance was gene dosage dependent. However, Vale and Lima (1995) attributed control of immunity in variety Macaibo to a single recessive gene.

An unusual interaction between CPMV and CPSMV has been reported from Canada (Eastwell and Kalmar 1997). In certain cowpea cultivars immune to CPMV, coinoculation of CPMV with CPSMV strain DG reduced severity and delayed symptoms normally induced by CPSMV. In cultivars susceptible to both viruses, coinoculation delays development of symptoms in response to CPSMV. The data by Eastwell and Kalmar (1997) revealed that the presence of CPMV in the inoculum resulted in a concomitant delay in synthesis of CPSMV coat protein and replication of CPSMV RNA and restricted the transport of CPSMV out of infection centers.

### ***Cowpea parasitic nematodes***

The comprehensive list of nematodes parasitic on cowpea compiled by Caveness and Ogunfowora (1985) contained 51 species in 23 genera. Florini (1997) indicated nine species reported on cowpea between 1985 and 1995. Although there have been a reasonable number of publications on plant parasitic nematodes between 1995 and 2000, about 50% of these publications have been devoted to *Meloidogyne* spp., with the rest focusing mostly on *Rotylenchulus reniformis* and *Heterodera cajani*. Other species previously recorded on cowpea but referred to rather incidentally are *Paratrichodorus* minor, *Pratylenchus* sp., *Xiphinema* sp., *Criconemella* spp., *Hoplolaimus pararobustus*, and *Aphasmatylenchus straturatus*.

*Aphasmatylenchus straturatus* and *A. variabilis* were found in Senegal by Baujard and Martiny (1995a). The two species appeared incapable of entering anhydrobiosis necessary for survival of dry conditions of the Sahel. Only *A. straturatus* was pathogenic in cowpea

but relatively high soil populations of more than 1000 nematodes per plant were required. Similarly, *Hoplolaimus pararobustus* was pathogenic in cowpea inducing reduction in cowpea root fresh weight also at relatively high soil populations (Baujard and Martiny 1995b). The rest of this section reviews appropriate publications on *Meloidogyne incognita*, *Rotylenchulus reniformis*, and *Heterodera cajani*.

### **Meloidogyne spp.**

By far the most important of the species of *Meloidogyne* pathogenic in cowpea is *M. incognita* (Sarmah and Sinha 1995; Khan et al. 1996). *M. javanica* has also been reported, but it is far less pathogenic than *M. incognita* (Puruthi et al. 1995). To identify resistance sources, several researchers have screened cowpea genotypes for their reaction to *Meloidogyne* spp. In India, Subramaniyan et al. (1997) identified only three out of 37 lines as resistant to *M. incognita*. By contrast eight out of nine cultivars were rated as resistant in Cuba (Rodriguez et al. 1996); in Venezuela one out of eight varieties was resistant to *Meloidogyne* spp (Renato et al. 1995). A related study on genetics of inheritance (Roberts et al. 1996) indicated that resistance in IT84S-2049 is governed by one dominant nuclear gene.

Attempts have been made to evaluate several plant-derived materials for the control of *Meloidogyne* spp. in cowpea. In Nigeria, Onifade and Fawole (1996) demonstrated that extract from *Anacardium occidentale* was the most efficacious against *M. incognita*, the least effective being the extract from *Gmelina arborea*. A pot culture study in India has shown that adding chopped green leaves of neem (*Azadirachta indica*) and of *Eupatorium* sp. effectively controlled *M. incognita*. Similarly, in Egypt, incorporating different types of crop residue into the soil about one week before soil inoculation with *M. incognita* was more effective than residue incorporated at the time of soil inoculation (Youssef and Amin 1997). Efforts at biological control include those of Azmi (1995) who obtained good control of *M. incognita* with predacious nematodes in India as well as those of Youssef and Ali (1998) who controlled *M. incognita* with a mixture of three species in three genera of native blue green algae. As expected, nematicides have been screened for the control of *Meloidogyne* spp. For example, of several nematicides evaluated in India as seed treatment for the control of *M. incognita*, carbosulfan at 0.05 or 0.1% soak for 6 hours or monocrotophos at 0.1% soak (also for 6 hours) was effective (Kumar 1996). In Egypt, fenamiphos 10% granular was more effective than soil amendment with organic matter (residue) derived from both cereals and legumes (Youssef and Amin 1997).

Interaction between *Meloidogyne* spp. on the one hand and beneficial microorganisms and other soilborne plant pathogens on the other were reported during the period under review. Kassab and Ali (1996) investigated interactions among *M. incognita*, *Rotylenchulus reniformis*, *Rhizoctonia solani*, and *Rhizobium*. They showed additive interaction (resulting in reduced germination and seed emergence) between *R. solani* and any of the nematodes. Inoculation with *M. incognita* alone slightly promoted plant growth and nodulation, both of which were suppressed by inoculation with *R. reniformis* alone. *R. solani* alone severely damaged plant growth and suppressed nodulation and when inoculated with *M. incognita*, there was increase in both galling and nematode fecundity. Combined inoculation of *R. solani* and *R. reniformis* resulted in *R. solani* being parasitic but not pathogenic to cowpea, without affecting life cycle and fecundity of *R. reniformis*. An experiment in the USA studied the interactions between virulent *M. incognita* and *Fusarium* wilt on resistant cowpea genotypes (Roberts et al. 1995). It was shown that

infection by *M. incognita* did not predispose wilt-resistant genotypes to wilt disease. In wilt-susceptible genotypes, wilt occurred in nematicide-treated plots and was exacerbated by nematode infection in nontreated plots, regardless of the presence of wilt resistance gene. The yield of wilt-resistant genotypes was reduced by about 17%, on the average, as a result of nematode infection while that of wilt-susceptible genotypes was reduced by 37–65% because of combined effects of nematode and wilt infections.

Interactions between *Meloidogyne* spp. and mycorrhizal fungi have also received attention. Santhi et al. (1995) studied the interactions between *M. incognita* and three species of the mycorrhizal fungal genus, *Glomus* (*G. fasciculatus*, *G. versiforme*, and *G. etunicatus*). Their data revealed that *G. fasciculatus* was the most effective at reducing the nematode population in the soil. The influence of three levels of phosphorus on the interaction between VAM fungi and *M. incognita* has been investigated in India (Santhi and Sundarababu 1995). It was found that plants with VAM were more resistant to *M. incognita* than those without; there was a positive correlation between nematode levels and nematode populations and a negative correlation between phosphorus levels and the VAM spore population and root colonization.

### **Rotylenchulus reniformis**

The work published on cowpea–*R. reniformis* pathosystem dwelt mostly with host–parasite interactions and control. Cowpea genotypes have been shown to be good hosts of *R. reniformis* in India (Rao and Ganguly 1996) and Egypt (Amin and Youssef 1997; Kassab and Ali 1996). Control of *R. reniformis* by soil amendments with organic matter has been reported for chopped leaves of neem and *Eupatorium* in India (Ajith and Sheela 1996) and for various crop residues in Egypt (Youssef and Amin 1997). Nematicides have also been evaluated for the control of *R. reniformis* and effective controls reported for fenamiphos and monocrotophos applied as seed treatments in India (Rathore and Yadav 1996) and for soil-applied granular formulations of Aldicarb 10G and Carbofuran 3G, also in India (Rathore 1995).

### **Heterodera cajani**

Only a few publications on *H. cajani* were apparently produced during the last five years. It has been demonstrated in India that cowpea is an efficient host of *H. cajani* (Sharma et al. 1996). Cowpea's host efficiency has been exploited in the control of *H. cajani* on pigeon pea in which cowpea is used as a green manure incorporated into the soil at four weeks after sowing, thereby inducing reduction of populations of juvenile stages of *H. cajani* and the consequent increase in grain yield of pigeon pea (Rathore 1995).

The hatching and infectivity of second juvenile stage of *H. cajani* under varying soil moisture, relative humidity, and storage period were investigated in India (Gaur et al. 1996). Cysts were stored at between 0 and 100% relative humidity (RH) for up to three weeks or in moist or air-dried soil for up to 12 months. It was found that desiccation reduced but did not completely inhibit hatching of cyst. Eggs within cysts withstood extremes of desiccation. Cysts stored in moist soil for up to 12 months had a greater percentage cyst hatch in cowpea root diffusate than those from air-dried soil. Egg hatch occurred up to first four months of storage in moist compared to first two months in air-dried soil.

## Parasitic flowering plants

Among crop plants, cowpea is perhaps unique in being severely attacked by two species in two genera of parasitic angiosperms, namely *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (L.) Benth. Both were included in the review by Singh and Emechebe (1997) but only *S. gesnerioides* featured in the reviews by Lane et al. (1997) and Allen et al. (1998). Although both parasites constitute severe constraints to cowpea production in most of the growing regions in sub-Saharan Africa, *S. gesnerioides* is considered the more important of the two (Emechebe et al. 1991; Lagoke et al. 1994). The cowpea strain of *S. gesnerioides* attacks only cowpea but there are wild strains that attack leguminous shrubs while two other strains attack tobacco and sweetpotato in certain parts of Africa. Populations of *A. vogelii* on cowpea cause serious damage to groundnut, moderate infections in bambara groundnut, and occasional infection of soybean.

Research effort on cowpea *Striga* published between mid-1995 and mid-2000 has been devoted to HPR, histopathology, sources of suicidal germination, and the physiology of the effect of N on *Striga* infection of cowpea. Reiss and Bailey (1998) studied the process of infection of cowpea by *S. gesnerioides*. They showed that penetration of host cortical cells by the cone-shaped *Striga* endophyte is accomplished through intercellular growth facilitated by gentle dissolution of the middle lamella.

Pathogenic variation within and among populations of *S. gesnerioides* does not appear to have changed during the period under review, since there is no evidence of the existence of more than the five races described by Lane et al. (1997). However Race 4 (so called Zakpota strain) has been detected in Kazaure, Gumel, and Birnin Kudu in Jigawa State of Nigeria (Emechebe et al. 1999) while the newest race (Race 5) has been found in Maradi (Niger) by Toure et al. (1998) in addition to its previously documented occurrence in Benin, Burkina Faso, Cameroon, and Nigeria (Lane et al. 1996). Fortunately sources of resistance gene to race 5 (landraces 87-2 and APL1) have been identified (Moor et al. 1995) and have been incorporated into popular varieties (B.B. Singh 1999, personal communication).

The strategy for *Striga* control is the reduction of the population of *Striga* seeds in the soil. One way of accomplishing this is to induce *Striga* seeds to germinate in the absence of the host with the subsequent death of the *Striga* seedling. In this respect, Berner and Williams (1998) evaluated cultivars of more than 20 crop species for their capacity to induce germination of seeds of *S. gesnerioides* in vitro. They found that genotypes of all *Vigna* spp. evaluated as well as some genotypes of *Cajanus cajan*, *Lablab purpureus*, *Sphenostylis stenocarpa*, and *Sorghum bicolor* induced germination of seeds of *S. gesnerioides*. However, it has been suggested by Berner and Williams (1998) that *S. gesnerioides* control involving rotation with nonhost cultivars has potential for success only if these cultivars are selected with the *Striga* isolate(s) from the locality of intended deployment of the nonhost cultivar. Ethylene gas has been used to stimulate germination of *S. asiatica* in the absence of the host. In a recent report, Berner et al. (1999) presented data that suggested that some ethylene-producing strains of *Pseudomonas syringae* pv. *glycinea* were more effective than ethylene in stimulating germination of seeds of three species of *Striga*, including *S. gesnerioides*. Although this is scientifically interesting, it is unlikely to have any direct practical application in the control of any of the species since the bacterium is the pathogen of soybean bacterial blight, a serious pathogen of soybean—an important crop in production systems of the areas of potential use.



An estimate of the yield loss due to *Striga* infection of cowpea under natural conditions was provided by Muleba et al. (1997). They studied yield losses in cowpea genotypes of varying susceptibility to cowpea *Striga* under *Striga*-infested and *Striga*-free conditions. They found that yield losses in *Striga*-infested plots varied from 3.1% at the experiment station to 44.2% in farmers' fields. Also, depending on the susceptibility of the cowpea genotypes to *Striga*, the yield loss varied from 3.1% to 36.5%. These losses are attributed to the adverse effects of *Striga* infection on the host. The effect of *Striga* infection on cowpea photosynthesis was reported by Hibberd et al. (1996). They showed that the allometric relationship between shoot and root dry weight was similar in parasitized plants relative to the control plants, as was the proportion of the dry matter partitioned into leaf, stem, and root tissues. However, infected plants failed to make any significant investment of dry matter in pods. The rate of photosynthesis of the youngest, fully expanded leaf of infected plants was significantly lower than that of control plants. The lower rate of photosynthesis was not attributed to stomatal limitation, a loss of chlorophyll, or to an accumulation of carbohydrates. The depression of photosynthesis in the young leaves was transient. As control leaves aged, photosynthesis declined. This also occurred in *Striga* infected plants but to a lesser extent, resulting in higher rate of photosynthesis in mature leaves when compared to those of uninfected plants.

## References

- Adebitan, S.A. 1996. Effect of phosphorus and weed interference on anthracnose of cowpea in Nigeria. *Forage Research* 22(1): 11–19.
- Adebitan, S.A. 1998. Record of new host plants for *Sphaceloma* on cowpea in Nigeria. *Mycopathologia* 143(1): 47–51.
- Adebitan, S.A., B. Fawole, and G.L. Hartman. 1996. Effect of plant spacing and cropping pattern on brown blotch (*Colletotrichum truncatum*) of cowpea. *Tropical Agriculture* 73: 275–280.
- Adebitan, S.A. and T. Ikotun. 1996. Effect of plant spacing and cropping pattern on anthracnose (*Colletotrichum lindemuthianum*) of cowpea. *Fitopatologia Brasileira* 21(1): 5–12.
- Adejumo, T.O., T. Ikotun, and D.A. Florini. 1999. Biological control of *Protomyces phaseoli*, the causal agent of leaf smut of cowpea. *Journal of Phytopathology* 147(6): 371–375.
- Afouda, L.A.C. 1999. Approach to biological control of *Macrophomina phaseolina* (Tassi) Goid, causal agent of charcoal rot of cowpea (*Vigna unguiculata* [L.] Walp.) and development of serological methods for its detection. PhD dissertation, Georg-August University, Goettingen. Cuvillier Verlag, Goettingen. 139 pp.
- Ajith, K. and M.S. Sheela. 1996. Utilization of green leaves of neem and *Eupatorium* for the management of soil organisms in bhindi and cowpea. *India Journal of Nematology* 26(2): 139–143.
- Allen, D.J. and J.M. Lenne. 1998. Diseases as constraints to production of legumes in agriculture. Pages 1–61 in *The pathology of food and pasture legumes*, edited by D.J. Allen and J.M. Lenne. CAB International, Wallingford, UK.
- Allen, D.J., G. Thottappilly, A.M. Emechebe, and B.B. Singh. 1998. Diseases of cowpea. Pages 267–324 in *The pathology of food and pasture legumes*, edited by D.J. Allen and J.M. Lenne. CAB International, Wallingford, UK.
- Amadi, J.E. 1995. Chemical control of *Cercospora* leaf spot disease of cowpea (*Vigna unguiculata* [L.] Walp.). *Agrosearch* 1(2): 101–107.
- Amadioha, A.C. 1999. Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Archives of Phytopathology and Plant Protection* 32(2): 141–149.
- Amin, W.A. and M.M.A. Youssef. 1997. Host status effect of cowpea and sunflower on the populations of *Meloidogyne javanica* and *Rotylenchulus reniformis*. *Anzeiger für Schadlingskunde, Pflanzenschutz, Umweltschutz* 70(4): 75–76.

- Amusa, N.A. and R.U. Okechukwu. 1998. Reaction of selected cowpea (*Vigna unguiculata* [L.] Walp.) breeding lines to *Xanthomonas campestris* pv. *vignicola*. Tropical Agricultural Research and Extension 1(2): 162–164.
- Anderson, E.J., A.S. Kline, T.E. Morelock, and R.W. McNew. 1996. Tolerance to blackeye cowpea mosaic potyvirus not correlated with decreased virus accumulation or protection from cowpea stunt disease. Plant Disease 80(8): 847–832.
- Arjunan, G. and T. Raguchander. 1996. Effect of seed treatment on root rot of cowpea. Indian Journal of Pulses Research 9(1): 83–84.
- Aveling, T.A.S. and A. Adandonon. 2000. First report of pre-and post-emergence damping-off of cowpea caused by *Pythium ultimum* in South Africa. Plant Disease Reporter 84(8): 922.
- Azmi, M.I. 1995. Control of root knot and root-lesion nematodes on cowpea and maize through predacious nematode complex in pots. Advances in Agricultural Research in India 3: 108–118.
- Bankole, S.A. and A. Adebajo. 1996. Biocontrol of brown blotch of cowpea caused by *Colletotrichum truncatum* with *Trichoderma viride*. Crop Protection 15(7): 633–636.
- Bankole, S.A. and A. Adebajo. 1998. Efficacy of some fungal and bacterial isolates in controlling wet rot disease of cowpea caused by *Pythium aphanidermatum*. Journal of Plant Protection in the Tropics 11(1): 37–43.
- Barbosa, M.A.G., S.J. Michereff, R.L.R. Mariano, and E. Maranhao. 1995. Biocontrol of *Rhizoctonia solani* in cowpea by seed treatment with fluorescent *Pseudomonas* spp. Summa Phytopathologica 21(2): 151–157.
- Bashir, M. and R.O. Hampton. 1995. Purification and electron microscopy of some isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic potyvirus. Pakistan Journal of Botany 27(1): 243–249.
- Bashir, M. and R.O. Hampton. 1996. Serological and biological comparisons of blackeye cowpea mosaic and cowpea aphid-borne mosaic potyvirus isolates in *Vigna unguiculata* (L.) Walp. germplasm. Journal of Phytopathology 144(5): 257–263.
- Bashir, M., R.O. Hampton, and B. Muhammad. 1995. Antiserum production against cowpea aphid-borne mosaic virus and standardization of enzyme-linked immunosorbent assays. Sarhad Journal of Agriculture 11(4): 505–512.
- Baujard, P. and B. Martiny. 1995a. Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 4. The genus *Aphasmatylenchus* Sher. Fundamental and Applied Nematology 18(4): 355–360.
- Baujard, P. and B. Martiny. 1995b. Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 6. *Hoplolaimus pararobustus* (Schuurmans Stekhoven and Teunissen, 1938) Sher, 1963 and comparison with *Hoplolaimus seinhorsti* Luc, 1958. Fundamental and Applied Nematology 18(5): 435–444.
- Berner, D.K. and O.A. Williams. 1998. Germination stimulation of *Striga gesnerioides* seeds by hosts and nonhosts. Plant Disease 82(11): 1242–1249.
- Berner, D.K., N.W. Schaad, and B. Volksch. 1999. Use of ethylene-producing bacteria for stimulating of *Striga* spp. seed germination. Biological Control 15(3): 274–282.
- van Boxtel, J., B.B. Singh, G. Thottapilly, and A.J. Maule. 2000. Resistance of cowpea (*Vigna unguiculata* [L.] Walp.) breeding lines to Blackeye cowpea mosaic and Cowpea aphid-borne mosaic potyvirus isolates under experimental conditions. Journal of Plant Disease and Protection 107(2): 197–204.
- Bradbury, J.F. 1986. *Xanthomonas* Dowson 1939, 187. Pages 198–260 in Guide to plant pathogenic bacteria. CAB International Mycological Institute, Slough, England.
- Caveness, F.E. and A.O. Ogunfowora. 1985. Nematological studies worldwide. Pages 273–285 in Cowpea research, production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Chang, C.A., T.T. Yang, T.M. Tsan, and C.C. Chen. 1994. Production and application of virus free seeds to control virus diseases of asparagus beans. Plant Protection Bulletin (Taipei) 36(4): 313–325.
- Cherian, S., T.B. Anilkumar, and V.V. Shulladmath. 1996a. Evaluation of slow rusting in cowpea germplasm. Mysore Journal of Agricultural Sciences 30(4): 374–379.

- Cherian, S., T.B. Anilkumar, and V.V. Sulladmath. 1996b. Slow rusting resistance in cowpea. *Mysore Journal of Agricultural Sciences* 30(2): 153–158.
- Dahal, G. and S.E. Albrechtsen. 1996. Some studies in cowpea aphid-borne mosaic and pea seed-borne mosaic potyviruses in Nepal. *International Journal of Pest Management* 42(4): 337–344.
- D'Silva, I. and M.C. Heath. 1997. Purification and characterization of two novel hypersensitive response-inducing specific elicitors produced by the cowpea rust fungus. *Journal of Biological Chemistry* 272(7): 3924–3927.
- Eastwell, K.C. and G.B. Kalmar. 1997. Characterizing the interference between two comoviruses in cowpea. *Journal of the American Society for Horticultural Science* 122(2): 163–168.
- Edema, R., E. Adipala, and D.A. Florini. 1997. Influence of season and cropping system on occurrence of cowpea diseases in Uganda. *Plant Disease* 81(5): 465–468.
- Emechebe, A.M. 1980. Scab disease of cowpea (*Vigna unguiculata*) caused by a species of the fungus *Sphaceloma*. *Annals of Applied Biology* 96: 11–16.
- Emechebe, A.M. 1981. Brown blotch of cowpea in northern Nigeria. *Samaru Journal of Agricultural Research* 1(1): 20–26.
- Emechebe, A.M. and D.A. Florini. 1997. Shoot and pod diseases of cowpea induced by fungi and bacteria. Pages 176–192 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Emechebe, A.M. and D. McDonald. 1979. Seed-borne pathogenic fungi and bacteria of cowpea in northern Nigeria. *PANS* 25(4): 401–404.
- Emechebe, A.M. and S.A. Shoyinka. 1985. Fungal and bacteria diseases of cowpea in Africa. Pages 173–192 in *Cowpea research, production and utilization*, edited by S.R. Singh and K.O. Rachie, John Wiley and Sons, Chichester, UK.
- Emechebe, A.M., B.B. Singh, O.I. Leleji, I.D.K. Atokple, and J.K. Adu. 1991. Cowpea *Striga* problems and research in Nigeria. Pages 18–28 in *Combating Striga in Africa*, edited by S.K. Kim. IITA, Ibadan, Nigeria.
- Emechebe, A.M., J.P. Voh, O.O. Olufajo, and M.C. Dike. 1999. Report of 1998 Activities of PEDUNE-Nigeria. IAR/ABU, Zaria, Nigeria.
- Fernando, W.G.D and R.G. Linderman. 1997. The effect of mycorrhizal (*Glomus intraradices*) colonization on the development of root and stem rot (*Phytophthora vignae*) of cowpea. *Journal of the Natural Science Council of Sri Lanka* 25(1): 39–47.
- Filho, F.M.A., O.R. Paguio, J.L. Sherwood, and C.M. Deom. 2000. Mapping a symptom determinant of cowpea chlorotic mottle virus. *Phytopathology* 90(6) Supplement: 125.
- Florini, D.A. 1997. Nematodes and other soilborne pathogens of cowpea. Pages 193–206 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Gaur, H.S., J. Beans, and R.N. Perry. 1996. Effect of storage in vitro and in soil on the hatch from cysts of pigeonpea cyst nematode, *Heterodera cajani*. *Fundamental and Applied Nematology* 19(6): 537–544.
- Gillaspie, A.G. Jr., M.R. Hajimorad, and S.A. Ghabrial 1998. Characterization of a severe strain of cucumber mosaic cucumovirus seedborne in cowpea. *Plant Disease* 82(4): 419–422.
- Gillaspie, A.G. Jr, S.E. Mitchell, G.W. Stuart, and R.F. Bozarth. 1999. RT-PCR method for detecting cowpea mottle carmovirus in *Vigna* germplasm. *Plant Disease* 83(7): 639–643.
- Grange, N.L.A. and T.A.S. Aveling. 1998. First report of *Alternaria cassiae* on cowpea. *Plant Disease* 82(10): 1171.
- Gubba, A. 1994. Identification of cowpea viruses in Zimbabwe. *Zimbabwe Journal of Agricultural Research* 32(2): 149–155.

- Hacker, D.L. 1995. Identification of a coat protein binding site on southern bean mosaic virus RNA. *Virology* (New York) 207(2): 562–565.
- Hacker, D.L. and K. Sivakumara. 1997. Mapping and expression of southern bean mosaic virus genomic and subgenomic RNAs. *Virology* (New York) 234(2): 317–327.
- Hadiastono, T. 1996. A mosaic virus on blackeye cowpea (*Vigna unguiculata* [L.] Walp.). *Agrivita* 19(3): 118–120.
- Hampton, R.O., G. Thottappilly, and H.W. Rossel. 1997. Viral diseases of cowpea and their control by resistance-conferring genes. Pages 159–175 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Haque, M.S., M.L. Rahman, and M.A. Malek. 1994. Effect of fungicides and number of sprays on *Cercospora* leaf spot of cowpea. *Bangladesh Journal of Plant Pathology* 10(1–2): 3–4.
- Heath, M.C. 1998. Involvement of reactive oxygen species in the response of resistant (hypersensitive) or susceptible cowpeas to the cowpea rust fungus. *New Phytologist* 138(2): 251–263.
- Hibberd, J.M., W.P. Quick, M.C. Press, and J.D. Scholes. 1996. The influence of the parasitic angiosperm *Striga gesnerioides* on the growth and photosynthesis of its host, *Vigna unguiculata*. *Journal of Experimental Botany* 47(297): 507–512.
- Huguenot, C., M.T. Furneaux, J.A. Clare, and R.I. Hamilton. 1996. Improved diagnosis of cowpea aphid-borne mosaic virus in Africa. *Archives of Virology* 141(1): 137–145.
- Huguenot, C., M.T. Furneaux, and R.I. Hamilton. 1997. Further characterization of cowpea aphid-borne mosaic and blackeye cowpea mosaic potyviruses. Pages 231–239 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Hunter, A.G., O.L. Chambliss, and J.C. Williams. 1996. Simultaneous screening for resistance to blackeye cowpea mosaic virus and root knot nematode in southernpea. *HortScience* 31(7): 1217–1218.
- Iceduna, L.C., E. Adipala, and M.W. Ogenga-Latigo. 1994. Evaluation of 80 cowpea lines for resistance to scab, *Sphaceloma* sp. *African Crop Science Journal* 2: 207–217.
- Jang, H.S., Y.D. Choi, and C.H. Kim. 1996. Nucleotide sequence and infectivity of cucumber mosaic virus strain B RNA2 involved in resistance breakage on cowpea. *Molecules and Cells* 6(6): 704–711.
- Jenkins, A.M. 1931. Lima bean scab caused by *Elsinoe*. *Journal of Agricultural Research* 42: 13–23.
- Jong, W., K. Mise, A. Chu, and P. Ahlquist. 1997. Effects of coat protein mutations and reduced movement protein expression on infection spread of cowpea chlorotic mottle virus and its hybrid derivatives. *Virology* (New York) 232(1): 167–173.
- Kale, J.K. and K.H. Anahosur. 1994. Evaluation of cowpea genotypes for rust resistance. *Karnataka Journal of Agricultural Sciences* 7(1): 87–88.
- Kale, J.K. and K.H. Anahosur. 1996. Chemical control of cowpea rust. *Karnataka Journal of Agricultural Sciences* 9(1): 179–181.
- Kannan, N.R. and S. Doraiswamy. 1994. Screening cowpea entries for seed-borne infection of CAMV and to study the weed hosts of the virus. *Madras Agricultural Journal* 81(11): 637–638.
- Kassab, A.S. and M.K. Ali. 1996. Interaction among *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Rhizoctonia solani*, and *Rhizobium* on cowpea. *Annals of Agricultural Sciences* (Cairo) 41(1): 521–531.
- Kasteel, D.T.J., J. Wellink, R.W. Goldbach, and J.M.W. van Lent. 1997. Isolation and characterization of tubular structure of cowpea mosaic virus. *Journal of General Virology* 78(12): 3167–3170.

- Khan, M.R., M.W. Khan, and A.A. Khan. 1996. Effect of *Meloidogyne incognita* on dry weight, root gall and root nodulation of chickpea and cowpea cultivars. *Test of Agrochemicals and Cultivars* 17: 70–71.
- Khatri-Chhetri, G., K. Wydra, and K. Rudolph. 1998a. Characterization of *Xanthomonas campestris* pv. *vignicola* strains by metabolic fingerprints (BIOL.OG system). *International Congress of Plant Pathology*, Edinburgh 1998. Abstract 2.2.103.
- Khatri-Chhetri, G., K. Wydra, and K. Rudolph. 1998b. Development of semi-selective medium for quick and easy isolation of *Xanthomonas campestris* pv. *vignicola*, incitant of cowpea bacteria blight and pustule. *Mitteilung Biologische Bundesanstalt* 357: 247.
- Kim, C., P. Palukaitis, and C.H. Kim. 1997. The plant defence response to cucumber mosaic virus in cowpea is elicited by the viral polymerase gene and affects virus accumulation in single cells. *EMBO Journal* 16: 4060–4068.
- Kline, A.S. and E.J. Anderson. 1997. First report of cowpea aphid-borne mosaic potyvirus from cowpea grown commercially in the US. *Plant Disease* 81(8): 959.
- Konate, G. and B.J. Neya. 1996. Rapid detection of cowpea aphid-borne mosaic virus in cowpea seed. *Annals of Applied Biology* 129(2): 261–266.
- Konate, G. and J. Ouedraogo. 1988. Cowpea pathology in Burkina Faso, with particular emphasis on viral diseases. Pages 51–52 in *State of cowpea research in semi-arid zones of West and Central Africa*, edited by N. Muleba and A.M. Emechebe. IITA/SAFGRAD, Ouagadougou, Burkina Faso.
- Kumar, S. 1996. Efficacy of systemic insecticides as seed soaking for the control of *Meloidogyne incognita* on cowpea. *Madras Agricultural Journal* 83(8): 538–539.
- Lagoke, S.T.O., J.Y. Shebayan, G. Weber, O. Olufajo, K. Elemo, J.K. Adu, A.M. Emechebe, B.B. Singh, A. Zaria, A. Awad, L. Ngawa, G.O. Olaniyan, S.O. Olafare, and A.A. Adeoti. 1994. Survey of *Striga* problems and evaluation of *Striga* control methods and packages in crops in the Nigerian savanna. Pages 91–120 in *Improving Striga management in Africa*. Proceedings, Second General Workshop of Pan-African *Striga* Control Network (PASCON), edited by S.T.O. Lagoke, R. Hoevers, S.S. M'boob, and R. Traboulsi, 23–29 June 1991, Nairobi, Kenya. FAO/PASCON, Accra, Ghana.
- Lane, J.A., T.M.H. Moore, D.V. Child, and K.F. Cardwell. 1996. Characterization of virulence and geographic distribution of *Striga gesnerioides* on cowpea in West Africa. *Plant Disease* 80(3): 299–301.
- Lane, J.A., T.M.H. Moore, D.V. Child, and J.A. Bailey. 1997. Variation in virulence of *Striga gesnerioides* on cowpea: new sources of crop resistance. Pages 225–230 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Latha, K.R., D. Alice, R.O. Singh, and S.R. Durai. 1997. Effect of Zn on the development of root rot of summer pulses. *Madras Agricultural Journal* 84(6): 340–342.
- Latunde-Dada, A.O., R.J. O'Connell, C. Nash, R.J. Prong, J.A. Luis, and J.A. Bailey. 1996. Infection process and identity of the hemibiotrophic anthracnose fungus (*Colletrichum destructivum*) from cowpea (*Vigna unguiculata*). *Mycological Research* 100(9): 1133–1141.
- Latunde-Dada, A.O., R.J. O'Connell, C. Nash, and J.A. Lucas. 1999. Stomatal penetration of cowpea (*Vigna unguiculata*) leaves by *Colletrotrichum* species causing latent anthracnose. *Plant Pathology* 48(6): 777–785.
- Leina, M.J., T.K. Tan, and S.M. Wong. 1996. Resistance of *Hibiscus esculentus* L. and *Vigna sinensis* (L.) Endl. to *Pseudocercospora* and plant peroxidase activity in relation to infection. *Annals of Applied Biology* 129(2): 197–206.
- Lekkerkerker, A., J. Wellink, P. Juan, J. van Lent, R. Goldbach, and A.B. Kammen. 1996. Distinct functional domains in the cowpea mosaic virus movement protein. *Journal of Virology* 70(8): 5658–5661.

- Lin, M.C., H.P. Chen, Y.F. Wang, and W.Z. Zhang. 1995. Evaluation of cowpea varieties resistant to cowpea leaf mould (*Cercospora cruenta* Sacc.). *Crop Genetic Resources* 4: 36–37.
- Lin, M.T. and G.P. Rios. 1985. Cowpea diseases and their prevalence in Latin America. Pages 199–204 in *Cowpea research, production and utilization* edited by K.O. Rachie and S.R. Singh. John Wiley and Sons, Chichester, UK.
- Mahabeer, S., J.P. Agnietri, and M. Singh. 1995. Screening of cowpea genotypes against *Macrophomina phaseolina*. *Indian Journal of Mycology and Plant Pathology* 25(3): 324.
- Maramba, P. 1983. Plant pathology notes. *Zimbabwe Agricultural Journal* 80(1): 23.
- Maringa, I.K., D. Giga, and P. Maramba. 1985. Cowpea production constraints and research in Zimbabwe. *Tropical Grain Legume Bulletin* 30: 9–14.
- Moore, T.H.M., J.A. Lane, D.V. Child, G.H. Arnold, J.A. Bailey, and G. Hofman. 1995. New sources resistance of cowpea (*Vigna unguiculata*) to *Striga gesnerioides*, a parasitic angiosperm. *Euphytica* 84(3): 165–174.
- Muhammad, B., R.O. Hampton, and M. Bashir. 1996. Sources of genetic resistance in cowpea (*Vigna unguiculata* [L.] Walp.) to cowpea aphid-borne mosaic potyvirus. *European Journal of Plant Pathology* 102(5): 411–419.
- Muleba, N., J.T. Ouedraogo, and J.B. Tignegre. 1997. Crop yield loss attributed to *Striga* infestations. *Journal of Agricultural Science* 129(1): 43–48.
- Mungo, C.N., A.M. Emechebe, and D.A. Florini. 1998a. Isolation of *Sphaceloma* sp. from cowpea plant parts using eight media. *Crop Protection* 17(4): 341–343.
- Mungo, C.N., A.M. Emechebe, and D.A. Florini. 1998b. The effect of cropping history and the role of cowpea debris in the epidemiology of cowpea scab. *Plant Pathology* 47(5): 595–600.
- Munoz, M.A. and M.P.J. Tamayo. 1994. The presence of *Choanephora cucurbitarum* in cowpea (*Vigna unguiculata* [L.] Walp.). *ASCOLFI-Informa* 20(1): 4–5.
- Muqit, A., M.S. Haque, and M.M. Hossain. 1996. Reaction of cowpea lines against *Sclerotium rolfsii*. *Bangladesh Journal of Plant Pathology* 12(1–2): 63.
- Nagaraju, N. and K.V.K. Murthy. 1997. Studies on the relationship between cowpea mosaic virus and its vector *Myzus persicae* Sulz. *Mysore Journal of Agricultural Sciences* 31(2): 170–174.
- Nagaraju, U. and K.V. Keshavamurthi. 1998. Varietal reaction of cowpea cultivars to mosaic disease of cowpea. *Current Research—University of Agricultural Sciences (Bangalore)* 27(1): 10–11.
- Nakawuka, C.J. and E. Adipala. 1997. Identification of sources and inheritance of resistance to *Sphaceloma* scab in cowpea. *Plant Disease* 81(12): 1395–1399.
- Nakayama, M., K. Matsuura, and T. Okuno. 1996. Production of salicylic acid in tobacco and cowpea plants by a systemic fungicide ferimzone and induction of resistance to virus infection. *Journal of Pesticide Science* 21(1): 69–72.
- Nasu, Y., A. Karasawa, S. Hase, and Y. Ehara. 1996. *Cry*, the resistance locus of cowpea to cucumber mosaic virus strain Y. *Phytopathology* 86(9): 946–951.
- Noronha, M.A., S.J. Michereff, and R.L.R. Mariano. 1995. Effect of cowpea seed treatment with *Bacillus subtilis* on *Rhizoctonia solani* control. *Fitopatologia Brasileira* 20(2): 174–178.
- Noronha, M.A., S.J. Michereff, and R.L. Mariano. 1998. Selection of common beans (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) germplasms resistant to *Rhizoctonia solani*. *Boletim Micologica* 13(1–2): 111–116.
- Onifade, A.K. and B. Fawole. 1996. Effect of some plant extracts on the pathogenicity of *Meloidogyne incognita* on cowpea. *Global Journal of Pure and Applied Sciences* 2 (1): 9–15.
- Patel, P.N. 1985. Fungal, bacterial and viral diseases of cowpea in the USA. Pages 205–213 in *Cowpea research, production and utilization*, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Patel, P.N. and J.K. Jindal. 1982. Distinguishing reactions of the bacterial blight and pustule organisms of cowpea on pods of *Phaseolus vulgaris*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 89(7): 406–409.

- Paz, C.D., A.A. Lima, G. Pio-Ribeiro, F.M. Assis Filho, G.P. Andrade, and M.F.B. Goncalves. 1999. Purification of an isolate of cowpea severe mosaic obtained in Pernambuco, production of anti-serum and determination of sources of resistance in cowpea. *Summa Phytopathologica* 25 (4): 285.
- Premala, J., A.A. Brunt, and P. Jeyanandarajah. 1996. Blackeye cowpea mosaic potyvirus, the cause of a severe disease of vegetable cowpea (*Vigna unguiculata* ssp. *seguipedalis*) cv. Polonmae in Sri Lanka. *Tropical Science* 36(3): 129–137.
- Puruthi, I.J., R.J. Jain, and D.C. Gupta. 1995. Relative susceptibility of a few vegetable crops to two species of root knot nematodes. *Haryana Journal of Agricultural Science* 24(1): 76–78.
- Rahman, M.L., M.S. Haque, A. Muqit, K.B. Alam, and S. Ali. 1994. Response of *Sclerotium rolfsii* to different fungicides. *Bangladesh Journal of Plant pathology* 10(1–2): 35–36.
- Rakesh, S., M.K. Bhatnagar, and R. Shah. 1994a. Influence of inoculum concentration and multiplication of *Xanthomonas campestris* pv. *vignicola* on cowpea. *Legume Research* 17(3–4): 154–156.
- Rakesh, S., M.K. Bhatnagar, and R. Shah. 1994b. Survival of *Xanthomonas campestris* pv. *vignicola* causing bacterial blight of cowpea. *Indian Journal of Mycology and Plant Pathology* 24(3): 206–208.
- Rakesh, S., M.K. Bhatnagar, and R. Shah. 1995. Effect of plant age and inoculation methods on bacterial blight development in cowpea. *Indian Journal of Mycology and Plant Pathology* 25(3): 314–315.
- Ram, S., S. Hari, D.S. Dodan, R. Singh, and H. Singh. 1995. Sensitivity of *Rhizoctonia solani* isolates causing seedling mortality of mung to different fungicides. *Plant Disease Research* 10(1): 41–43.
- Ramadose, S. 1994. Effect of fungicides and insecticides on the growth and sclerotic production of *Macrophomina phaseolina* (Tassi) Goid. *Madras Agricultural Journal* 81(1): 21–23.
- Rangaiah, S. 1997. Inheritance of resistance to *Uromyces phaseolus* in *Vigna unguiculata* (L.) Walp. *Crop Improvement* 24(2): 251–252.
- Rao, A.L.N. 1997. Molecular studies on bromovirus capsid protein. III. Analysis of cell-to-cell movement competence of coat protein defective variants of cowpea chlorotic mottle virus. *Virology* (New York) 232(2): 385–395.
- Rao, G.M.V.P. and S. Ganguly. 1996. Host preference of six geographical isolates of refiner nematode, *Rotylenchulus reniformis*. *Indian Journal of Nematology* 20(1): 19–22.
- Rathore, B.S. 1995. Effect of granular nematicides against reniform nematode, *Rotylenchulus reniformis* on cowpea. *Indian Journal of Mycology and Plant Pathology* 25(3): 309–310.
- Rathore, B.S. and B.S. Yadav. 1996. Effect of chemical seed soaking on plant growth and reproduction of *Rotylenchulus reniformis* in cowpea. *Indian Journal of Mycology and Plant Pathology* 26(3): 281–283.
- Ratnoo, R.S., K.L. Jain, and M.K. Bhatnagar. 1997. Effect of atmospheric temperature on the development of ashy stem blight of cowpea. *Journal of Mycology and Plant Pathology* 27(1): 90–91.
- Reeves, J.C. 1997. Potentially important seedborne pathogens of economic crops in Northeast Asia. Pages 73–79 in *Seed health testing, progress towards the twenty-first century*, edited by W.S. Wu and J.D. Hutchins. CAB International, Wallingford, UK.
- Reiss, G.C. and J.A. Bailey. 1998. *Striga gesnerioides* parasitizing cowpea: development of infection structures and mechanisms of penetration. *Annals of Botany* 81(30): 431.
- Renato, C.P., A. Higuera, and A.M.P. Casassa. 1995. Response of some cowpea accessions to *Meloidogyne* spp. *Revista de la Facultad de Agronomia, Universidad del Zulia* 12(4): 485–490.
- Roberts, P.A., J.D. Ehlers, A.E. Hall, and W.C. Matthews. 1997. Characterization of new resistance to root knot nematodes in cowpea. Pages 207–214 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International

- Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Roberts, P.A., C.A. Frate, W.C. Matthews, and P.P. Osterili. 1995. Interactions of virulent *Meloidogyne incognita* and *Fusarium* wilt on resistant cowpea genotypes. *Phytopathology* 85(10): 1288–1295.
- Roberts, P.A., W.C. Matthews, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to the *RK* locus of cowpea. *Crop Science* 36(4): 889–894.
- Rodriguez, I., M.G. Rodriguez, L. Sanchez, and A. Iglesias. 1996. Expression of resistance to *Meloidogyne incognita* in cowpea cultivars (*Vigna unguiculata*). *Revista de Protection Vegetal* 11(1): 63–65.
- Rodriguez, V.J.L.B., M. Menezes, R.S.B. Coelho, and P. Miranda. 1997. Identification of resistance sources on genotypes of cowpea (*Vigna unguiculata*) to *Macrophomina phaseolina* (Tass.) Goid. under greenhouse conditions. *Summa Phytopathologica* 23(2): 170–172.
- Roy, K.W. and S. Ratnayake. 1997. First report of *Phomopsis longicolla* infection of cowpea pods and seeds in Mississippi. *Plant Disease* 81(6): 693.
- Ryerson, D.E. and M.C. Heath. 1996. Inheritance of resistance to the cowpea rust fungus in cowpea cultivar Calico Growder. *Canadian Journal of Plant Pathology* 18(4): 384–391.
- Santhi, A., S. Rajeswari, and R. Sundarababu. 1995. Effect of three species of VAM viz *Glomus fasciculatus*, *G. versiforme*, *G. etunicatus* and root knot nematode *Meloidogyne incognita* on cowpea growing in different types of soil. *International Journal of Tropical Plant Diseases* 13(1): 63–68.
- Santhi, A. and R. Sundarababu. 1995. Effect of phosphorus on the interaction of vesicular arbuscular mycorrhizal fungi with *Meloidogyne incognita* on cowpea. *Nematologia Mediterranea* 23(2): 263–265.
- Santos, A.A., M.A.W. Quindere, and M.B. Melo. 1997. Evaluation of cowpea genotypes for resistance to cowpea smut (*Entyloma vignae*). *Fitopatologia Brasileira* 22(1): 77–78.
- Sarmah, B. and A.K. Sinha. 1995. Pathogenicity of *Meloidogyne incognita* on cowpea. *Plant Health* 1: 12–14.
- Schneider, W.L., A.E. Greene, and R.F. Allison. 1997. The carboxy terminal two-thirds of the cowpea chlorotic mottle bromovirus capsid protein is incapable of virion formation yet supports systemic movement. *Journal of Virology* 71(6): 4862–4865.
- Sharma, S.B., T.J. Rego, M. Mchiuddin, and V.N. Rao. 1996. Regulation of population densities of *Heterodera cajani* and other plant-parasitic nematodes by crop rotations on vertisols in semi-arid tropical production systems in India. *Journal of Nematology* 28(2): 244–251.
- Shoyinka, S.A., R.F. Bozarth, J. Rees, and H.W. Rossel. 1978. Cowpea mottle virus: a seedborne virus with distinctive properties infecting cowpea in Nigeria. *Phytopathology* 68: 693–699.
- Sikirou, R. 1999. Epidemiological investigations and development of integrated control methods of bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola*. PhD thesis. University of Gottingen, Germany. 218 pp.
- Sikirou, R.K., K. Wydra, and K. Rudolph. 1998a. Inoculum source of *Xanthomonas campestris* pv. *vignicola*, incitant of cowpea bacterial blight and pustule, and identification of other hosts besides *Vigna unguiculata*. International Congress of Plant Pathology, 9–16 August 1998, Edinburgh, UK. Abstract 6.67.
- Sikirou, R.K., K. Wydra, and K. Rudolph 1998b. Survival of *Xanthomonas campestris* pv. *vignicola*, incitant of cowpea bacterial blight and pustule on weeds and in soil and identification of other hosts besides *Vigna unguiculata*. *Mitteilung Biologische Bundesanstalt* 357: 223.
- Singh, B.B. and A.M. Emechebe. 1997. Advances in research on cowpea *Striga* and *Alectra*. Pages 215–223 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Sivakumara, K., B.C. Foulter, and D.L. Hacker. 1998. Identification of viral genes required for cell-to-cell movement of southern bean mosaic virus. *Virology* (New York) 252(2): 376.



- Smith, J.E., L. Christen, and T.A.S. Aveling. 1999a. Infection process of *Colletotrichum dematium* on cowpea stems. *Mycological Research* 103(2): 230–234.
- Smith, S.N., D.M. Helms, and S.R. Temple. 1999b. The distribution of *Fusarium* wilt of blackeyed cowpeas within California caused by *Fusarium oxysporum* f.sp. *tracheiphilum* race 4. *Plant Disease* 83(7): 694.
- Stofella, P.J., R.C. Bullock, and R.M. Sonoda. 1990. Influence of pesticide spray schedules on growth and yields of cowpeas. *Proceedings of Inter-American Society for Tropical Horticulture* 34: 83–87.
- Subramaniam, S., A.K. Faziullah, G. Rajendran, and S. Vadivelu. 1997. Reaction of some mung, cowpea, lablab and tomato genotypes to the reniform and root knot nematodes. *Indian Journal of Nematology* 27(1): 130–131.
- Sunder, S., S.K. Sharma, A.S. Taya, and O.P. Sheoran. 1996. Effect of age and quality of inoculum, temperature and pH of substrate on the pathogenic behaviour of *Rhizoctoria solani*. *Plant Disease Research* 11(1): 107–110.
- Thammaiah, N. and A.N.A. Khan. 1995a. Effect of seed treatment on *Xanthomonas campestris* pv. *vignicola* causal agent of bacterial blight of cowpea. *Advances in Agricultural Research in India* 4: 93–102.
- Thammaiah, N. and A.N.A. Khan. 1995b. Studies on control of *Xanthomonas campestris* pv. *vignicola* (Burkh.) dye in cowpea seeds by hot water treatment. *Advances in Agricultural Research in India* 3: 194–198.
- Thammaiah, N., A.N.A. Khan, and E. John. 1995. Effects of extracts of *Adhatoda zeylanica* on *Xanthomonas campestris* pv. *vignicola* causing bacterial blight of cowpea. *Advances in Agricultural Research in India* 4: 109–117.
- Thottappilly, G., O.P. Sehgal, and H.W. Rossel. 1993. Characteristics of a cowpea chlorotic mottle virus isolate from Nigeria. *Plant Disease* 77: 60–63.
- Toure, M., A. Oliver, B.R. Ntare, J.A. Lane, and C.A. Pierre. 1998. Reaction of cowpea (*Vigna unguiculata*) cultivars to *Striga gesnerioides* races from Mali and Niger. *Canadian Journal of Plant Science* 78(3): 477–480.
- Umaharan, P., R.R. Ariyanayagam, and S.O. Haque. 1997a. Resistance to cowpea severe mosaic virus, determined by three dosage-dependent genes in *Vigna unguiculata* (L.) Walp. *Euphytica* 95(1): 49–95.
- Umaharan, P., R.R. Ariyanayagam, and S.O. Haque. 1997b. Identification of resistance to cowpea severe mosaic virus (Trinidad isolate) in cowpea (*Vigna unguiculata* [L.] Walp.). *Tropical Agriculture* 74(4): 324–328.
- Ushamali, C., K. Rajappan, and K. Gangadharan. 1997a. Inhibition of *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *tracheiphilum* by antagonists under in vitro conditions. *Plant Disease Research* 12(2): 167–170.
- Ushamali, C.K., Rajappan, and K. Gangadharan. 1997b. Control of *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *tracheiphilum* by antagonists under field conditions. *Plant Disease Research* 12(2): 122–129.
- Ushamali, C., K. Rajappan, and K. Gangadharan. 1997c. Suppression of charcoal rot and wilt pathogens of cowpea by botanicals. *Plant Disease Research* 12(2): 113–117.
- Vale, C.C. and J.A. Lima. 1995. The inheritance of immunity in *Vigna unguiculata* Macaibo to cowpea severe mosaic virus. *Fitopatologia Brasileira* 20(1): 30–32.
- Vauterin, L., B. Hoste, K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* 45: 472–489.
- Vauterin, L., J. Rademaker, and J. Swings. 2000. Synopsis on the taxonomy of the genus *Xanthomonas*. *Phytopathology* 90(7): 677–682.
- Verdier, V., K. Assigbetse, G. Khatri-Chhetri, K. Wydra, K. Rudolph, and J.P. Geiger. 1998. Molecular characterisation of the incitant of cowpea bacterial blight and pustule, *Xanthomonas campestris* pv. *vignicola*. *European Journal of Plant pathology* 104: 595–602.

- Verma, J.S. and S.N. Mishra. 1989. Evaluation of improved lines from IITA in humid-subtropical India. *Tropical Grain Legume Bulletin* 36: 38–39.
- Verver, J., J. Wellink, J. van Lent, K. Gopinath, and A. van Kammen. 1998. Studies on the movement of cowpea mosaic virus using the jellyfish green fluorescent protein. *Virology* (New York) 242(1): 22–27.
- Wikoff, W.R., J.O. Tsai-Chao, T.S. Baker, J.E. Johnson, and G.J. Wang. 1997. The structure of cucumber mosaic virus, cryoelectron microscopy, x-ray crystallography, and sequence analysis. *Virology* (New York) 232(1): 91–97.
- Williams, R.J. 1975. Diseases of cowpea (*Vigna unguiculata* [L.] Walp.) in Nigeria. *PANS* 21(3): 253–267.
- Xu, H.X. and M.C. Heath. 1998. Role of calcium in signal transduction during the hypersensitive response caused by basidiospore derived infection of the cowpea rust fungus. *Plant Cell* 10(4): 585–597.
- Youssef, M.M.A. and M.S. Ali. 1998. Management of *Meloidogyne incognita* infecting cowpea by using native blue green algae. *Anzeiger für Schadlingskunde Pflanzenschutz, Umweltschutz* 71: 15.
- Youssef, M.M.A. and W.A. Amin. 1997. Effect of soil amendment in the control of *Meloidogyne javanica* and *Rotylechulus reniformis* infection on cowpea. *Pakistan Journal of Nematology* 15: 55–63.
- Zeng, Y., Z. Wang, and C. Zhao. 1999. Studies on the techniques for evaluation of resistance of cowpea seedlings to rust disease. *Journal of South China Agricultural University* 20(2): 23–27.

## Development of sex pheromone traps for monitoring the legume podborer, *Maruca vitrata* (F.) (Lepidoptera: Pyralidae)

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### Abstract

The Natural Resources Institute (NRI) and the International Institute of Tropical Agriculture (IITA) are collaborating to develop sex pheromone traps as monitoring tools for *Maruca vitrata*. The principal component of the pheromone is (*E,E*)-10,12-hexadecadienal. Our trapping experiments in cowpea fields in Benin have shown that the optimal synthetic blend also contains small amounts of (*E,E*)-10,12-hexadecadien-1-ol and (*E*)-10-hexadecenal. Polyethylene vial lures containing 0.1 mg of pheromone attracted more males than other combinations of dose or dispenser. Lures showed no loss of effectiveness for up to four weeks in the field. A water-trap made from a plastic jerry can was superior to commercial funnel- and sticky-trap designs and 120 cm was the optimum height for traps. Females comprised up to 50% of total catches with synthetic lures, though almost none were attracted to traps baited with live females. Preliminary observations indicated a temporal coincidence between catches in traps placed outside cowpea fields and the appearance of larvae in fields a few days later. Thus pheromone trap catches may predict larval infestations.

### Introduction

The legume podborer, *Maruca vitrata* (F.) (syn. *M. testulalis*) (Lepidoptera: Pyralidae), is a pantropical pest of legume crops, particularly cowpea (Jackai 1995), pigeonpea (Shanower et al. 1999), and beans (Abate and Ampofo 1996). In West Africa without control measures, flower infestation rates by *M. vitrata* of up to 80% were reported by Afun et al. (1991) and seed damage rates of 50% by Dreyer et al. (1994).

Although the basic biology of *M. vitrata* has been studied extensively (Taylor 1967; Singh et al. 1990; Jackai et al. 1990; Onyango and Ochieng-Odero 1993), much remains to be understood concerning the behavior of this pest in the field, which has hindered development of IPM strategies in Africa (Jackai 1995) and Asia (Shanower et al. 1999). Pheromone-baited traps for *M. vitrata* could provide tools for monitoring the activity and movements of adults that would assist researchers in this respect. Bottenberg et al. (1997) provided some data relating to the population dynamics and migration of *M. vitrata* at three locations in West Africa, based on light trap catches. However, light

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traps are difficult and expensive to maintain, and catches of the target species have to be sorted from among other insects. Pheromone traps could be deployed more easily, cheaply, and in greater numbers than light traps. Moreover, pheromone traps are specific for the species of interest. Effective traps could provide simple alternatives to pest scouting by farmers or extension workers to time application of control measures.

Adati and Tatsuki (1999) recently reported (*E,E*)-10,12-hexadecadienal (EE10,12-16:Ald) to be an EAG-active component of the extract from female *M. vitrata* abdominal tips. Synthetic EE10,12-16:Ald was shown to be attractive to male moths in laboratory bioassays. The corresponding alcohol, EE10,12-16:OH, was also noted as being present at 3–4% of the aldehyde. Although no behavioral data were presented in relation to this compound, it was said to produce no increase in attraction. No field testing of the compounds was carried out. Previous analytical work carried out by the Natural Resources Institute (NRI) has confirmed the presence of EE10,12-16:Ald and EE10,12-16:OH as major and minor blend components, respectively. Results also suggested the presence of a third pheromone component, and subsequent laboratory and field bioassays indicated this was probably (*E*)-10-hexadecenal (E10-16:Ald) (Downham and Hall 2000, NRI unpublished data). Cross mating and cross attraction experiments with strains from Benin, India, Nigeria, Sri Lanka, and Taiwan, together with analysis of gland extracts from females of these strains, indicated that there is no geographic variation in the natural pheromone blend of *M. vitrata*.

In this paper we report the first successful field trapping experiments that used a blend of these compounds to catch *M. vitrata*. Experiments to develop further a practical trapping system are also reported.

## Materials and methods

### *Trap and lure optimization experiments*

Trapping experiments were carried out during 1998 and 1999 within cowpea fields at the IITA research station near Cotonou, Republic of Benin. Unless otherwise noted, traps were suspended from sticks using wire at a height of approximately 1.0–1.2 m. Synthetic lures were replaced every two weeks and, unless otherwise noted, they were shielded from sunlight to minimize isomerization by aluminum foil wrapped around them to leave only the lowermost surface exposed. The isomeric purity of pheromone components EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald used in lures was > 99%. Trap catches were counted daily and trapped moths discarded at that time. Insecticides were not sprayed in the fields.

In the first experiment, four pheromone blends were evaluated. These were EE10,12-16:Ald alone or in combination with one or both of the two minor components, EE10,12-16:OH and E10-16:Ald, both of which were present at a level of 5% relative to the EE10,12-16:Ald. These synthetic blends were presented in polyethylene vial and rubber septa dispensers as lures in white, sticky, delta traps (Agrisense-BCS, Pontypriidd, UK). For each of these, two doses, 0.01 mg or 0.1 mg, were compared, making 16 treatment combinations. These were compared with traps containing two virgin females confined to small wire-mesh cages and with unbaited controls. Females were two days old when placed in traps and were replaced every two days. Sticky card inserts in delta traps were replaced on a weekly basis. The experiment consisted of three cowpea fields, forming replicate blocks; in each, traps were positioned in a grid formation with 10-m spacing.

Four subsequent experiments included a comparison of lure age and shielding, two of different trap designs and one of trap height. The lures used in each of these were 0.1-mg polyethylene vials. Sticky, delta traps (Fig. 1a) were used in the lure age and shielding experiment; green plastic funnel traps (Fig. 1b) (Agrisense-BCS, Pontypridd, UK), with DDVP insecticide strips to kill trapped moths, were used in one trap design experiment and the trap height comparison. Four water-trap designs (Figs. 1c–1f), each constructed from cheap, locally obtained materials, were also evaluated in the trap design comparisons. A water-pan (Fig. 1c) trap was made from a green plastic bowl (20-cm diameter) and plate held 5 cm apart with steel wire. Others were made from a 1.5-liter clear plastic bottle (Fig. 1d) and 2-l and 5-l white plastic jerry cans (Figs. 1e, 1f) in which four windows had been cut from the sides. Lures were suspended within the center of each trap. A little soap powder was added to the water within each trap to reduce surface tension, and vegetable oil to reduce evaporation. In the lure age and shielding experiment, shielded and unshielded lures were pre-aged for two or four weeks before use by exposing them in sticky, delta traps. Each experiment was carried out to a randomized complete-block design with five replications. Traps within a replicate block were set out in lines or rectangular formations, the exact layout depending on the number of treatments being compared. Individual traps were positioned 20 m apart. Blocks were at least 50 m apart and were usually situated in separate fields.

During the trapping experiments, it is possible that there were some interactions between traps within replicate blocks. This may have occurred as individual pheromone plumes overlapped and moths, initially attracted by the plume of one trap, passed on to the plumes of other traps. This would have acted to blur treatment differences. However, the random positioning of treatments within blocks and night-to-night variation in wind direction would have meant that no systematic biases occurred.

For statistical analysis the total catches by each trap over the respective trapping periods were used. With the blend experiment and the lure age/shielding experiment, analysis involved the raw data, since these met the normality and constant variance assumptions. However, it proved necessary to transform data of the trap height (square root) and trap design ( $\log_{10}[\times + 1]$ ) experiments. Analysis of variance was carried out using Genstat 5 for Windows (release 4.1). Where this indicated statistically significant effects, treatment means were separated using the least significant difference (LSD) at the 5% level.

### ***Observations relating pheromone trap catches to light trap catches and larval infestations***

IITA operates a light trap (500 W mercury-vapor bulb) at its Cotonou station. During the relevant period this was situated several hundred meters from any experimental cowpea fields. Catches of *M. vitrata* were recorded on a daily basis and compared to those in pheromone traps forming part of the trap and lure optimization experiments. Weekly inspections for larvae were carried out in the fields containing traps from the optimization experiments; all individuals on four randomly selected plants per field were counted. On 22 September 1998, before the second cropping season began, a ring of 20 sticky, delta pheromone traps was established around the perimeter of the IITA station. These traps were baited with polyethylene vial lures containing 0.1 mg of the 3-component blend (100:5:5 ratio). They were placed 150 m apart and at least 80 m from the nearest cowpea field. Data from these traps were also compared to the light trap and other data.



Figure 1a. Sticky delta trap.



Figure 1b. Agrisense-BCS funnel trap.

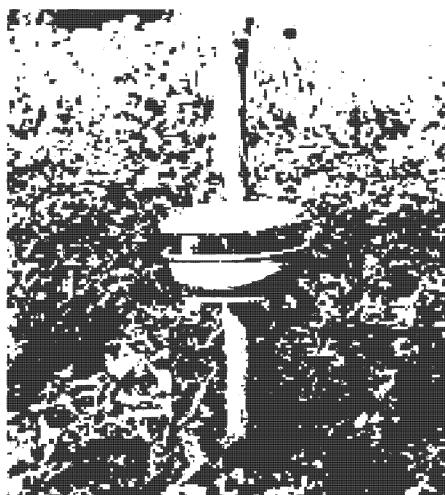


Figure 1c. Water-pan trap.



Figure 1d. Plastic bottle water trap (1.5 liters).



Figure 1e. Two-liter bottle water trap.



Figure 1f. Five-liter jerry can water trap.

In a separate set of observations, conducted from mid-January to mid-March 2000, two pheromone traps (5-l jerry can design—see trap design experiments) were monitored three times/week in each of 10 on-farm plots. Plots were situated in the villages of Agonguey, Agbonou, and Wosounmé in the Ouémé valley in southeast Benin. The nearby river allows cowpea to be grown here in what is the off-season elsewhere in Benin. The size of plots varied from 400 to 600 m<sup>2</sup>. Plots were not treated with insecticides but were situated within larger blocks that were treated. The cowpea variety in each case was Chawé Daho, which has a growing season of 90 days. Sowing dates were 30 November–15 December 1999, flowering commenced 9–25 January 2000, and harvest was 25 February–5 March 2000. At weekly intervals from 19 January to 21 February, 20 flowers per plot were inspected for the presence of larvae.

## Results

### *Trap and lure optimization experiments*

In the pheromone blend experiment, traps baited with lures containing all three of the proposed components, EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald, caught significantly more male *M. vitrata* moths than those baited with one or two component blends or live females ( $P < 0.05$ ), all of which attracted similar numbers of male moths. No males were captured in unbaited control traps (Table 1). Although the polyethylene vial dispensers loaded with 0.1 mg pheromone attracted slightly more males than other combinations of dose or dispenser (Table 2), there was no significant overall effect of dispenser type or dose ( $P > 0.05$  LSD). About 20% of total catches in traps baited with synthetic lures were female moths although almost no females were attracted to live females or unbaited controls. The trends in respect of different blends, doses, and dispensers were similar to those for males (Tables 1 and 2).

When the attractiveness of lures of different ages was compared, separate analyses of variance indicated highly significant effects in respect of captures of both sexes ( $P < 0.01$ ) (Table 3). Four to six-week-old lures were significantly less attractive to males than 0–2 and 2–4-week-old lures ( $P < 0.05$ ); in respect of females, 0–2-week-old lures were significantly more attractive than both older sets of lures ( $P < 0.05$ ). Analyses of variance showed that male captures were not influenced by shielding of the lures ( $P = 0.75$ ), but female captures with shielded lures were significantly greater than with unshielded lures ( $P < 0.01$ ). Captures of female moths made up 14% of the total in this experiment.

**Table 1.** Mean catches/trap of *M. vitrata* in the blend experiment at IITA, Cotonou, Benin, June–August 1998 (catches for synthetic blends averaged across dose and dispenser type).

| Lure or ratio of components <sup>†</sup> | Males <sup>†</sup> |     | Females <sup>†</sup> |     |
|--|--------------------|-----|----------------------|-----|
|  | Mean               | SE  | Mean                 | SE  |
| 100:0:0                                  | 7.0 bc             | 1.4 | 1.3 cd               | 0.4 |
| 100:5:0                                  | 5.3 c              | 0.9 | 1.8 bc               | 0.4 |
| 100:0:5                                  | 8.9 b              | 1.1 | 2.9 b                | 0.6 |
| 100:5:5                                  | 33.1 a             | 2.4 | 5.3 a                | 0.9 |
| 2 × virgin females                       | 5.8 c              | 0.8 | 0.2 d                | 0.1 |
| Blank, control                           | 0.0 d              | 0.0 | 0.0 d                | 0.0 |

<sup>†</sup>(E,E)-10,12-16:Ald: (E,E)-10,12-16:OH: (E)-10-16:Ald.

<sup>†</sup>Means within a column followed by a common letter were not significantly different ( $P > 0.05$ , LSD following ANOVA).

**Table 2.** Mean catches per trap of *M. vitrata* with synthetic dispensers in the blend experiment at IITA, Cotonou, Benin, June–August 1998 (catches averaged across different blends).

| Lure dose/dispenser type | Males <sup>†</sup> |     | Females <sup>†</sup> |     |
|--------------------------|--------------------|-----|----------------------|-----|
|                          | Mean               | SE  | Mean                 | SE  |
| 0.01 mg vials            | 12.3               | 3.3 | 2.5                  | 0.5 |
| 0.1 mg vials             | 16.8               | 4.2 | 2.7                  | 0.6 |
| 0.01 mg septa            | 13.3               | 3.4 | 3.2                  | 0.9 |
| 0.1 mg septa             | 12.0               | 3.8 | 3.0                  | 1.0 |

<sup>†</sup> There was no significant effect of dose or dispenser type on male or female catches ( $P > 0.05$ , F-ratio ANOVA).

**Table 3.** Mean catches/trap of *M. vitrata* with lures of different ages, shielded or not shielded from sunlight, at IITA, Cotonou, Benin, August–November 1999.

| Lure age (weeks) | Shielding<br>Yes/No | Males  |     | Females |     |
|------------------|---------------------|--------|-----|---------|-----|
|                  |                     | Mean   | SE  | Mean    | SE  |
| 0–2              | Yes                 | 11.8 a | 1.0 | 3.6 a   | 0.8 |
| "                | No                  | 12.0 a | 2.0 | 1.8 b   | 0.6 |
| 2–4              | Yes                 | 11.4 a | 1.5 | 1.6 b   | 0.7 |
| "                | No                  | 9.8 a  | 1.9 | 0.8 b   | 0.2 |
| 4–6              | Yes                 | 5.0 b  | 0.8 | 1.4 b   | 0.4 |
| "                | No                  | 7.6 ab | 1.4 | 0.4 b   | 0.2 |



In both trap design experiments, significant treatment effects were observed ( $P < 0.05$ ). In the first comparison, the sticky, delta trap attracted the fewest moths of both sexes (Table 4). Three to four times more males were captured by the Agrisense-BCS funnel trap than the delta trap, but the locally constructed water-pan trap was most effective in capturing females (three times more than the delta trap). The sticky, delta trap was also less effective than two other locally constructed water traps in the second experiment. In this experiment, for both sexes, the 5-l and 2-l jerry can designs proved superior to both the delta trap and the 1.5-l bottle design (Table 5). Overall percentage captures of females in the two experiments were 46% and 35%.

The trap height experiment indicated that 120 cm was optimal in respect of catches of males (Table 6). Mean catches of males at this height were significantly greater than at 20 and 170 cm ( $P < 0.05$ ), though not at 70 cm. Overall catches of females were around 11% of the total, and there were no significant differences in respect of trap height.

**Table 4.** Mean catches/trap of *M. vitrata* in the first trap design experiment at IITA, Cotonou, Benin, October–December 1998.

| Trap design   | Males  |     | Females |     |
|---------------|--------|-----|---------|-----|
|               | Mean   | SE  | Mean    | SE  |
| Sticky, delta | 3.0 b  | 1.6 | 3.2 b   | 1.5 |
| Water-pan     | 7.6 ab | 3.4 | 9.0 a   | 3.5 |
| Funnel        | 11.0 a | 4.0 | 6.4 ab  | 2.7 |

**Table 5.** Mean catches/trap of *M. vitrata* in the second trap design experiment at IITA, Cotonou, Benin, September–November 1999.

| Trap design   | Males  |     | Females |     |
|---------------|--------|-----|---------|-----|
|               | Mean   | SE  | Mean    | SE  |
| 5-l jerry     | 13.0 a | 1.8 | 7.4 a   | 1.3 |
| 2-l jerry     | 10.8 a | 2.0 | 6.0 ab  | 1.7 |
| Sticky, delta | 4.0 b  | 0.8 | 1.4 c   | 0.5 |
| 1.5-l bottle  | 5.0 b  | 1.1 | 2.8 bc  | 0.6 |

**Table 6.** Mean catches/trap of *M. vitrata* at different heights aboveground at IITA, Cotonou, Benin, July–October 1999.

| Height (cm) | Males  |     | Females |     |
|-------------|--------|-----|---------|-----|
|             | Mean   | SE  | Mean    | SE  |
| 20          | 5.6 bc | 1.2 | 0.2 a   | 0.2 |
| 70          | 6.8 ab | 0.6 | 1.4 a   | 0.4 |
| 120         | 10.4 a | 1.4 | 0.6 a   | 0.4 |
| 170         | 3.4 c  | 1.3 | 1.2 a   | 1.0 |

### **Observations relating pheromone trap catches to light trap catches and larval infestations**

Catches in the light trap were always much greater than in individual pheromone traps, and while males predominated in pheromone trap catches, females tended to form the majority in the light traps. Within each type of trap, temporal patterns of catches of each sex were similar, so that catches of one sex accurately reflected the presence of the other.

During two seasons of on-station trials at IITA-Cotonou, a general observation was made that the timing of catches in the light trap and those in pheromone traps within fields did not correspond closely. However, there was a better temporal correspondence between the light trap catches and those in perimeter traps (Fig. 2). This was notable at the start of the second season of 1998. Following several weeks of zero catches in the light trap and the perimeter pheromone traps, the latter detected the first small peak of moths at exactly the same time (29 October) as the light trap, although there appeared to be little subsequent quantitative correlation.

During this period, the first appearance of moths in traps in cowpea fields was at least 12 days after catches were first noted in the light trap and perimeter pheromone traps. These initial within-field catches were 33–50 days after the fields were sown. The first crop inspection, eight days after the initial catches in perimeter traps, showed that larvae were already present in each of three fields sampled at that time; but this was several days before within-field catches in two of the fields and simultaneous with the first catches in a third. Representative data for one field are shown in Figure 3 and can be compared to Figure 2. Trap catches within fields in the second season were confined to periods of 8–12 days.

Results from the on-farm observations in the Ouémé valley are summarized in Figure 4. Although overall catches were relatively low—rarely exceeding an average of 0.5 moths per trap per count—the timing of their onset across all plots was consistent. In eight of the 10 plots, the first catches were noted on 28 January, while first catches occurred in the remaining plots on the subsequent count three days later. Catches were evenly distributed across all 10 plots and three village sites until the end of February, when they began to decline. Males and females were trapped in approximately equal numbers. Larvae were only found on two dates: 9 and 14 February. On the first occasion they were noted in four plots, on the second they were observed in seven plots. Since some of the larvae were late instars it is probable that eggs were laid five to ten days after the first adults were trapped.

## **Discussion**

From the results of the trap and lure optimization experiments an effective and practical trapping system for *M. vitrata* has now been developed for the first time. The best pheromone blend is a mixture of EE10,12-16:Ald, EE10,12-16:OH, and E10-16:Ald in the ratio 100:5:5. Although no significant differences were evident in respect of dose or dispenser, the 0.1-mg polyethylene vials would be expected to show the greatest longevity of the lures tested on the basis of dose and release rate characteristics. Our results indicate no loss of attractiveness for up to four weeks under field conditions. Therefore, these lures have now been adopted as standard for use in further work. The best trap height is 120 cm and the most effective traps are those produced from locally available plastic jerry cans. Not only are these relatively much cheaper than imported, commercial designs (US\$0.30–0.80 as

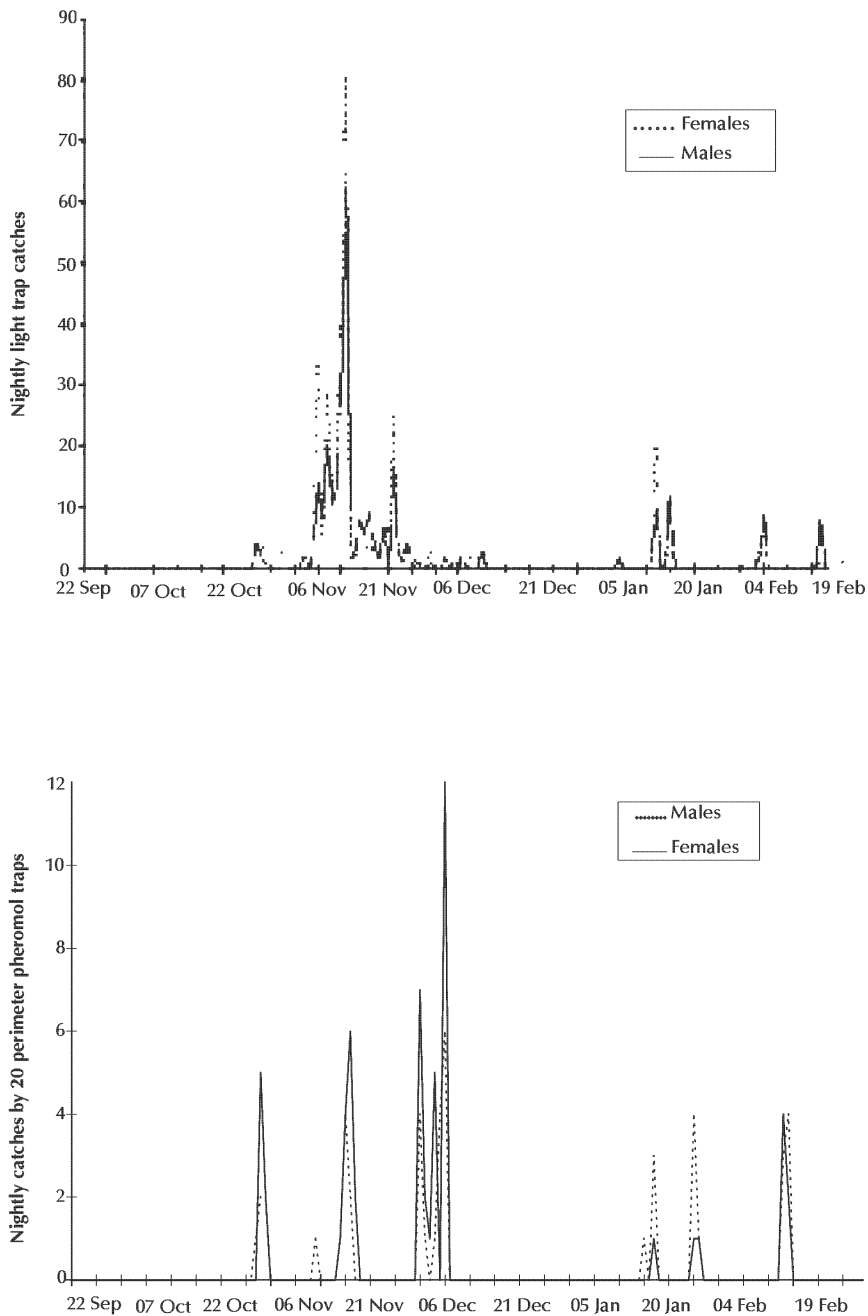


Figure 2. *M. vitrata* catches in a light trap (top) and in 20 pheromone traps in noncrop areas around the perimeter of the IITA station (bottom) during and after the second cropping season in 1998.

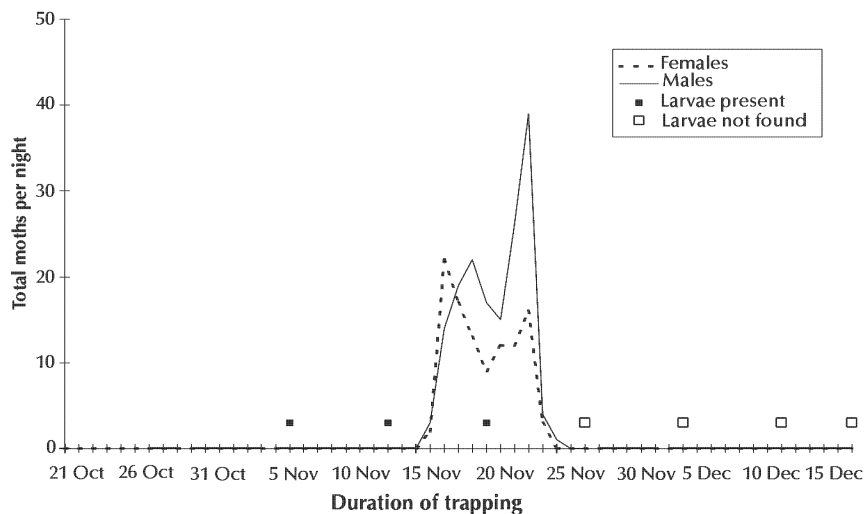


Figure 3. Total catches of *M. vitrata* by seven pheromone traps in field C3, forming one block of a pheromone blend experiment during the second cropping season in 1998.

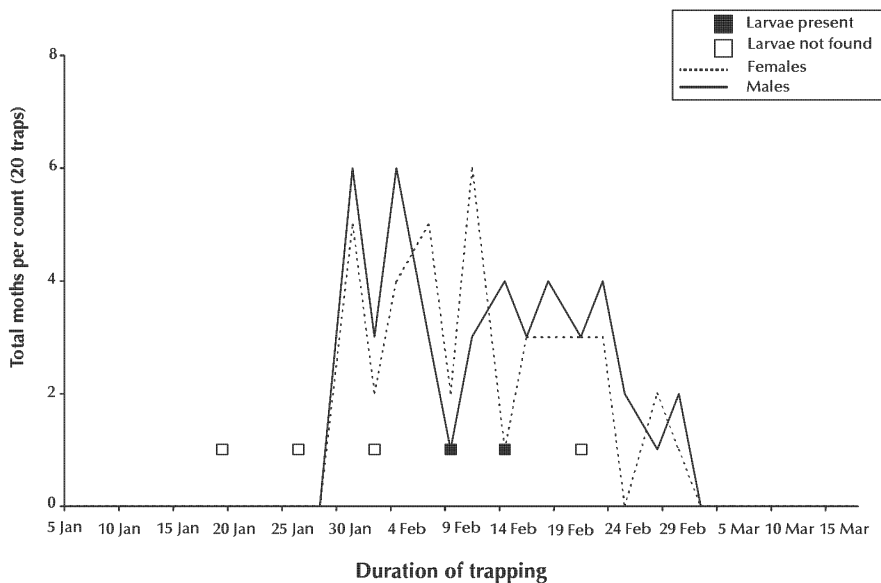


Figure 4. Total catches of *M. vitrata* by 20 pheromone traps in 10 on-farm plots in the Ouémé valley, Benin, 2000.

against approximately US\$3.00 for sticky, delta traps and more for plastic funnel traps), they are easy to construct and robust in use.

Further work on trap and lure optimization is required. Experiments are planned to determine the effect of trap color and frequency of checking on trap catches. An experiment concerning isomeric purity of the pheromone blend components is currently underway and the results will be of particular relevance. If a lower level of purity can be used without a marked loss of attraction, it will be possible to reduce the cost of lure production to around US\$0.50 per lure. This will be important in helping to ensure the economic viability of pheromone trap monitoring of *M. vitrata*.

The consistent capture of significant numbers of female moths with synthetic sex pheromone lures is, to the best of our knowledge, unprecedented. It could suggest an incomplete pheromone blend, but extensive analytical work with the natural pheromone, to be reported elsewhere, has failed to find any evidence for further blend components (M.C.A. Downham and D.R. Hall, unpublished data). Furthermore, incomplete pheromone blends generally produce lower catches of males, rather than co-attraction of both sexes. Thus a better explanation may lie in some unknown aspect of the species' mating behavior or ecology, and further work to explain the phenomenon would be very helpful. Regardless of explanation, catches of females may actually improve the predictive power of traps, since they should more accurately reflect local population events than males alone.

With regard to the practical use of traps for monitoring purposes, results to date indicate real potential. In the on-station trials at Cotonou, catches in pheromone traps outside cowpea fields preceded larval infestations in fields; in contrast, catches in traps within the fields occurred only after larvae appeared. Thus it seemed that traps near but outside fields might be better able to predict pest attacks. However, a subsequent trial at a different time of year in farmers' fields suggested that within-field traps could give early warning of larval infestations. Resolving the question of the best positioning of traps to detect immigrating moths will be the task of further work currently in progress at several on-farm sites around Benin, and soon to be extended to Ghana. This work is being carried out in association with the West African regional PRONAF (Projet de Niébé pour l'Afrique) project, a partnership between IITA and various NARS that aims to promote the transfer and implementation of research on cowpea to subsistence farmers. It will be necessary to determine the relationship between larval infestations and catches by pheromone traps with confidence. Whether any good quantitative correlation between catches and damage exists remains to be seen, but it seems likely that pheromone traps could be used as the basis for timing the application of control measures.

Afun et al. (1991) found that by using action thresholds based on larval/flower infestation rates to time insecticide applications on cowpea, the number of sprays could be reduced, relative to a calendar-based approach, with no loss of control and at reduced cost. Ultimately it is hoped that pheromone trap-based action thresholds could be used in conjunction with other promising sustainable control methods being developed through PRONAF, e.g., neem-based insecticides (W. Hammond, pers. comm.; see also Bottenberg and Singh 1996). Furthermore, Bottenberg (1995) found that many farmers are unable to link the adult stage of *M. vitrata* with the highly destructive larval stage. Pheromone traps could also serve a training role by assisting in making up this gap in knowledge.

## References

- Abate, T. and J.K.O. Ampofo. 1996. Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* 41: 45–73.
- Adati, T. and S. Tatsuki. 1999. Identification of the female sex pheromone of the legume pod borer, *M. vitrata* and antagonistic effects of geometrical isomers. *Journal of Chemical Ecology* 25: 105–115.
- Afun, J.V.K., L.E.N. Jackai, and C.J. Hodgson. 1991. Calendar and monitored insecticide application for the control of cowpea pests. *Crop Protection* 10: 363–370.
- Bottenberg, H. 1995. Farmers' perceptions of crop pests and pest control practices in rainfed cowpea in Kano, Nigeria. *International Journal of Pest Management* 41: 195–200.
- Bottenberg, H. and B.B. Singh. 1996. Effect of neem leaf extract applied using the 'broom' method, on cowpea pests and yield. *International Journal of Pest Management* 42: 207–209.
- Bottenberg, H., M. Tamò, D. Arodokoun, L.E.N. Jackai, B.B. Singh, and O. Youm. 1997. Population dynamics and migration of cowpea pests in northern Nigeria: implications for integrated pest management. Pages 271–284 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Dreyer, H., J. Baumgärtner, and M. Tamò. 1994. Seed damaging field pests of cowpea (*Vigna unguiculata* L. Walp.) in Benin: occurrence and pest status. *International Journal of Pest Management* 40: 252–260.
- Jackai, L.E.N. 1995. Integrated pest management of borers of cowpea and beans. *Insect Science and its Application* 16: 237–250.
- Jackai, L.E.N., R.S. Ochieng, and J.R. Raulston. 1990. Mating and oviposition behavior in the legume pod borer, *Maruca testulalis*. *Entomologia Experimentalis et Applicata* 56: 179–186.
- Onyango, F.O. and J.P.R. Ochieng-Odero. 1993. Laboratory rearing of the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on a semi-synthetic diet. *Insect Science and its Application* 14: 719–722.
- Shanower, T.G., J. Romeis, and E.M. Minja. 1999. Insect pests of pigeonpea and their management. *Annual Review of Entomology* 44: 77–96.
- Singh, S.R., L.E.N. Jackai, J.H.R. Dos Santos, and C.B. Adalla. 1990. Insect pests of cowpeas. Pages 43–90 in *Insect pests of tropical legumes*, edited by S.R. Singh. John Wiley and Sons, Chichester, UK.
- Taylor, T.A. 1967. The bionomics of *Maruca testulalis* Gey. (Lepidoptera: Pyralidae), a major pest of cowpeas in Nigeria. *Journal of the West African Science Association* 12: 111–129.

## 2.4

# Evaluation of a novel technique for screening cowpea varieties for resistance to the seed beetle *Callosobruchus maculatus*

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### Abstract

A novel method for screening cowpea varieties for resistance to the postharvest insect pest *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) was compared to conventional screening techniques at the International Institute for Tropical Agriculture (IITA) laboratory in Ibadan, Nigeria. The new technique assesses seed resistance by measuring larval feeding activity via electronic sensors. Initial small-scale trials demonstrated that the method could be successfully applied in the laboratory with a potential saving in time and effort. The results were used to design a larger scale device that was able to screen 32 varieties of cowpea in 19 days using a methodology designed to identify only the most resistant cowpea varieties. Resistant varieties were identified but some problems were encountered with analysis of the results. Practical application of the technique for large-scale resistance screening is discussed.

### Introduction

The “biomonitor” technique, which uses ultrasonic transducers to detect sounds made by insect larvae feeding within seeds, was first described by Shade, Ferguson, and Murdock (1990). The sounds were counted automatically and used as a measure of insect feeding activity. Work at the Natural Resources Institute (NRI) (Devereau et al. 1999) has investigated the use of this technique for screening varieties of cowpea, *Vigna unguiculata* (L.) Walp., for resistance to the bean weevil *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). A device was developed which could simultaneously monitor eight cowpeas, each containing one insect, and a methodology was developed that detected significant differences between susceptible and resistant cowpea varieties by comparing feeding activity between 14 and 15 days after oviposition.

This paper describes two sets of trials undertaken at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, to further develop the technique. The first set of trials tested the ability of the technique to differentiate between four cowpea cultivars of different but known susceptibility. The time and effort required in comparison to the conventional screening method were also measured. The results were used to develop a

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larger scale device designed to be both faster than conventional screening and to require less staff time. This new device was tested in the second set of trials.

## Materials and methods

### Biomonitor system

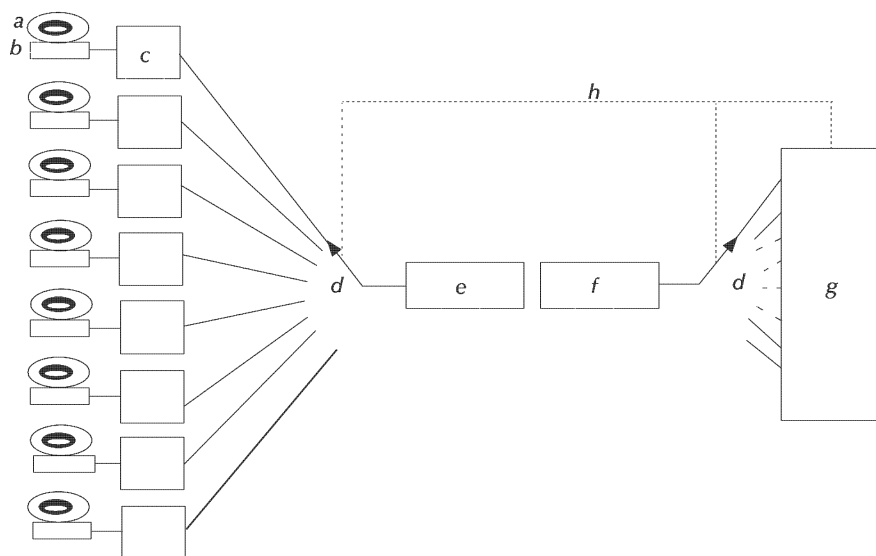
The system used in the first set of trials, shown schematically in Figure 1, was essentially the same as that used by Shade et al. (1990). It was housed in a small metal cabinet to help screen it from electrical interference. Each of the eight transducers was monitored for five minutes in turn, with the number of counts recorded automatically by a datalogger (Delta-T Devices, UK). Each sensor was therefore monitored for five out of every forty minutes, resulting in 36 readings being recorded from each sensor during the 24-hour monitoring period.

The system was placed in the culture room at IITA in which insects were being maintained for the trial. The temperature and relative humidity (rh) were  $26 \pm 2^\circ\text{C}$  and 70–80%.

For the second trial a larger scale biomonitor device was designed in an attempt to approach a practical level of throughput. The number of sensors was increased to 32 and the monitoring time was reduced to one minute per channel, giving a reading every 32 minutes from each sensor, i.e., 45 readings per 24 hours.

### Insect population

The laboratory population of *C. maculatus* that has been maintained at IITA since 1973 with regular crossing using local (Ibadan market) insects was used in the study. The insects were cultured on dry seeds of a susceptible cowpea cultivar, either Ife Brown, IT84D-715,



**Figure 1.** Block diagram of the biomonitor. a = infested cowpea; b = ultrasonic transducer; c = pre-amplifier; d = electronic switch; e = filter/amplifier; f = trigger; g = datalogger; h = clock signal (for electronic switch operation).



or IT82E-889. They were maintained under laboratory conditions until adults started to emerge after about 21–26 days. During the trials cultures were initiated every five days to provide a regular supply of 0–6-day-old adults.

### ***Cowpea varieties***

The four cowpea varieties used in the first trial were Ife Brown, IT87D-1827, TVu 2027, and IT84S-2246-4. Ife Brown is used at IITA as a susceptible reference, and TVu2027 was established by Singh and Jackai (1985) as the only accession in IITA's germplasm collection to show resistance. Ofuya and Credland (1995) measured the relative susceptibilities of cowpea varieties including Ife Brown, TVu2027, and IT84S-2246-4. The latter two had significantly longer ( $P \leq 0.05$ ) development periods than Ife Brown, but only TVu2027 showed lower percentage adult emergence. Recent work at IITA (L. Jackai, personal communication.) showed that IT87D-1827 was a susceptible variety and also suggested that TVu2027 showed less resistance to the strain of *C. maculatus* used at IITA than to other strains of the species.

Thirty-three cowpea varieties were used in the second trial. Conventional screening (Singh and Jackai 1985) was used to establish the relative susceptibility of 24 of these varieties, which are listed with the results in Table 4. The 32 varieties screened using the biomonitor included all those screened conventionally with the exception of variety Ngouya Local, as well as varieties IT-534, IT81D-994, IT433-1, IT82E-25, TVu1509, TVu6867, Vicam1, TVu11011, and TVu3000.

### ***Infestation method***

Cowpeas were brought out of cold storage and allowed to return to room temperature in the laboratory 24 hours before infestation. Clean, unbroken cowpeas were placed in a single layer inside labelled plastic CORNING® 35 mm tissue culture dishes (35 × 10 mm) with one cowpea variety per dish. The dishes were then introduced into a culture jar containing hundreds of 0–6-day-old adults for 1 hour between 9am and 10am for oviposition (Ofuya and Credland 1995). Dishes were removed from the culture after 55 minutes and the insects removed using a vacuum generator during the remaining 5 minutes.

Excess eggs were removed from the exposed cowpeas after 24 hours to leave one egg on the cheek of each seed. Cowpeas with no eggs or with eggs laid in the wrong area were discarded. The remaining cowpeas were left in the laboratory for 14 days, after which they were ready for evaluation on the biomonitor. At this time the seeds were inspected to ensure that the eggs had hatched and that the larvae had penetrated the seed. Seeds not showing penetration were discarded.

### ***Experimental design***

A randomized block design was used for the first trial. Cowpeas of all four varieties were exposed simultaneously to oviposition on consecutive days, using fresh cowpeas each day, to form a series of infested cowpea sets. Exactly 14 days after oviposition on the first cowpea set, two replicates of each variety were selected, placed at random on one of the eight biomonitor sensors and monitored for 24 hours. After 24 hours they were removed, the data collected, and the process repeated using the next set of cowpeas. This was repeated for eight days. The data were analyzed after five days (10 replicates of each treatment) and eight days (16 replicates of each treatment) with each day treated as a block to account for variations in, for example, parent insect age, laboratory conditions, etc.

A randomized design was used for the second set of trials using the 32-channel device. This device was designed to detect significant differences between eight cowpea varieties during one 24-hour monitoring period using only four replicates of each variety, so no blocking was required. The susceptible and resistant references, Ife Brown and TVu2027, were included in each set of eight cowpeas. All eight varieties were exposed to oviposition and monitored as before, with the cowpeas placed at random on the 32 sensors.

### Data analysis

The mean and peak activity over 24 hours, as counts per five minutes or counts per minute for the 32-channel device, were calculated for each replicate. The statistical package SPSS for Windows was used to analyze this data graphically and using ANOVA, for which a  $\log_{10}$  transformation was used. Contrasts between each cowpea variety and the reference varieties were then made.

### Time and effort requirement

The time and staff effort required for screening using both conventional and biomonitor techniques were estimated by IITA staff as the trials were conducted.

## Results

### First trials

#### Screening results

Figures 2 and 3 show the mean and peak feeding activity for each of the four cowpea varieties after five days, i.e., with 10 replicates per treatment. The susceptible varieties showed much larger ranges of feeding activity than the resistant varieties. ANOVA performed on these data showed significant differences ( $F_{(3,32)} = 25.26, P < 0.001$ , and  $F_{(3,32)} = 22.49, P < 0.001$ ) due to cowpea variety for mean and peak feeding activity, respectively.

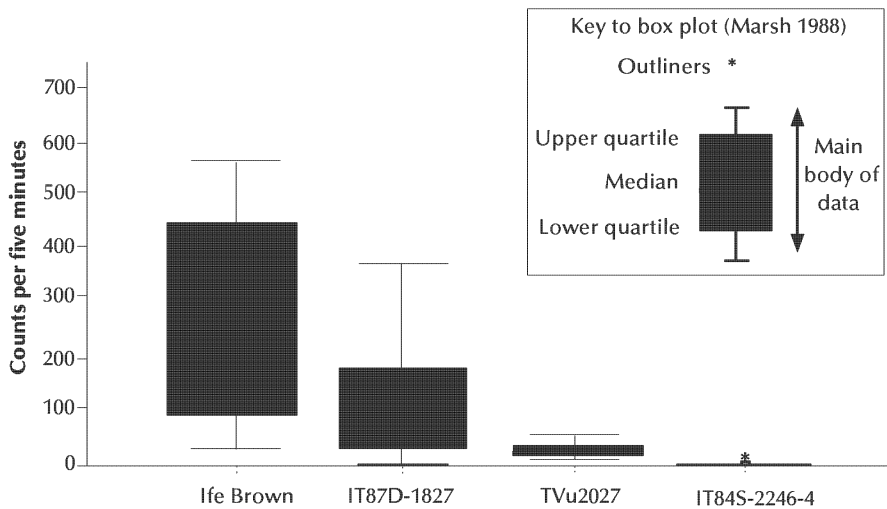
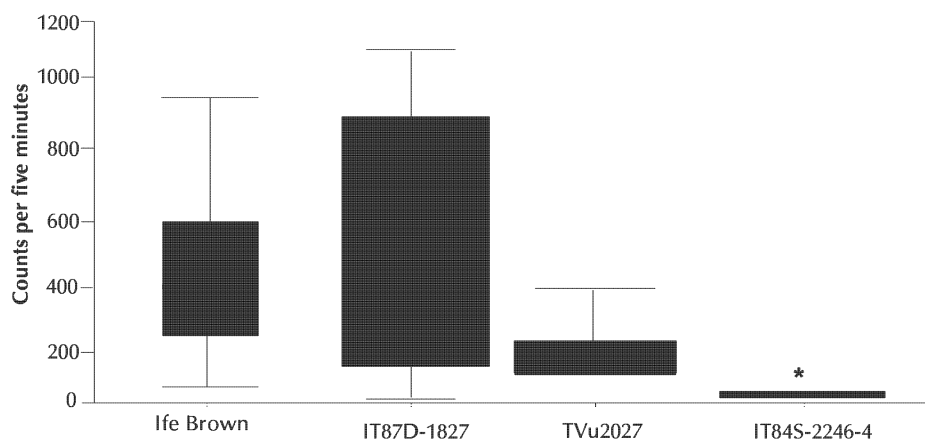


Figure 2. Mean activity (counts per five minutes) after five days for the four cowpea varieties in the first trials.



**Figure 3.** Mean peak activity (counts per five minutes) after five days (key as in Figure 2).

Table 1 records the mean and peak feeding activity after five days, and shows that varieties IT84S-2246-4 and TVu2027 exhibited significantly lower mean feeding activity ( $t_{32} = 8.43$ ,  $P < 0.00001$ ; and  $t_{32} = 3.38$ ,  $P = 0.002$ , respectively) when compared with Ife Brown, while IT87D-1827 showed a difference which was less significant ( $t_{32} = 2.32$ ,  $P = 0.026$ ). For peak feeding activity, varieties IT84S-2246-4 and TVu2027 again showed significantly lower activity ( $t_{32} = 7.67$ ,  $P < 0.00001$ ; and  $t_{32} = 2.78$ ,  $P = 0.009$ , respectively), but IT87D-1827 did not differ from Ife Brown.

The larva in one replicate of IT87D-1827 was inactive during the whole monitoring period, in contrast to all other individuals in cowpeas of that variety. This had a large effect on the results, reducing the mean activity feeding values for variety IT87D-1827 by 13 counts per five minutes and peak activity feeding values by 46.4 counts.

Table 2 shows the mean and peak feeding activity after eight days, i.e., from 16 replicates per treatment. The results are very similar to those after five days.

### ***Time and effort requirements***

Both methods required an initial 28 days for preparation of insect cultures. The conventional method then required a further 64 days to complete, while the biomonitor took 19 days.

The conventional bioassay and the biomonitor technique needed almost identical amounts of staff effort, 21.1 and 21.2 hours, respectively, to reach a conclusion. This included preparation of insect cultures, oviposition, and collection and analysis of results.

## **Second trials**

### ***Screening results***

The screening methodology used was designed to identify only the most resistant varieties, allowing susceptible varieties to be rapidly eliminated and resistant varieties to be subjected to further, more detailed analysis.

The number of replicates needed to show significant differences between varieties was estimated using a formula given by Sokal and Rohlf (1981). This suggested that four

**Table 1.** Mean and peak activity (counts per five minutes) and  $\log_{10}$  transformation of mean and peak activity for four cowpea varieties tested at IITA. Standard error of the difference between transformed mean and mean peak activity = 0.198 and 0.18, respectively.

| Cowpea variety | Mean activity | Transformed mean activity | Peak activity | Transformed peak activity |
|----------------|---------------|---------------------------|---------------|---------------------------|
| Ife Brown      | 248.2         | 2.21                      | 445.5         | 2.56                      |
| IT87D-1827     | 119.7         | 1.75                      | 425.4         | 2.34                      |
| TVu2027        | 37.6          | 1.54                      | 123.4         | 2.06                      |
| IT84S-2246-4   | 4.97          | 0.54                      | 21.5          | 1.18                      |

**Table 2.** Mean activity (counts per five minutes) and  $\log_{10}$  transformation of mean and peak activity for each cowpea variety after eight days. Standard error of the difference between transformed means = 0.198 and 0.18, respectively.

| Cowpea       | Mean activity (counts per five minutes) | Transformed mean activity | Mean peak activity (counts per five minutes) | Transformed mean peak activity |
|--------------|---|---------------------------|--|--------------------------------|
| Ife Brown    | 234.2                                   | 2.12                      | 412.6  | 2.49                           |
| IT87D-1827   | 99.3                                    | 1.66                      | 329.1  | 2.19                           |
| TVu2027      | 45.6                                    | 1.59                      | 182.8  | 2.13                           |
| IT84S-2246-4 | 26.9                                    | 0.70                      | 54.9   | 1.25                           |

replicates would be required to show a significant difference at the 5% level between the mean activities of Ife Brown and the most resistant variety, IT84S-2246, in 90% of experiments, and this was selected as the criterion or threshold for indicating resistance in the second trial. By increasing the number of biomonitor sensors to 32 and using four replicates, eight cowpea varieties could be monitored per day.

Figures 4a–4e show the boxplots of mean feeding activity from the five sets of cowpeas monitored during the second trial. There were clearly contrasting levels of activity, with many varieties showing very low activity, suggesting resistance. Peak feeding activity showed a very similar pattern for most varieties with the exception of IT82E-25 (Figure 4d) which showed a relatively higher range of peak activity than mean activity.

Table 3 shows the mean and peak feeding activity for the set of cowpeas shown in Figure 4a. ANOVA for this data showed significant differences due to cowpea variety ( $F_{(7,24)} = 6.727$ ,  $P < 0.001$  and  $F_{(7,24)} = 6.73$ ,  $P < 0.001$ , respectively) for the  $\log_{10}$  transformed mean and peak data. Only variety IT82E-716 showed significantly lower feeding activity at the 5% level than Ife Brown, and was therefore identified as the only resistant variety in the batch. The other sets of cowpeas, i.e., those shown in Figures 4b–4e, were similarly analyzed. Varieties IT89KD-245, IT-534, IT84S-2246, and IT81D-994 all showed significant lower feeding activity than Ife Brown at the 5% level and were therefore identified as resistant.

Table 4 shows the results of the conventional screening trials. Not all of the varieties that were identified as resistant by the biomonitor method, i.e., those that showed a significantly lower level of activity than Ife Brown, were screened conventionally. Those that were included IT89KD-245, IT84S-2246, and IT82E-716, and these varieties were all

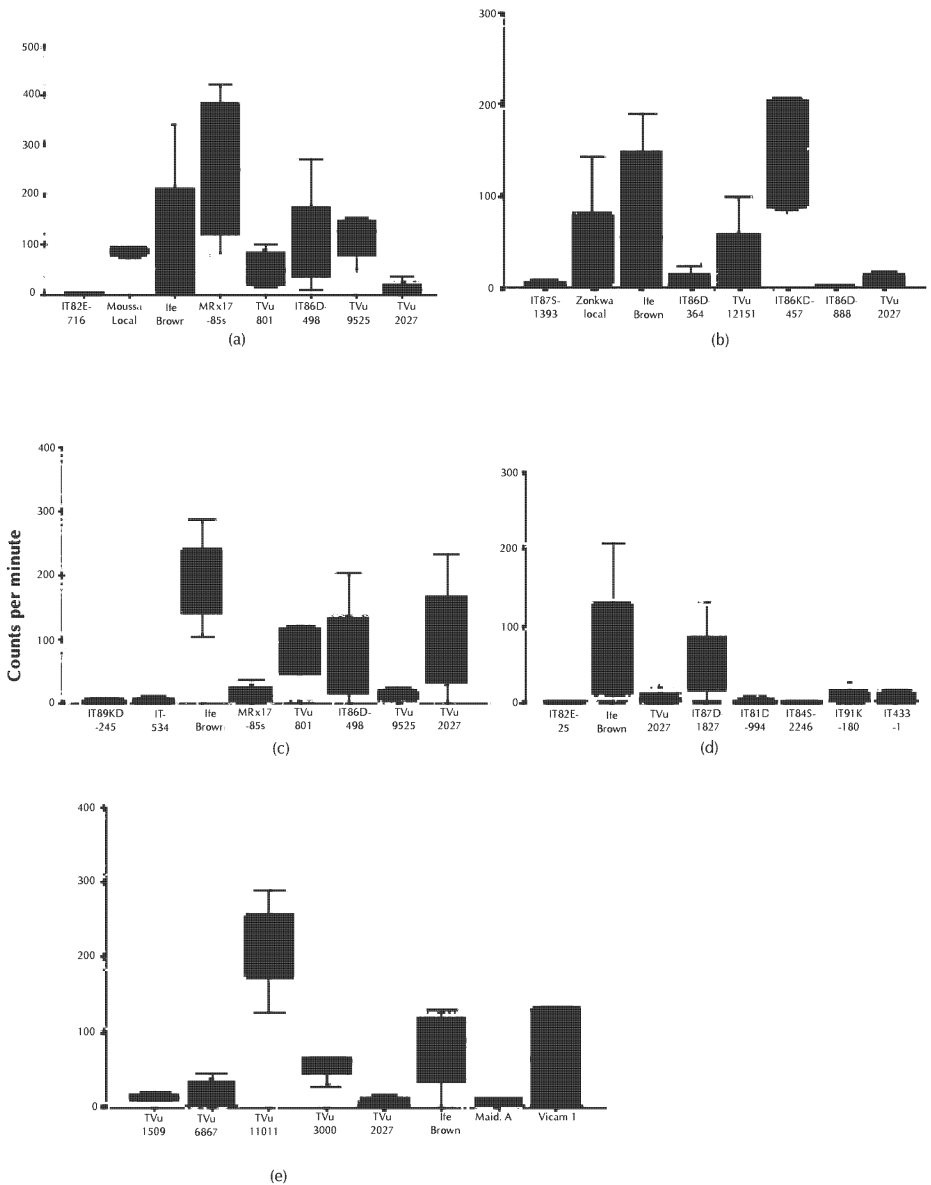
**Table 3.** Mean and peak activity for four replicates of eight cowpea varieties between 14 and 15 days after oviposition. S.e.d. between transformed means = 0.41 and 0.38, respectively.

| Cowpea variety | Mean activity<br>(counts per<br>minute) | Transformed<br>mean activity | Peak activity<br>(counts per<br>minute) | Transformed<br>peak activity |
|----------------|---|------------------------------|---|------------------------------|
| MRx17-85S      | 250.9                                   | 2.32                         | 577.5                                   | 2.70                         |
| TVu 9525       | 112.4                                   | 2.01                         | 266.0                                   | 2.38                         |
| Ife Brown      | 107.7                                   | 1.19                         | 185.0                                   | 1.69                         |
| IT86D-498      | 104.4                                   | 1.75                         | 176.0                                   | 2.07                         |
| Moussa Local   | 84.4                                    | 1.92                         | 162.8                                   | 2.21                         |
| TVu 801        | 52.2                                    | 1.59                         | 114.8                                   | 1.97                         |
| TVu 2027       | 12.1                                    | 0.79                         | 48.3                                    | 1.26                         |
| IT82E-716      | 1.08                                    | $3.4 \times 10^{-2}$         | 4.0                                     | 0.46                         |

**Table 4.** Relative susceptibility of cowpea varieties determined by conventional bioassay at IITA. Total development time (TDT) = mean development time per insect. Growth index (GI) = ([in % adult emergence]/TDT). Susceptibility index = (GI test material/GI Ife Brown)  $\times$  100.

| Cowpea variety | Adult<br>emergence<br>(%) | Total<br>development<br>time | Growth<br>index | Susceptibility<br>index |
|----------------|---------------------------|------------------------------|-----------------|-------------------------|
| Ife Brown (SC) | 100.0                     | 23.1                         | 0.20            | 100.0                   |
| Moussa L.      | 100.0                     | 23.7                         | 0.19            | 97.4                    |
| IT89KD-457     | 100.0                     | 24.0                         | 0.19            | 96.3                    |
| TVu 13731      | 100.0                     | 24.1                         | 0.19            | 95.7                    |
| MRx17-85S      | 100.0                     | 24.3                         | 0.19            | 95.1                    |
| IT91K-180      | 100.0                     | 24.4                         | 0.19            | 94.7                    |
| TVu 9525       | 95.0                      | 24.1                         | 0.19            | 94.6                    |
| IT86D-888      | 100.0                     | 24.8                         | 0.19            | 93.3                    |
| Ngouya L.      | 100.0                     | 25.0                         | 0.18            | 92.5                    |
| MRx10-85S      | 79.2                      | 23.9                         | 0.18            | 91.8                    |
| IT86D-400      | 87.5                      | 24.6                         | 0.18            | 90.7                    |
| TVu 12151      | 87.5                      | 24.9                         | 0.18            | 89.7                    |
| TVu 801        | 83.3                      | 24.7                         | 0.18            | 89.2                    |
| Zonkwa L.      | 91.7                      | 25.5                         | 0.18            | 88.8                    |
| IT87D-1827     | 81.3                      | 27.0                         | 0.16            | 82.2                    |
| IT89KD-245     | 100.0                     | 30.9                         | 0.15            | 74.6                    |
| IT86D-364      | 62.5                      | 29.0                         | 0.14            | 71.0                    |
| IT82E-716      | 83.8                      | 31.8                         | 0.14            | 70.4                    |
| Maiduguri B    | 59.8                      | 30.2                         | 0.14            | 69.1                    |
| IT87D-697-2    | 90.0                      | 33.3                         | 0.13            | 67.6                    |
| IT84S-2246     | 81.7                      | 33.4                         | 0.13            | 66.1                    |
| TVu 2027       | 68.8                      | 36.2                         | 0.11            | 57.5                    |
| IT86D-498      | 23.8                      | 32.0                         | 0.09            | 45.8                    |
| IT87S-1393     | 26.7                      | 39.0                         | 0.09            | 43.8                    |

in the lower half of Table 4, i.e., they were less susceptible, though they showed higher susceptibility than variety TVu2027. Other varieties with low susceptibility in Table 4 were not shown to be resistant by the biomonitor method however. Figure 4 shows that most of the varieties in the lower half of Table 4 showed low levels of feeding activity in



**Figure 4.** Boxplots of mean feeding activity (counts per minute) for five sets of eight cowpea varieties monitored between 14 and 15 days after oviposition (key as in Figure 2).

comparison to insects in Ife Brown, but they were not found to be significantly different due to one or two replicates of Ife Brown being inactive during monitoring of each set of cowpeas. The other main disagreements were variety IT86D-498, which was the second most resistant variety according to the conventional screening but showed high levels of feeding activity on the biomonitor (Figure 4a), and varieties IT86D-888 and IT91K-180 which were of high susceptibility according to conventional screening but showed low levels of feeding activity on the biomonitor (Figures 4b and 4d, respectively).

### ***Time and effort requirements***

The 24 cowpea varieties screened by the conventional technique required approximately 95 hours of effort and took 64 days to complete. The biomonitor by contrast required 36 hours of effort and took 19 days to reach a conclusion for 32 varieties.

## **Discussion**

The first trial showed clear differences between feeding activity in the cowpea varieties using the biomonitor method. These corresponded to the known susceptible status of these varieties. It also confirmed that variety TVu2027, when tested using IITA insects, was not the most resistant variety.

There was however the problem caused by variability of the insects' development rates. The monitoring period of 24 hours after 14 days' development was designed to correspond to the highly active fourth instar larvae in susceptible varieties and provide a significant contrast to the low activity of larvae in resistant varieties. However, for variety IT87D-1827, the fourth instar started or stopped during the monitoring period in some cases and caused some of the moult period before the fourth instar or the pupal period after the fourth instar to be monitored. As no feeding activity occurs during these periods of development this caused the mean activity to be reduced. Including the peak activity in the analysis helped to identify when this had happened.

One replicate of IT87D-1827 showed no larval feeding activity throughout monitoring, and this had a relatively large effect on the mean feeding activity levels. This could have been caused by the fourth instar finishing before monitoring began or starting after it had finished for this replicate, or by the larva dying between penetration and monitoring for reasons which may have been related or unrelated to susceptibility. Because of this uncertainty, it is difficult to justify removing the point from the analysis, especially as a similar situation in a resistant variety would have been easily overlooked or attributed to resistance.

A similar amount of effort was required to conduct both the biomonitor and conventional techniques during the first trial. The biomonitor was much faster though, reaching a conclusion in less than a third of the time of the conventional method. This is a distinct advantage as cowpea breeders need to know the resistant status of new varieties as soon as possible after harvest. Another advantage of the new method was that data is collected automatically and downloaded directly to a computer for analysis, which is less laborious and less prone to error than manually recorded results.

The methodology for the second trial was designed to be as fast as the first trial but to require less effort. This was achieved by increasing the capacity of the biomonitor to 32 channels and by reducing the number of replicates to four, a level that would only identify the most resistant cowpea varieties. The varieties identified as resistant in the second trial

were among those identified as having lower susceptibility by conventional screening, but other varieties shown to be resistant by conventional screening were not identified by the biomonitor technique. The problem in most cases was that, despite some varieties showing consistently low levels of feeding activity typical of resistance, one or two replicates of the susceptible variety Ife Brown to which they were being compared showed no feeding activity during monitoring despite their known susceptibility. Removing these inactive replicates from the analysis would allow more varieties to be identified as resistant, but, as discussed above, this is hazardous as the cause of the inactivity was not determined and could have been due to a number of reasons. When such low feeding activity occurs in the susceptible reference it would be sensible to repeat the trial.

Three cowpea varieties, IT86D-498, IT86D-888, and IT91K-180, showed complete disagreement between the biomonitor results and conventional screening. It was possible that the results from either method were incorrect—given the number of tests being made and the natural variability in susceptibility there will always be some errors or anomalies in screening results. Relatively susceptible varieties being identified in error as resistant is not a serious problem provided it does not occur too often, as the more detailed screening that should follow the initial rapid screen will identify these varieties. Failing to detect a resistant variety is more serious however. The methodology needs to be designed to minimize the risk of this happening. It is also possible that resistance or susceptibility was not manifest in these varieties in the same way as in other varieties. Further, detailed investigation will be needed to establish whether this is the case or not.

In conclusion, although the biomonitor technique was successful on a small scale with clear advantages of reduced time and effort over conventional screening, further work is necessary to ensure its reliability for large-scale screening. The degree of replication used was too small given the amount of variability which occurred, so this should be increased and the criterion used for identifying resistance revised. It may also be necessary to modify the monitoring period to take into account variability in larval development time. To achieve these modifications while retaining a practical level of throughput will require a device with a larger capacity, and this is being addressed by current work at NRI.

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## References

- Devereau, A.D., P.F. Credland, J. Appleby, and L. Jackai. 1999. Rapid screening of grain for insect resistance. Pages 18–26 in *Proceedings of the 7<sup>th</sup> International Working Conference on Stored-Product Protection*, edited by J. Zuxun, L. Quan, L. Yongsheng, T. Xianchang and G. Lianghua, 14–19 October 1998, Beijing. Sichuan Publishing House of Science and Technology, Chengdu, P.R. China.
- Marsh C. 1988. *Exploring data*. Polity Press, Cambridge, Massachusetts, USA. 385 pp.
- Ofuya, T.I. and P.F. Credland. 1995. Responses of three populations of the seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), to seed resistance in selected varieties of cowpea, *Vigna unguiculata* (L.) Walp. *Journal of Stored Product Research* 31: 17–27.



- Sokal, R.R. and F.J. Rohlf. 1981. Biometry. 2<sup>nd</sup> edition. W.H. Freeman and Company, New York, USA. 859 pp.
- Shade, R.E., E.S. Fergason, and L.L. Murdock. 1990. Detection of hidden insect infestations by feeding generated ultrasonic signals. *American Entomologist* 36(3): 231–234.
- Singh, S.R. and L.E.N. Jackai. 1985. Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. Pages 217–231 *in* Cowpea research production and utilization, edited by S.R. Singh and K.O. Richie. John Wiley and Sons, New York, USA.

## Detection of fumonisin B1 in cowpea seeds

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### Abstract

Cowpeas (*Vigna unguiculata* [L.] Walp) are important nutritious legume crops for many subsistence farmers and rural communities. In tropical and subtropical Africa, cowpeas are often stored at high relative humidities and high ambient temperatures and are susceptible to fungal contamination. Some of these fungi produce mycotoxins, which can have adverse effects on the health of both farm animals and humans. Eight cowpea seed samples from four different cultivars were analyzed for the *Fusarium* mycotoxins, fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>. Samples were extracted with methanol/water (70:30) and cleaned up on strong anion exchange solid phase extraction cartridges. High-performance liquid chromatography with precolumn derivatization using o-phthalaldehyde was used for the detection and quantification of fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>. The analyses revealed that all eight samples were contaminated with fumonisin B<sub>1</sub> at levels ranging between 81 and 1002 ng/g, whereas fumonisins B<sub>2</sub> and B<sub>3</sub> were not detected. It is believed that this is the first report of the natural occurrence of fumonisin B<sub>1</sub> in cowpea seeds. Since none of the known fumonisin-producing fungi were isolated from the cowpea seeds, it is necessary to identify which species are responsible for toxin production.

### Introduction

Cowpeas (*Vigna unguiculata* [L.] Walp.) are regarded as popular and important indigenous African legume crops by many rural communities living in less developed countries of tropical and subtropical Africa. They are grown as a pulse, vegetable, fodder, and as a cover crop (Ushamalani et al. 1998). Cowpeas are mainly consumed as a favorite food-stuff in the form of dried seeds, either as flour or split (Johnson and Raymond 1964; van Wyk and Gericke 2000). They are a good source of carbohydrates, vitamins, and protein, providing more than half of plant protein in human diets in some areas of the semiarid tropics (Singh et al. 1997; Tuan and Phillips 1992).

It is well known, however, that cowpea seeds are susceptible to fungal contamination when poorly stored at high relative humidities and high ambient temperatures (Esuruoso 1975; Hitokoto et al. 1981; Seenappa et al. 1983). It is also under these conditions that certain fungi may produce toxic secondary metabolites, namely mycotoxins (van Warmelo 1967). The ingestion of mycotoxins in contaminated agricultural products can lead to detrimental health problems for humans and farm animals (Desjardins and Hohn 1997; Moss 1996). Mycotoxins exhibit properties of acute, subacute, and chronic toxicity, leading to interference with the functioning of various body systems (Coker 1994;

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Saber et al. 1998). Furthermore, they are capable of causing mutations and deformities in developing embryos (Saber et al. 1998).

Fumonisin is the most recently characterized mycotoxin that has major significance in human health (Moss 1996). They are primarily produced by *Fusarium verticillioides* (Sacc.) Nirenberg, *Fusarium proliferatum* (Matsushima) Nirenberg, and *Fusarium nygamai* Burgess and Trimboli (Coker 1994; Marasas 1994). Fumonisin is acutely toxic to the liver and kidneys (Desjardins and Hohn 1997). They are amino polyalcohols that inhibit the activity of sphingosine N-acetyltransferase that leads to the accumulation of toxic sphingoid bases (Desjardins and Hohn 1997). Various fumonisins have been isolated and characterized (Musser 1996), of which fumonisin B<sub>1</sub> (FB<sub>1</sub>), fumonisin B<sub>2</sub> (FB<sub>2</sub>), and fumonisin B<sub>3</sub> (FB<sub>3</sub>) are the most important analogs found in contaminated maize (Shepherd et al. 1996). FB<sub>1</sub> and FB<sub>2</sub> are known to be toxicologically significant. FB<sub>1</sub> has been known to cause leukoencephalomalacia (LEM), a fatal brain disease in horses (Coker 1994; Desjardins and Hohn 1997; Marasas 1996) and pulmonary edema syndrome (PES) in pigs (Marasas 1996). FB<sub>1</sub> is also toxic to the central nervous system, liver, pancreas, kidneys, and lungs in numerous animal species (Coker 1994). Furthermore, it is a cancer promoter and initiator in rat liver, hepatotoxic to horses, pigs, rats, and vervet monkeys, and phytotoxic to several plants (Marasas 1995; 1996). Lastly, FB<sub>1</sub> has been statistically linked to the incidence of human esophageal cancer rates in Transkei, South Africa, and China (Marasas 1996). FB<sub>1</sub> has been classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC) which considers it to be possibly carcinogenic to humans (Vainio et al. 1993).

There are various reports concerning mycotoxins associated with legume seeds, including chickpea (*Cicer arietinum* L.) (Ahmad and Singh 1991), lupine (*Lupinus* spp. L.) (Abdel-Hafez 1984), pea (Saber et al. 1998), and various types of beans (El-Kady et al. 1991; Saber 1992; Tseng and Tu 1997). There is, however, little literature regarding cowpea seeds and mycotoxins. Seenappa et al. (1983) found cowpea samples to be susceptible to *Aspergillus parasiticus* Speare infection, and in subsequent aflatoxin contamination. There is no report, however, concerning the presence of fumonisins in cowpea seeds.

This paper deals with the detection and quantification of the *Fusarium* toxins, specifically FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> in cowpea seeds.

## Materials and methods

### Apparatus

- Liquid chromatography—Waters 6000 A pump (Waters Corp., Milford, MA 01757, USA) and Rheodyne injector.
- Fluorescence detector—Waters Fluorescence 474 set at 335 nm (excitation) and 440 nm (emission) (Waters Corp., Milford, MA 01757, USA).
- Column—Phenomenex Ultracarb 5 ODS (20) (150 × 4.6 mm id.).
- Integrator—Borwin Chromatography Software 1.22 (JMBS Developments, France).
- Solid-phase extraction (SPE) columns—Chromabond® Strong anion exchange (SAX) cartridges, 6 ml capacity, containing 500 mg SiOH (Machery-Nagel, Düren, D-52313, Germany).
- SPE manifold—12-place vacuum manifold (Lida).

- Reacti-Therm™ Heating module (Pierce, Rockford, IL 61105, USA).
- Reacti-Vap™ Evaporator (Pierce, Rockford, IL 61105, USA).

### **Reagents**

Fumonisin B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> standards were obtained from PROMEC, Medical Research Council, Tygerberg, South Africa. All other reagents and solvents were obtained from Merck (Darmstadt, D-64271, Germany).

### **Seed samples**

Four cultivars (Bechwana White, Glenda, Iron Grey, and Rhino) were used. Approximately 100 g each of cowpea seeds were received from A. Haasbroek from the Agricultural Research Council (ARC), Grain Crops Institute, Potchefstroom, South Africa. The seeds were harvested from experimental fields at the institute and were kept in cold storage (approximately 5 °C) for four months prior to the analyses.

### **Determination of seedborne fungi**

One hundred seeds were randomly chosen from each sample. Prior to plating, 50 seeds from each sample were surface sterilized in 1% sodium hypochlorite for 1 min. The remaining 50 seeds from each sample were not surface sterilized. The seeds were plated on malt extract agar (MEA) consisting of 15 g malt extract (diastase free), 17 g Bacto agar, 1000 ml distilled H<sub>2</sub>O, and 0.125 g novobiocin. The plates were incubated at 25 °C for two to seven days. The fungi were isolated, identified with the aid of various references (Samson et al. 1981; Nelson et al. 1983; Watanabe 1994), and recorded. The *Fusarium* spp. were identified by Dr J.P. Rheeder of PROMEC, Medical Research Council, Tygerberg, South Africa.

### **Sample preparation, extraction, and clean up**

Cowpea seeds of the four different cultivars were used as samples. The sample extraction and clean up were based on the method described by Sydenham et al. (1992) and were carried out at the Department of Botany, University of Pretoria, Pretoria, South Africa. Approximately 50 g of seeds from each sample were ground using a coffee grinder and 20 g of the ground seeds weighed. After adding 100 ml 70% (v/v) methanol, the ground samples were homogenized for 3 min at 5000 rpm using a hand-held mixer. The samples were then centrifuged for 10 min at 4000 rpm and filtered through Whatman No. 4 filter paper. The pH of the filtrate was measured and adjusted with 0.1 M NaOH to between pH 5.8 and 6.5. Clean up and extraction of the filtrate were carried out on strong anion exchange (SAX) cartridges attached to a solid phase extraction (SPE) manifold. Prior to adding 10 ml of the filtrate, the SAX cartridges were conditioned by washing successively with 5 ml 100% methanol followed by 5 ml 70% (v/v) methanol, whilst maintaining a flow rate of 1 ml/min. Cartridges were then washed with 5 µl 70% (v/v) methanol and 3 ml 100% methanol. This was followed by elution with 10 µl 1% (v/v) methanolic acetic acid at a flow rate of 1 ml/min and the eluate collected in vials. Eluates then were evaporated to dryness in vials on a Reacti-Therm heating module and evaporator at 50 °C under a slight stream of nitrogen (AFROX). The collection vials were washed with methanol and the additional methanol was evaporated until a dry residue formed. The dry residues were maintained at 4 °C until used for high performance liquid chromatography.

### High Performance Liquid Chromatography (HPLC)

The HPLC analyses were undertaken at PROMEC, Medical Research Council, Tygerberg, South Africa. A derivatization agent, *o*-phthaldialdehyde (OPA), was added to both the standards and samples prior to HPLC. This is necessary since fumonisins are unable to absorb either UV or visible light and are unable to fluoresce. OPA derives the fluorescent products from the fumonisins (Sydenham et al. 1992). OPA (225 µl) was added to 25 µl of the standard and 10 µl was injected into the HPLC, whilst 150 ml OPA was added to 100 µl of the sample (which had been redissolved in 200 µl CH<sub>3</sub>CN:H<sub>2</sub>O) and 50 µl was injected into the HPLC (Sydenham et al. 1992).

### Results and discussion

The percentage of fungi isolated from each sample was higher in the untreated seeds than in the surface-sterilized seeds (Table 1). The most fungi was isolated from Iron Grey (98% infection) followed by Rhino with 94% infection, Bechwana White with 92% infection, and Glenda with 88% infection. In the surface-sterilized seeds, the most fungi were isolated from Rhino (68% infection) followed by Iron Grey (52% infection). Glenda and Bechwana White had low counts of fungal colonies (8 and 4% infection, respectively). The most common fungi found included members of the genera, *Aspergillus* and *Phoma*, present in both surface-sterilized and untreated seeds in all four samples. *Aspergillus glaucus* Link ex. Gray was the predominant species, present in three samples, followed by both *Aspergillus flavus* Link ex. Fries and *Aspergillus niger* van Tieghem. Seenappa et al. (1983) reported that all cowpea samples analyzed were susceptible to *Aspergillus* infection and subsequent aflatoxin production.

**Table 1. Percentages of fungi isolated from four cultivars of cowpea seeds.**

| Fungi                          | Cultivar       |                |                |    |       |    |           |    |
|--------------------------------|----------------|----------------|----------------|----|-------|----|-----------|----|
|                                | Glenda         |                | Bechwana White |    | Rhino |    | Iron Grey |    |
|                                | + <sup>a</sup> | - <sup>b</sup> | +              | -  | +     | -  | +         | -  |
| <i>Aspergillus flavus</i>      | 4              | 10             | -              | 26 | -     | -  | -         | 2  |
| <i>A. glaucus</i>              | -              | 4              | -              | -  | 8     | 8  | 40        | 68 |
| <i>A. niger</i>                | -              | 18             | -              | 14 | -     | -  | 4         | 2  |
| <i>Chaetomium</i> sp.          | 2              | 2              | -              | -  | -     | -  | 2         | 2  |
| <i>Cladosporium</i> sp.        | -              | 18             | -              | 14 | -     | -  | 2         | -  |
| <i>Diplodia</i> sp.            | -              | 4              | -              | -  | -     | -  | -         | -  |
| <i>Fusarium chlamydosporum</i> | -              | -              | -              | 2  | -     | -  | -         | -  |
| <i>F. equiseti</i>             | -              | 2              | -              | -  | 2     | 10 | -         | -  |
| <i>F. graminearum</i>          | -              | -              | -              | -  | -     | 2  | -         | -  |
| <i>F. sambucinum</i>           | -              | -              | -              | -  | -     | 2  | -         | -  |
| <i>F. scirpi</i>               | -              | -              | -              | -  | 6     | -  | -         | -  |
| <i>F. subglutinans</i>         | -              | -              | -              | 2  | -     | -  | -         | -  |
| <i>Penicillium</i> sp.         | -              | 4              | -              | -  | -     | 32 | -         | 16 |
| <i>Phoma</i> sp.               | 2              | 14             | 4              | 28 | 52    | 36 | 2         | -  |
| <i>Trichothecium roseum</i>    | -              | 2              | -              | 2  | -     | -  | -         | 2  |
| Other                          | -              | 10             | -              | 4  | -     | 4  | 2         | 6  |
| Total % infection              | 8              | 88             | 4              | 92 | 68    | 94 | 52        | 98 |

<sup>a</sup>surface-sterilized seeds, <sup>b</sup>untreated seeds.

Six *Fusarium* species were isolated; *Fusarium equiseti* (Corda) Sacc. appeared to be dominant. Four of these *Fusarium* species were present in the Rhino seeds, two in the Bechwana White sample, and one in the Glenda sample. An interesting occurrence can be noted here. While the most important fumonisin-producing species are *F. verticillioide*s and *F. proliferatum* (Coker 1994; Marasas 1994), neither of these two species were isolated from the samples. However, Esurioso (1995) recorded *F. verticillioide*s on nearly all cowpea samples (81) examined. Other *Fusarium* species known to produce high concentrations of other mycotoxins but not fumonisins, including *F. equiseti*, *F. sambucinum* Fuckel, and *F. subglutinans* (Wollenw. and Reink.) Nelson, Toussoun, and Marasas were isolated. Further research is required to identify the fungal species present on cowpea seeds responsible for the fumonisin production. Other fungal genera isolated from the samples included *Chaetomium*, *Cladosporium*, *Penicillium*, and *Trichothecium* spp. *Penicillium* spp. are also known to produce mycotoxins including ochratoxins (Moss 1996) and citrinin (Pitt 1998).

From the eight samples analyzed for *Fusarium* toxins, specifically FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub>, FB<sub>1</sub> was found to be present in all the samples (Table 2), while FB<sub>2</sub> and FB<sub>3</sub> were not detected. The highest concentration of FB<sub>1</sub> was found in the Rhino A cultivar (1002 ng/g), followed by Rhino B (213 ng/g), Bechwana White A (178 ng/g), Glenda A and B (161 ng/g), and Iron Grey A (127 ng/g). Levels below 100 ng/g were detected in Bechwana White B and Iron Grey B. This is the first report of the natural occurrence of FB<sub>1</sub> on cowpea seeds.

Since large quantities of cowpea seeds are produced and consumed in tropical and subtropical countries (Seenappa et al. 1983) and in the light of the various toxicological consequences as a result of fungal mycotoxin contamination, a potential health risk exists for both humans and animals. It is thus essential that care be taken when seeds are stored such that fungal infestation and subsequent mycotoxin production can be effectively controlled and prevented. There are various reports concerning the antifungal activity of essential plant oils (Adegoke and Odesola 1996; National Research Council 1992) which can be used as an alternative approach to controlling and preventing fungal contamination of cowpea seeds.

**Table 2. Fumonisin concentrations in cowpea seed cultivars.**

| Cultivar         | Fumonisin concentration (ng/g) |                 |                 |
|------------------|--------------------------------|-----------------|-----------------|
|                  | FB <sub>1</sub>                | FB <sub>2</sub> | FB <sub>3</sub> |
| Bechwana White A | 178                            | 0               | 0               |
| Bechwana White B | 81                             | 0               | 0               |
| Glenda A         | 161                            | 0               | 0               |
| Glenda B         | 161                            | 0               | 0               |
| Iron Grey A      | 127                            | 0               | 0               |
| Iron Grey B      | 99                             | 0               | 0               |
| Rhino A          | 1002                           | 0               | 0               |
| Rhino B          | 213                            | 0               | 0               |

## References

- Abdel-Hafez, S.I.I. 1984. Mycoflora of bean, broad bean, lentil, lupine and pea seeds in Saudi Arabia. *Mycopathologia* 88: 45–49.
- Ahmad, S.K. and P.L. Singh. 1991. Mycofloral changes and aflatoxin contamination in stored chickpea seeds. *Food Additives and Contaminants* 8: 723–730.
- Adegoke, G.O. and B.A. Odesola. 1996. Storage of maize and cowpea and inhibition of microbial agents of biodeterioration using the powder and essential oil of lemon grass (*Cymbopogon citratus*). *International Biodeterioration and Biodegradation* 37: 81–84.
- Coker, N.R.I. 1994. Biodeterioration of grain and the risk of mycotoxins. Pages 27–38 in *Grain storage techniques: evolution and trends in developing countries*, edited by D.L. Proctor. FAO Agricultural Services Bulletin 109, Rome, Italy.
- Desjardins, A.E. and T.M. Hohn. 1997. Mycotoxins in plant pathogenesis. *Molecular Plant-Microbe Interactions* 10: 147–152.
- El-Kady, I.A., S.S.M. El-Maraghy, and A.A. Zohri. 1991. Mycotoxin production on different cultivars and lines of broad bean (*Vicia faba* L.) seeds in Egypt. *Mycopathologia* 113: 165–169.
- Esurioso, O.F. 1975. Seed-borne fungi of cowpea (*Vigna unguiculata*) in Western Nigeria. *Nigerian Journal of Plant Produce* 2: 87–90.
- Hitokoko, H., S. Morozumi, T. Wauke, S. Sakai, and H. Kurata. 1981. Fungal contamination and mycotoxin-producing potential of dried beans. *Mycopathologia* 73: 33–38.
- Johnson, R.M. and W.D. Raymond. 1964. The chemical composition of some tropical food plants II. Pigeon peas and cowpeas. *Tropical Science* 6: 68–73.
- Marasas, W.F.O. 1994. *Fusarium*. Pages 522–530 in *Food-borne disease handbook: diseases caused by viruses, parasites and fungi*, edited by Y.H. Hui, J.R. Gorham, K.D. Murrell, and D.O. Cliver. Marcel Dekker Inc, New York, USA.
- Marasas, W.F.O. 1995. Fumonisin: their implication for human and animal health. *Natural Toxins* 3: 193–198.
- Marasas, W.F.O. 1996. Fumonisin: history, worldwide occurrence and impact. Page 3 in *Fumonisin in food*, edited by L. Jackson. Plenum Press, New York, USA.
- Moss, O.M. 1996. Mycotoxins. *Mycological Research* 100: 513–523.
- Musser, S.M. 1996. Quantification and identification of fumonisins by liquid chromatography/mass spectrometry. Pages 65–74 in *Fumonisin in food*, edited by L. Jackson. Plenum Press, New York, USA.
- National Research Council. 1992. *Neem: a tree for solving global problems*. National Academy Press, Washington DC, USA. Pages 53–55.
- Nelson, P.E., T.A. Tousson, and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press, University Park, Pennsylvania, USA. 89 pp.
- Pitt, J.I. 1998. Toxigenic aspergillus and penicillium species. In *Mycotoxin prevention and control in food grains* edited by R.L. Semple, A.S. Frio, P.A. Hicks, J.V. Lozare. Food and Agriculture Organization of the United Nations (FAO) and the Information Network on Post-Harvest Operations (INPhO). Bangkok, Thailand. <http://www.fao.org/inpho/vlibrary/x0036e/x0036e00.html>
- Saber, S.M. 1992. Fungal contamination, natural occurrence of mycotoxins and resistance for aflatoxin accumulation of some broad bean (*Vicia faba* L.) cultivars. *Journal of Basic Microbiology* 32: 249–258.
- Saber, M.S., M.B. Aboul-Nasr, and O.M.O. El-Maghraby. 1998. Contamination of pea (*Pisum sativum* L.) seeds by fungi and mycotoxins. *African Journal of Mycology and Biotechnology* 6: 53–64.
- Samson, R.A., E.S. Hoekstra, and C.A.N. Van Oorschot. 1981. *Introduction to food-borne fungi*. Centraalbureau voor Schimmelcultures, Netherlands. 190 pp.

- Seenappa, M., C.L. Keswani, and T.M. Kundy. 1983. Aspergillus infection and aflatoxin production in some cowpea (*Vigna unguiculata* [L.] Walp) lines in Tanzania. *Mycopathologia* 83: 103–106.
- Shephard, G.S., P.G. Thiel, S. Stockenstrom, and E.W. Sydenham. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. *Journal AOAC International* 79: 671–687.
- Singh, B.B., D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. 1997. Pages x–xii in *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Sydenham, E.W., G.S. Shephard, and P.G. Thiel. 1992. Liquid chromatographic determination of fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> in foods and feeds. *Journal AOAC International*. 75: 313–317.
- Tseng, T.C. and J.C. Tu. 1997. Mycoflora and mycotoxins in adzuki and mung beans produced in Ontario, Canada. *Microbios Letters* 90: 87–95.
- Tuan, Y.H. and R.D. Phillips. 1992. Nutritional quality of hard-to-cook and processed cowpea. *Journal of Food Science* 68: 1371–1374.
- Ushamalani, C., K. Rajappan, and K. Gangadharan. 1998. Seed-borne mycoflora of cowpea (*Vigna unguiculata* [L.] Walp.) and their effect on seed germination under different storage conditions. *Acta Phytopathologica et Entomologica Hungarica* 33: 285–290.
- Vainio, H., E. Heseltine, and J. Wilburn. 1993. Report on an IARC working group meeting on some naturally occurring substances. *International Journal of Cancer* 53: 535–537.
- Van Warmelo, K.T. 1967. The fungus flora of stock feeds in South Africa. Onderstepoort *Journal of Veterinary Research* 34: 439–450.
- Van Wyk, B-E. and N. Gericke. 2000. *People's plants: a guide to useful plants of southern Africa*. Briza Publications, Pretoria, South Africa. 30 pp.
- Watanabe, T. 1994. *Pictorial atlas of soil and seed fungi*. CRC Press Inc., Boca Raton, FA, USA. Pages 159–399.



## Breeding cowpea varieties for resistance to *Striga gesnerioides* and *Alectra vogelii*

B.B. Singh<sup>1</sup>

### Abstract

Two parasitic flowering plants, *Striga gesnerioides* (Wild.) Vatke and *Alectra vogelii* (Benth.), cause substantial yield reduction in cowpea in the dry savannas of sub-Saharan Africa. *Alectra* is more prevalent in the northern Guinea savanna and southern Sudan savanna of West Africa, as well as in East and southern Africa whereas *Striga* is mostly found in West and Central Africa. However, both are fast spreading beyond these limits. Collaborative studies with national and regional programs have revealed the presence of five strains of *S. gesnerioides* of which strain 1 is presently found in Burkina Faso, strain 2 in Mali, strain 3 in Nigeria and Niger, strain 4 in Benin Republic, and strain 5 in Cameroon. A local landrace, B 301 from Botswana, confers complete resistance to *Striga* and *Alectra* in Burkina Faso, Cameroon, Mali, Niger, and Nigeria. However, it has moderate levels of resistance to the strain from Benin Republic. Other lines such as IT81D-994, IT89KD-288, 58-57, and Gorom local confer complete resistance to strains from Benin Republic and Burkina Faso. Therefore, crosses were made among the selected complementary parents and a number of new varieties have been developed with combined resistance to *Alectra* as well as all the five strains of *Striga*. Most of these lines also serve as a false host for *S. hermonthica* reducing its seed bank in the soil when grown as an intercrop or in rotation with cereals.

### Introduction

Cowpea is the most important food legume in West and Central Africa and this region represents over 66% of the 12.5 million ha grown worldwide. It contains about 25% protein and so is a cheap source of protein in the daily diet of rural and urban populations. Its haulms are also an important source of nutritious fodder for the livestock in the dry savannas (Bressani 1985; Singh et al. 1997; Tarawali et al. 1997). However, the average yield of cowpea is very low due to numerous biotic and abiotic constraints. Of these, two parasitic flowering plant species, *Striga gesnerioides* and *Alectra vogelii*, cause considerable yield reduction in cowpea (Emechebe et al. 1991). *Striga* causes severe damage to cowpeas in the Sudano-Sahelian belt whereas *Alectra* is more prevalent in the Guinea savanna and Sudan savanna covering most parts of West and Central Africa. *Alectra* is also widespread in East and southern Africa. The *Striga* infection in cowpea (Fig. 1) is more devastating in areas with sandy soils, low fertility, and low rainfall. Both parasites are difficult to control because they produce large numbers of seed and up to 75% of the crop damage is done before they emerge from the ground. Therefore, concerted efforts are being made to develop improved cowpea varieties with combined resistance to both parasites.

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## Sources of resistance and strain variation in cowpea *Striga*

Varietal differences with respect to *Striga* infection in cowpea were first noticed in 1981 in Burkina Faso, and two lines, Suvita-2 and 58-57, were found to be completely resistant (IITA 1982, 1983). However, the results of subsequent regional trials revealed that these lines were not resistant to *Striga* in Niger and Nigeria indicating strain variation in cowpea *Striga* (Aggarwal 1985). Further screening of new lines at several locations in West and Central Africa showed that IT82D-849 (breeding line from IITA) and B 301 (a landrace from Botswana) were completely resistant to *Striga* (Fig. 2) in Burkina Faso, Cameroon, Mali, Nigeria, and Niger. B 301 had earlier been identified as being resistant to *Alectra* in Botswana (Riches 1989) and it was found to be resistant to *Alectra* in Nigeria also. However, IT82D-849 and Suvita-2 were found to be highly susceptible to *Alectra*. Subsequently, several other lines were identified which had moderate to high levels of resistance to both *Striga* and *Alectra*. These included IT86D-534, IT81D-994, IT86D-371, IT84D-666 (Singh and Emechebe 1991), and Tvu 9238, TVu 11788, TVu 12415, TVu 12432, and TVu 12470 (Singh 1994). The *Striga* seeds germinate and the radicles attach to the roots of resistant and susceptible plants (Fig. 3) but the resistant roots do not permit haustorium development (Fig. 4.). The *Striga* seedling dies leaving the resistant plants completely healthy and productive. On the other hand, there is a normal development of haustorium on roots of susceptible varieties (Fig. 5) permitting *Striga* to parasitize cowpea plants and cause up to 100% yield reduction (Fig. 6).

Several of these resistant lines were tested at Zakpota in the coastal savanna of Benin Republic where severe *Striga* infestation had been reported. All the TVu lines as well as IT86D-534, IT86D-371, and IT84D-666 were susceptible to the Zakpota strain, whereas B 301 and IT82D-849 showed moderate levels of resistance such that about 10% to 30% plants of these varieties show susceptibility. However, Suvita-2, 58-57 and IT81D-994 were completely resistant indicating that the Zakpota strain was different from strains from Burkina Faso and Nigeria. Systematic collection of *Striga* seed from different parts of West and Central Africa and testing against selected cowpea varieties revealed the presence of 5 strains (Lane et al. 1994; Lane et al. 1997). Of these, strain 1 is presently found in Burkina Faso, strain 2 in Mali, strain 3 in Nigeria and Niger, strain 4 in Benin Republic, and strain 5 in Cameroon. The host differentials for different strains are presented in Table 1. As evident from Table 1, B 301 and IT82D-849 are resistant to strains from Burkina Faso, Cameroon, Mali, and Nigeria, but moderately resistant to the Benin strain, which causes 10% to 30% susceptibility in these lines. IT81D-994 is resistant to all the strains except for the Nigerian strain. Suvita-2 is only resistant to Burkina Faso and Benin Republic strains, and 58-57 is resistant to strains from Benin, Burkina Faso, and Cameroon, but susceptible to strains from Mali and Nigeria. Thus, a combination of B 301 or IT82D-849 on one hand and IT81D-994 or Suvita-2 and 58-57 on the other will provide resistance to all the existing strains of *Striga*. These data also indicate that lines resistant to the Nigerian strain (strain 3) confer resistance to all the strains except the Zakpota strain. Therefore, testing of new lines in Nigeria and Benin Republic will be adequate for identification of lines with combined resistance to all five strains.

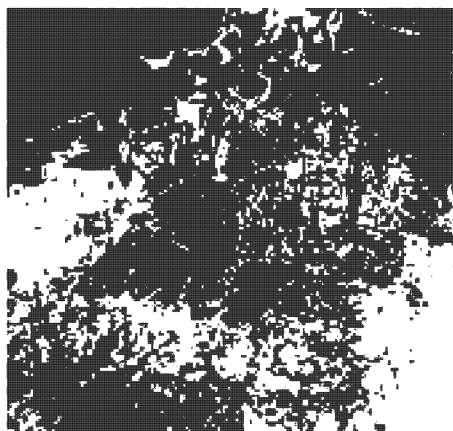


Figure 1. Cowpea *Striga* in the field.



Figure 2. *Striga* resistant and susceptible plants.

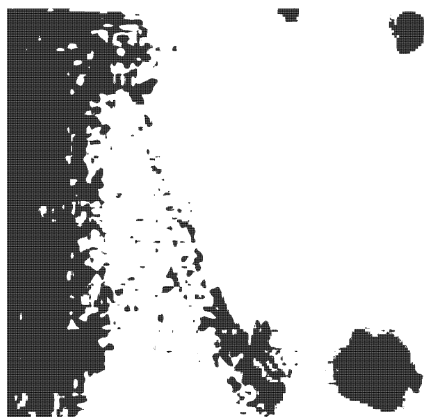


Figure 3. *Striga* attaching to cowpea root.



Figure 4. Resistant cowpea root kills *Striga*.



Figure 5. *Striga* parasitizing susceptible root.



Figure 6. *Striga* resistant and susceptible lines in the field.

**Table 1.** Host differentials for different strains of cowpea *Striga gesnerioides*.

| Cowpea variety | Reaction to <i>S. gesnerioides</i> strain <sup>†</sup> |   |   |    |   |
|----------------|--|---|---|----|---|
|                | 1  | 2 | 3 | 4  | 5 |
| Blackeye       | S  | S | S | S  | S |
| TVx 3236       | S  | S | S | S  | S |
| 58-57          | R  | S | S | R  | R |
| Suvita-2       | R  | S | S | R  | R |
| IT81D-994      | R  | R | S | R  | S |
| B 301          | R  | R | R | MR | R |
| IT82D-849      | R  | R | R | MR | R |

<sup>†</sup>Strain 1 occurs in Burkina Faso, 2 in Mali, 3 in Nigeria/Niger, 4 in Benin Republic, and 5 in Cameroon.

R = 100% plants resistant.

S = 100% plants susceptible.

### Effect of *Striga* infection on growth characters in susceptible and resistant cowpea varieties

Cowpea varieties with complete resistance to *Striga* stimulate germination and permit attachment of *Striga* radicles to their roots but the haustorium development is inhibited. The question has been raised whether the initial attachment of germinating *Striga* to resistant cowpea roots causes any shock to the plants and reduces plant growth even though further development of *Striga* is checked. Therefore, a set of 23 resistant and nine susceptible cowpea varieties were planted in pots infested with *Striga gesnerioides* seeds as well as in pots without *Striga* seeds and notes were taken on various parameters. As expected, the resistant varieties did not show any *Striga* emergence in both infested and noninfested pots and the susceptible varieties showed severe infestation (Table 2). The data on plant height, and shoot and root dry-matter of resistant varieties in infested pots did not differ significantly from the noninfested pots whereas the susceptible varieties suffered significant reduction in plant height as well as in dry matter in the infested pots. These results suggest that *Striga* attachment in resistant plants does not affect plant growth.

### Genetics of resistance to *Striga gesnerioides* and *Alectra vogelii*

Using the sources of resistance mentioned in the earlier section, systematic genetic studies were conducted to elucidate the nature of inheritance of resistance to *Striga* and *Alectra*. Singh and Emechebe (1990) reported that a single dominant gene, designated *Rsg<sub>1</sub>*, conditions resistance to *S. gesnerioides* in cowpea variety B301. Singh et al. (1993) found that duplicate dominant genes, designated *Rav<sub>1</sub>* and *Rav<sub>2</sub>* (resistant to *Alectra vogelii*) control resistance to *Alectra* in cowpea variety B 301. Atokple et al. (1993) demonstrated that the genes conditioning the resistance to *Striga* and *Alectra* in B301 are neither allelic nor linked. Atokple et al. (1995) reported the results of extensive allelism tests among cowpea lines resistant to *Striga* and *Alectra*. This work revealed that different genes are responsible for the *Striga* resistance exhibited by B 301, IT82D-849, and Suvita-2. Atokple et al. (1995) also reported that the single dominant gene conditioning *Alectra* resistance in IT81D-994 is not one of the two duplicate dominant genes conditioning resistance in B 301. They proposed the symbols *Rsg<sub>1</sub>*, *Rsg<sub>2</sub>*, and *Rsg<sub>3</sub>* for the genes conditioning resistance to

**Table 2. Effect of *Striga* infection on growth characters in susceptible and resistant cowpea varieties.**

| Growth character/plant               | Susceptible varieties |              | Resistant varieties |              |
|--------------------------------------|-----------------------|--------------|---------------------|--------------|
|                                      | Infected              | Not infected | Infected            | Not infected |
| No. of <i>Striga</i> plants emerged  | 8**                   | 0            | 0 NS                | 0            |
| No. of <i>Striga</i> plants attached | 13**                  | 0            | 0 NS                | 0            |
| Plant height at flow. (cm )          | 29*                   | 37           | 49 NS               | 50           |
| Plant height at mat. (cm)            | 36**                  | 61           | 59 NS               | 50           |
| Shoot dry matter at mat. g           | 5**                   | 7.7          | 8 NS                | 8.2          |
| Root dry matter at mat. g            | 1.8 NS                | 2.3          | 2.5 NS              | 2.9          |

\*\* = Significantly different (0.01); NS = not significantly different, flow. = flowering, mat. = maturity.

*Striga gesnerioides* in B 301, IT82D-849, and Suvita-2, respectively. They also proposed the symbols  $Rav_1$  and  $Rav_2$  for the genes conditioning resistance to *Alectra vogelii* in B 301, and the symbol  $Rav_3$  for the gene conditioning resistance to *Alectra* in IT81D994. The fact that the resistance in B 301 is due to a single dominant gene indicated that this gene confers resistance to four strains and the genes for resistance in Suvita-2 and IT81D-994 confer resistance to two to three strains including the Zakpota strain. Therefore, B 301 derived *Striga* resistant lines and Suvita-2 or other lines showing resistance to Zakpota strain can be used as complementary parents for breeding cowpea varieties resistant to all the 5 strains. Recently Ouedraogo et al. (2001) have confirmed monogenic inheritance of *Striga* resistance in Suvita-2 cowpea and they have also identified AFLP (amplified fragment length polymorphism) markers tightly linked to genes conditioning resistance to *Striga*. This will permit marker-assisted selection for *Striga* resistance and the eventual cloning and characterization of the genes conferring resistance to *Striga* in cowpea.

### Breeding cowpea for resistance to *Striga* and *Alectra*

A systematic breeding program for resistance to *Striga* and *Alectra* using B 301 as a resistance source, was undertaken in 1987. This line was crossed to a susceptible variety, IT84S-2246-4, which is otherwise a high yielding variety with resistance to aphid, bruchid, and several diseases. The  $F_1$  was backcrossed to IT84S-2246-4. From the resistant  $BC_1$ - $F_1$  plants,  $F_2$ ,  $F_3$ ,  $F_4$ ,  $F_5$ , and  $F_6$  progenies were developed and selected under suitable disease, insect, and *Striga/Alectra* pressures. This led to the selection of a number of  $F_6$  breeding lines, which are very similar to IT84S-2246 and have combined resistance to aphid, bruchid, thrips, *Striga*, *Alectra*, and several diseases. These were evaluated for yield and other characters in a replicated trial in 1991 and promising lines distributed to various national programs in Africa. Based on their performance in *Striga* infested fields, IT89KD-374-57 (Sangaraka) and IT89KD-245 (Korobalen) have been released in Mali; IT90K-76 and IT90K-82-2 have been released for general cultivation in Nigeria, and IT90K-59-2 in South Africa. The last four varieties have combined resistance to aphid, bruchid, thrips, *Striga*, and *Alectra*. These have been used as parents in the breeding program and a large number of *Striga*-resistant cowpea varieties have been developed. Performance of a few promising *Striga*-resistant varieties in the *Striga*-infested area of the Sahelian region is presented in Table 3. The *Striga* resistant varieties yielded about 50 to 100% more than the susceptible checks.

**Table 3. Performance of *Striga*-resistant cowpea varieties in the Sahel (Toumnia, Niger).**

| Cowpea variety                  | Yield (kg/ha) |        | No. of <i>Striga</i> /plot<br>(6 m²) |
|---------------------------------|---------------|--------|--------------------------------------|
|                                 | Grain         | Fodder |                                      |
| Early maturing varieties        |               |        |                                      |
| IT97K-499-8                     | 1467          | 891    | 0                                    |
| IT97K-499-39                    | 1274          | 891    | 0                                    |
| IT97K-497-2                     | 1166          | 807    | 0                                    |
| IT97-819-180                    | 1105          | 1420   | 0                                    |
| Dan Ila (susceptible)           | 529           | 1280   | 26                                   |
| SED                             | 199           | 214    | 2.3                                  |
| Early semideterminate varieties |               |        |                                      |
| IT97K-819-45                    | 1319          | 1447   | 0                                    |
| IT98K-205-29                    | 1150          | 1002   | 0                                    |
| IT94K-437-1                     | 1092          | 1030   | 0                                    |
| IT97K-499-38                    | 1029          | 1948   | 1                                    |
| IAR 48 (susceptible)            | 726           | 1058   | 23                                   |
| Dan Ila (susceptible)           | 546           | 1336   | 21                                   |
| SED                             | 160           | 183    | 5.0                                  |

### Breeding for resistance to multiple strains of *S. gesnerioides*

After the discovery of the Zakpota strain of *Striga* in Benin Republic, a large number of crosses were made between IT81D-994 and 58-57 with B 301 derived lines like IT90K-59 and IT90K-76 which are similar to B 301 with respect to *Striga* and *Alectra* resistance, but with higher yield and better seed quality. The segregating F<sub>2</sub> populations were first screened at Kano and then part of the seeds from resistant F<sub>3</sub> progenies were tested at Zakpota. The remnant seeds of resistant F<sub>3</sub> progenies at Zakpota were then planted at Kano and F<sub>4</sub> plants were selected. This was continued until the F<sub>6</sub> generation. This procedure had to be adopted because seeds of the Benin strain of *Striga* cannot be brought to Kano. The advanced breeding lines derived from this program were tested at several locations and selected lines distributed to national programs. The most promising lines were IT93KZ-4-3-1-7, IT93KZ-8-2-2-3-6, and IT93KZ-4-5-6-1-5 with over two t/ha grain yield with two sprays of insecticide and 100 kg/ha fertilizer (NPK 15-15-15).

Subsequently, the breeding procedure was simplified to minimize record keeping and save costs. The crosses are made between complementary parents and the segregation populations screened for resistance to *Striga* with artificial infestation at Kano (Nigeria) and at Babura (Nigeria) with natural infestation. The lines are also subjected to disease and insect pressure while advancing the generations. The selected F<sub>6</sub> lines are then tested at several locations including Zakpota where selection for resistance to the Zakpota strain is made. These lines are also tested at Samaru (Nigeria) for resistance to *Alectra*. This strategy has been very effective and a number of new breeding lines have been selected with combined resistance to all the strains of *Striga* and *Alectra*, as well as resistance to aphid, bruchid, thrips, viruses, and several diseases. The yield performance and level of resistance of the newly developed breeding lines at *Striga*-free (Minjibir) and *Striga*-infested (Babura) locations are indicated in Table 4. The results indicated that in a good environment (Minjibir), the yield potential of most of the lines is between 1500 and 2500 kg/ha. However, significant varietal differences were observed at a poor environment

**Table 4. Performance of selected improved cowpea varieties at Minjibir (less *Striga*) and Babura (severe *Striga*).**

| Variety       | Yield (kg/ha) |      | Yield (kg/ha) |      | <i>Striga</i> /plot (6m <sup>2</sup> ) |      |      |
|---------------|---------------|------|---------------|------|--|------|------|
|               | Grain         |      | Fodder        |      | Min.                                   | Bab. | Zak. |
|               | Min.          | Bab. | Min.          | Bab. | Min.                                   | Bab. | Zak. |
| IT97K-400-3   | 2761          | 207  | 1420          | 459  | 0                                      | 112  | 19   |
| IT97K-351-5   | 2559          | 0    | 2004          | 109  | 2                                      | 60   | 10   |
| IT97K-825-8   | 2362          | 855  | 2881          | 752  | 0                                      | 0    | 0    |
| IT97K-817-178 | 2311          | 642  | 2422          | 551  | 0                                      | 0    | 0    |
| IT97K-825-21  | 2310          | 931  | 3006          | 1461 | 0                                      | 0    | 0    |
| IT97K-499-35  | 2297          | 805  | 1587          | 676  | 0                                      | 3    | 1    |
| IT90K-277-2   | 2579          | 99   | 1378          | 134  | 1                                      | 122  | 10   |
| IT86D-719     | 2171          | 184  | 1879          | 159  | 8                                      | 167  | 2    |
| IT97K-826-86  | 855           | 1653 | 2088          | 626  | 0                                      | 2    | 0    |
| IT97K-819-154 | 1787          | 1552 | 1253          | 793  | 0                                      | 0    | 0    |
| IT97K-819-132 | 1519          | 1284 | 1420          | 1670 | 0                                      | 5    | 2    |
| Dan Ila       | 1415          | 223  | 1975          | 710  | 1                                      | 57   | 2    |
| SED           | 365           | 318  | 103           | 385  | 1.6                                    | 3    | 2    |

Min. = Minjibir, Bab. = Babura, Zak. = Zakpota.

(Babura) where soils are sandy, less fertile, and heavily infested with *Striga*. The *Striga*-resistant lines yielded between 642 and 1653 kg/ha but the *Striga* susceptible lines yielded from nothing to 223 kg/ha. The difference in the performance of *Striga*-resistant lines at Minjibir and Babura is due to low fertility at Babura and not due to *Striga*. It is interesting to note that a few *Striga* resistant lines such as IT97K-826-86, IT97K-819-154, and IT97K-819-132 yielded between 1384 and 1653 kg/ha grain with 2 sprays of insecticide even at Babura indicating their adaptability to poor soils and their ability to make efficient use of limited soil nutrients. A number of these lines have been multiplied and distributed to various national programs in cowpea international trials.

A *Striga*-resistant cowpea variety, IT 97K-499-38, was tested at eight farmers' fields in Benin Republic along with the respective local varieties grown by the farmers. The resistant variety, IT97K-499-38, performed as well as or better than local varieties at *Striga*-free locations but was much superior at *Striga*-infested locations (Table 5). The number of *Striga* in plots of local varieties (48m<sup>2</sup>) ranged from 1000–2000 and their grain yield ranged from 5 kg/ha to 220kg/ha whereas the number of *Striga* in the resistant variety ranged from 23–456 and the grain yield from 457–678 kg/ha. The fact that IT97K-499-38 showed some level of *Striga* infestation indicates that it is not immune to the *Striga* strain present in Benin Republic.

### Breeding for combined resistance to *Striga* and *Alectra*

Through planned crosses among complementary parents and screening of the derived breeding lines at Minjibir, Babura, and Zakpota for *Striga* and at Samaru for *Alectra*, a number of cowpea varieties have been developed which have combined resistance to all the strains of *Striga* as well as *Alectra*. Based on their resistance and yield performance in different trials, IT94K-437-1, IT94K-440-3, IT96D-748, IT97K-499-39, and IT97K-819-154 appear to be very promising (Table 6).

**Table 5. Performance of *Striga*-resistant varieties in the coastal savanna under low fertility without insecticide spray.**

| Location in<br>Benin Republic | Cowpea variety                    |               |                      |               |
|-------------------------------|-----------------------------------|---------------|----------------------|---------------|
|                               | IT97K-499-38                      |               | Local variety        |               |
|                               | No. of <i>Striga</i> <sup>†</sup> | Yield (kg/ha) | No. of <i>Striga</i> | Yield (kg/ha) |
| Mlinkpin                      | 0                                 | 787           | 47                   | 587           |
| Adjoko                        | 0                                 | 662           | 29                   | 650           |
| Maikpin                       | 0                                 | 775           | 23                   | 500           |
| Oukombe                       | 0                                 | 587           | 1461                 | 202           |
| Kodota                        | 57                                | 312           | 589                  | 50            |
| Aligodon                      | 70                                | 609           | 1053                 | 300           |
| Some                          | 437                               | 300           | 1526                 | 262           |
| Zakpota                       | 360                               | 225           | 2724                 | 5             |

<sup>†</sup>No. of *Striga* in 40 m<sup>2</sup> plots (single replicate).**Table 6. Reaction of improved cowpea breeding lines to *Striga gesnerioides* and *Alectra vogelii*.**

| Breeding line    | Emerged <i>Striga</i> /plot <sup>†</sup> |        | Emerged <i>Alectra</i> /plot |        |
|------------------|--|--------|------------------------------|--------|
|                  | Samaru                                   | Babura | Zakpota                      | Samaru |
| IT93K-596        | 0.0                                      | 1.8    | 4.5                          | 0.0    |
| IT93K-693-2      | 0.0                                      | 0.0    | 0.0                          | 0.0    |
| IT94K-437-1      | 0.0                                      | 0.0    | 0.0                          | 0.0    |
| IT94-440-3       | 2.0                                      | 0.0    | 3.0                          | 0.0    |
| IT95K-1090-12    | 0.0                                      | 0.3    | 0.0                          | 0.0    |
| IT95K-1091-3     | 0.0                                      | 1.7    | 0.0                          | 0.0    |
| IT96D-748        | 0.0                                      | 0.0    | 0.0                          | 0.0    |
| IT97K-499-39     | 0.0                                      | 0.0    | 1.0                          | 0.0    |
| IT97K-819-154    | 0.0                                      | 0.0    | 0.0                          | 0.0    |
| Tvx 3236 (check) | 12                                       | 10     | 35                           | 28     |

<sup>†</sup>Plot = 6 m<sup>2</sup>

## Screening cowpea, sorghum, and millet varieties as false hosts for *Striga* spp.

In view of the fact that cowpea is mostly planted as an intercrop with pearl millet and sorghum, it would be ideal to select cowpea varieties that can stimulate suicidal germination of *S. hermonthica* on one hand, and millet and sorghum varieties that can stimulate suicidal germination of *S. gesnerioides* on the other hand, thereby reducing the seed bank of both types of *Striga*. Therefore, a range of cowpea, millet, and sorghum varieties were tested from 1993 to 1995 for their ability to cause suicidal germination of *Striga* spp. Most of the cowpea varieties were able to cause from 65% to 80% suicidal germination of *S. hermonthica* (Table 7). Of these, IT90K-76, IT81D-994, and Suvita-2 are resistant to several strains of cowpea *Striga*. From a total of 55 sorghum varieties tested, only 6 could stimulate the germination of *Striga gesnerioides* and of these, varieties Yalan and BES were the best with about 60% germination which was close to that of cowpea variety, TVx 3236. Of the 50 millet varieties tested, none was able to cause significant germination of *S. gesnerioides*.



**Table 7. Percentage suicidal germination of *Striga hermonthica* by cowpea varieties and *Striga gesnerioides* by sorghum and millet varieties.**

| Host crop/variety | Suicidal germination (%) |
|-------------------|--------------------------|
| Cowpea            | <i>S. hermonthica</i>    |
| IT90K-277-2       | 82.3                     |
| IT81D-994         | 80.9                     |
| Suvita-2          | 80.9                     |
| IAR 48            | 71.4                     |
| IAR 1696          | 65.4                     |
| Sorghum           | <i>S. gesnerioides</i>   |
| Yalang            | 63.5                     |
| BES               | 60.0                     |
| ICSV 1007         | 48.0                     |
| 47 others         | 0.0                      |
| Pearl millet      | <i>S. gesnerioides</i>   |
| ICMV-15 89201     | 3.3                      |
| ICMV-15 94110     | 0.0                      |
| 48 other lines    | 0.0                      |

These results indicate that most of the cowpea varieties can cause suicidal germination of *S. hermonthica*. The new *Striga*-resistant cowpea breeding lines have been tested and they cause similar germination indicating that these varieties would be ideal for intercropping or as a rotation crop with millet and sorghum. Although some sorghum varieties have shown ability to cause suicidal germination of *S. gesnerioides*, it is desirable to screen more sorghum and millet varieties to identify lines that can cause higher levels of suicidal germination of *S. gesnerioides* and use these lines in the breeding program. Thus, there is a need for cowpea breeders and millet and sorghum breeders to work together to identify complementary combinations of millet–cowpea and sorghum–cowpea intercrops to minimize *Striga* infestation on both crops in the dry savannas.

## Conclusion

Cowpea suffers considerable damage due to *Striga* and *Alectra* and the yield reduction can be up to 100% in severe cases. Current annual losses due to these parasitic plants are estimated to be over US\$ 200 million in West and Central Africa where over 8 million ha of cowpea are grown mostly by smallholder farmers who cannot afford to control these parasites by chemical means. Development of cowpea varieties with combined resistance to both parasites is the cheapest and best method of reducing the losses due to these parasitic weeds. A great deal of progress has been made and a number of improved *Striga/Alectra* resistant cowpea varieties have been developed, which are fast becoming popular with the farmers.

## References

- Aggarwal, V.D. 1985. Cowpea–*Striga gesnerioides* research. Pages 335–340 in Cowpea research, production, and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Atokple, I.D.K., B.B. Singh, and A.M. Emechebe. 1993. Independent inheritance of *Striga* and *Alectra* resistance in cowpea genotype B301. Crop Science 33: 714–715.

- Atokple, I.D.K., B.B. Singh, and A.M. Emechebe. 1995. Genetics of resistance to *Striga* and *Alectra* in cowpea. *Journal of Heredity* 86: 45–49.
- Bressani, R. 1985. Nutritive value of cowpea. Pages 353–360 in *Cowpea research, production, and utilization*, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Emechebe, A.M., B.B. Singh, O.I. Leleji, I.D.K. Atokple, and J.K. Adu. 1991. Cowpea *Striga* problems and research in Nigeria. Pages 18–28 in *Combating Striga in Africa*, edited by S.K. Kim. IITA, Ibadan, Nigeria.
- IITA (International Institute of Tropical Agriculture). 1982. Screening for resistance to *Striga gesnerioides*. Page 148 in *Annual Report 1981*. IITA, Ibadan, Nigeria.
- IITA (International Institute of Tropical Agriculture). 1983. *Striga gesnerioides* resistance. Pages 69–70 in *Annual Report 1982*. IITA, Ibadan, Nigeria.
- Lane, J.A., T.H.M. Moore, D.V. Child, K.E. Cardwell, B.B. Singh, and J.A. Bailey. 1994. Virulence characteristics of a new race of the parasitic angiosperm *Striga gesnerioides* from southern Benin on cowpea. *Euphytica* 72: 183–188.
- Lane, J.A., T.H.M. Moore, D.V. Child, and J.A. Bailey. 1997. Variation in virulence of *Striga gesnerioides* on cowpea: new sources of crop resistance. Pages 225–230 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Ouedraogo, J.T., V. Maheshwari, D.K. Berner, C.A. St-Pierre, and P. Timko. 2001. Identification of AFLP markers linked to resistance of cowpea to parasitism by *Striga gesnerioides*. *Theoretical and Applied Genetics* 102: 1029–1036.
- Riches, C.R. 1989. The biology and control of *Alectra vogelii* in Botswana. PhD Thesis, University of Reading, UK.
- Singh, B.B. 1994. Collection and utilization of germplasm of cowpea resistant to *Striga* and *Alectra*. Pages 135–144 in *Plant genetic resource management in the tropics*. JIRCAS International Symposium 2. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.
- Singh, B.B. and A.M. Emechebe. 1990. Inheritance of *Striga gesnerioides*: resistance in cowpea genotype B 301. *Crop Science* 30: 879–881.
- Singh, B.B. and A.M. Emechebe. 1991. Breeding for resistance to *Striga* and *Alectra* in cowpea. Pages 303–305 in *Proceedings of 5<sup>th</sup> International Symposium on Parasitic Weeds*, edited by J.K. Ransom, L.J. Musselman, A.D. Worsham, and C. Parker, 24–30 June 1991, Nairobi, Kenya. The International Maize and Wheat Improvement Center (CIMMYT), Mexico, D.F. Mexico.
- Singh, B.B., A.M. Emechebe, and I.D.K. Atokple. 1993. Inheritance of *Alectra vogelii* resistance in cowpea, genotype B 301. *Crop Science* 33: 70–72.
- Singh, B.B., O.L. Chamblis, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Tarawali, S.A., B.B. Singh, M. Peters, and S.F. Blade. 1997. Cowpea haulms as fodder. Pages 313–325 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.



## **Section III**

# **Biotechnology for cowpea**



## 3.1

# Isolation, sequencing, and mapping of resistance gene analogs from cowpea (*Vigna unguiculata* L.)

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### Abstract

Cowpea (*Vigna unguiculata* L.) is a staple crop of significant economic importance worldwide and for many people in emerging areas of the globe, it is a major source of protein necessary for proper human nutrition. Degenerate oligonucleotide primers designed to recognize conserved regions within the nucleotide binding site (NBS) of known NBS-LRR-type resistance genes from various plant species were used in PCR amplification reactions to identify resistance gene analogs (RGAs) from the *Striga gesnerioides*-resistant cowpea cultivar Suvita-2. The PCR reaction products consisted of a group of related fragments approximately 500 bp in length which migrated as a single band during agarose gel electrophoresis. The nucleotide sequences of 50 different fragments were determined and their predicted protein sequences compared to each other and to the proteins encoded by known resistance genes and RGAs from other plant species. A total of eight different classes of RGAs were found in cowpea. Gel blot analysis revealed that each class recognized a different subset of genes in the cowpea genome. Several of the RGAs were associated with restriction fragment length polymorphisms, which allowed them to be placed on the cowpea genomic map. The potential for using these sequences to isolate their corresponding genes and the subsequent direct manipulation of disease and pest resistance through genetic engineering is discussed.

### Introduction

Cowpea (*Vigna unguiculata* L.) is a staple food crop of significant economic importance worldwide. In the semiarid and humid tropical regions of Africa, cowpea is a major source of protein and of considerable importance for human nutrition. It is estimated that cowpea is now cultivated on at least 12.5 million hectares, with an annual production of over 3 million tonnes worldwide (Singh et al. 1997). While cowpea is grown on some 80 000 hectares in the USA (Fery 1990, Ehlers and Hall 1997), Central and West Africa account for more than half of the cultivated area, followed by South America, Asia, East and South Africa (Singh et al. 1997). Cowpea production is limited by numerous insects, microbial and fungal diseases, and other pests including the parasitic angiosperms *Striga gesnerioides* and *Alectra volgetii* (Bashir and Haptom 1996; Singh and Emechebe 1997). Because of its widespread use, numerous initiatives have been undertaken to improve various agronomic and nutritional traits of cowpea. These initiatives include selective

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breeding programs aimed at screening wild and cultivated germplasm for sources of disease and pest resistance, improved plant cell culture and cell transformation methods, and gene isolation and characterization analysis for the direct manipulation of the cowpea genome through genetic engineering.

Genes conferring resistance to the major classes of plant pathogens, including bacteria, virus, fungi, and nematodes, have been isolated from a variety of plant species, including almost all of the agronomically important grasses and legumes (Baker et al. 1997; Gebhardt 1997; Hammond-Kosack and Jones 1997). The products of the resistance (R) genes have been suggested to act as receptors that specifically bind ligands encoded by the corresponding pathogen avirulence factors in a gene-for-gene recognition process (Baker et al. 1997; Hammond-Kosack and Jones 1997). The R-gene product/avirulence factor complex is thought to initiate a series of signaling cascades within the cell leading to disease resistance. Among the downstream cellular events that characterize the resistant state are rapid oxidative bursts, cell wall strengthening, the induction of defense gene expression, and rapid cell death at the site of infection (Morel and Dangl 1997).

From comparisons of the predicted protein sequences of cloned disease and pest resistance genes from various plants, researchers were able to identify common motifs in R-gene products from plants of diverse evolutionary origin working against a broad array of pathogens. The structural motifs present in the various R-gene products, when compared to other prokaryotic and eukaryotic proteins of known function, also suggest possible roles and cellular locations for the R-gene encoded proteins. Based on shared molecular features, the products of R-genes from various plants have now been grouped into several major classes (Parker and Coleman 1997; Hammond-Kosack and Jones 1997; Van der Beizen and Jones 1998; Pan et al. 2000).

The majority of plant-resistance genes encode cytoplasmic receptor-like proteins that contain a leucine-rich repeat (LRR) domain and a nucleotide triphosphate binding site (NBS). Included in this class of R-genes are the N gene from tobacco conferring resistance to tobacco mosaic virus (TMV) (Whitham et al. 1994), *Prf*, *I2CI*, and *Mi* from tomato (Milligan et al. 1998; Ori et al. 1997; Salmeron et al. 1996), *RPM1*, *RPS2*, *RPP5*, *RPS5*, *RPP1*, and *RPP8* from *Arabidopsis* (Bent et al. 1994; Botella et al. 1997; Grant et al. 1995; McDowell et al. 1998; Mindrinos et al. 1994; Parker et al. 1997; Warren et al. 1998), the rust resistance genes *M* and *L6* from flax (Anderson et al. 1997; Lawrence et al. 1995), *RGC2* from lettuce (Meyers et al. 1998), *Xa1* from rice (Yoshimura et al. 1998), and the nematode resistance locus of wheat, *Cre3* (Lagudah et al. 1997). Some members of this group contain domains near their amino terminus which have significant similarity to the *Drosophila* Toll or human interleukin receptor-like (TIR) region (Hammond-Kosack and Jones 1997; Whitham et al. 1994). In others, the amino-terminus of the protein contains coiled-coil (CC) motifs (Pan et al. 2000). Interestingly, TIR-NBS-LRR type resistance proteins appear to be found only in dicotyledonous plants, whereas CC-NBS-LRR type resistance genes are found in both monocots and dicots (Meyers et al. 1999; Pan et al. 2000).

The second subfamily includes the tomato *Cf-2*, *Cf-4*, *Cf-5*, and *Cf-9* genes which confer resistance to different races of the fungus *Cladosporium fulvum*. (Jones et al. 1994; Dixon et al. 1996, 1998; Thomas et al. 1997) and the sugar beet nematode resistance gene *HS<sup>pro-1</sup>* (Cai et al. 1997; Hammond-Kosack and Jones 1997). These R-genes encode putative transmembrane molecules with extracellular LRR domains. The rice *Xa21*

gene encodes a third class of R proteins (Song et al. 1995), which have a transmembrane segment, an extracellular LRR domain and an intracellular serine-threonine kinase. The bacterial blight resistance gene *Xa1* from rice also contains both the NBS and LRR but differs significantly from the *Xa21* protein (Yoshimura et al. 1998). *Pto*, which confers resistance to the bacterial pathogen *P. syringae* pv. tomato (Martin et al. 1993) constitutes yet another class of R gene. *Pto* contains a serine-threonine kinase domain but lacks both the LRR and NBS and requires *Prf* for function.

As a result of the high degree of sequence conservation among R-genes encoding NBS-LRR type proteins, various investigators have designed degenerate oligonucleotides for use in polymerase chain reaction (PCR) amplification reactions to clone resistance genes and resistance gene analogs (RGAs) from the genomes of diverse plants species, including soybean (Kanazin et al. 1996; Yu et al. 1996), common bean (Rivkin et al. 1999), *Arabidopsis* (Speulman et al. 1998, Aarts et al. 1998), and numerous other dicot and monocots (see Meyers et al. 1999; Pan et al. 2000). The term RGA is used throughout the text to denote cloned R-gene sequences for which no function has yet been assigned in the plant species. In some cases, it has been possible to map RGAs within a plant genome and show that they are linked to a known disease or pest resistance locus (Yu et al. 1996; Chen et al. 1998; Collins et al. 1998; Seah et al. 1998; Shen et al. 1998; Mago et al. 1999; Tada 1999). RGAs have not only been found that are linked to known single dominant R-loci, but also to quantitative trait loci (QTL) (Pflieger et al. 1999).

Yields of edible cowpea seed are severely reduced by infection of the roots by the parasitic angiosperm *Striga gesnerioides* (Aggarwal and Ouédraogo 1989; Aggarwal 1991). Attempts to control the parasite by altering cultural practices have not been effective and the use of chemical treatments have been economically impractical for most local farmers (Aggarwal and Ouédraogo 1989; Muleba et al. 1996, 1997; Singh and Emechebe 1997). The identification of local varieties with natural resistance and their incorporation into breeding programs has been the most successful strategy used to date for controlling the parasite (Singh and Emechebe 1997). The identification and cloning of resistance genes to this and other disease pathogens would contribute significantly to the future improvement of cowpea germplasm. Given the success of these previous investigators, we have used primers based on the conserved motifs of previously isolated disease resistance genes to amplify similar regions from the *Striga gesnerioides*-resistant variety Suvita-2 (Atokple et al. 1995; Touré et al. 1997, 1998). The different RGAs were subsequently cloned, sequenced, and several of them mapped onto the cowpea genetic map.

## Materials and methods

### *Plant growth and materials*

Seeds of the cowpea accession Suvita-2 were obtained from the USDA-ARS, Plant Genetic Resources Conservation Unit, University of Georgia (Griffin, Georgia). Breeding lines IT84S-2049, 524B, and 96 recombinant inbred lines (RILs) derived from the cross between these two lines were from Paul Gepts (University of California, Davis, California). IT84S-2049 is a breeding line developed at the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria, and is reported to have multiple disease and pest resistance (Menéndez et al. 1997). Cultivar 524B is a California black-eye type developed from a cross between cultivars CB5 and CB3, which encompass the genetic variability in cowpea in California. Plants used for DNA isolation were grown in pots



in the greenhouse, the leaves were harvested, frozen immediately in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  until use.

### **DNA isolation, PCR amplification, and RGA cloning**

Genomic DNA was isolated following the protocol of Varadarajan and Prakash (1991). Conserved regions within the NBS and hydrophobic regions of the tobacco *N* gene, *RPS2* of *Arabidopsis*, and *L6* of flax were used to design degenerate oligonucleotide primers for amplification of RGAs from cowpea (Kanazin et al. 1996). Two primers were made for these studies, Primer 1-5'-GGIGGIGTIGGIAAIACIAC-3' and Primer 2-5' A(A/G)IGCTA(A/G)IGGIA(A/G)ICC-3' (purchased from Gibco-BRL, Life Sciences). PCR amplification reactions were carried out with 200 ng of Suvita-2 genomic DNA in 100  $\mu\text{l}$  reactions containing 10 mM Tris-HCl, pH 8.3, 1.5 mM  $\text{MgCl}_2$ , 50 mM KCl, 1mM of each primer, 100  $\mu\text{M}$  dNTPs, and 2.5 units of Taq DNA polymerase. The initial step of the amplification reaction was denaturation at  $94^{\circ}\text{C}$  for 5 min, followed by 35 cycles of  $94^{\circ}\text{C}$  for 1 minute,  $45^{\circ}\text{C}$  for 1 minute,  $72^{\circ}\text{C}$  for 2 minutes, and a final extension at  $72^{\circ}\text{C}$  for 10 minutes. The PCR products were resolved on the 0.8% agarose gel, the DNA bands were purified using GeneClean (BIO101, Vista, California), and ligated into Bluescript SK (Stratagene, La Jolla, California) which was linearized by digestion with *EcoRV* and T-tailed (Marchuk et al. 1991) before use. The resulting plasmids were transformed into *E. coli* DH5 $\alpha$ . A total of 100 colonies were randomly selected, liquid cultures grown from each, and plasmid DNA isolated by the alkaline lysis method (Sambrook et al. 1989).

### **Nucleotide sequencing and analysis**

Nucleotide sequencing was carried out manually using the Sequenase Version 2.0 DNA sequencing kit according to the manufacturer's protocol (United States Biochemical, Cleveland, Ohio) or using the BigDye fluorescence labeling method and an ABI Prism 310 automated sequencer (PE Applied Biosystems, Forest City, California). The open reading frames for the nucleic acid sequences were obtained by using DNASTAR program (DNASTAR Inc., Madison, Wisconsin) and the nucleotide and predicted amino acid sequences of the various cDNAs were analyzed using BLAST and BLASTX sequence analysis programs (Altschul et al. 1990, 1997; Gish and States 1993). Protein sequence alignments were carried out using the PILEUP program (Genetics Computer Group Sequence Analysis Package, Version 9.0, Madison, Wisconsin) and the various R-gene sequences available in the NCBI (National Center for Biotechnology Information, Bethesda, MD) nucleotide and protein sequence database. Construction of dendrograms was done using PaupSearch and PaupDisplay programs (Genetics Computer Group Sequence Analysis Package, Version 9.0, Madison, Wisconsin). Manual adjustment of the sequence alignments was carried out as necessary.

### **Gel blot analysis of genomic DNA**

Gel blot analysis of genomic DNA was carried out as described by Gowda et al. (1996, 1999) using 10 $\mu\text{g}$  aliquots of genomic DNA digested with *EcoRI*, *EcoRV* or *HindIII*. Restriction digestion products were separated on 0.8% agarose gels in TAE buffer and then transferred to NytranPlus membranes (Schliecher and Schuell, Keene, New Hampshire) by alkaline capillary transfer (Sambrook et al. 1989). The blots were hybridized with [ $\alpha^{32}\text{P}$ ]-dCTP-labeled hybridization probes prepared from the inserts of the various RGA

clones (e.g., 432, 434, 436, 438, 445, 468, and 490). Pre-hybridization, hybridization, and washing of the membranes were done according to Gowda et al. (1996).

### **Segregation and linkage analysis**

In order to place the polymorphisms recognized on this study on the existing map of the cowpea genome, segregation of polymorphic fragments was carried out with 96 RILs derived from a cross between IT84S-2049 and 524B (Menéndez et al. 1997). Segregation of individual markers was analysed by chi-square test for goodness of fit to a 1 : 2 : 1 or 1 : 3 ratio. Linkage analysis was performed using MAPMAKER 3.0 program (Lander et al. 1987). The “group” command was used to determine linkage groups, pair-wise comparisons, and group markers. An LOD score of 3.0 or above and a maximum recombination frequency of 30% were specified. To determine the most likely order within a linkage group, the “compare” command was used and the best order was accepted based on a log-likelihood difference of two or more. The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequency into map distances in centimorgans (cM).

## **Results**

### **Cloning and nucleotide sequence analysis of cowpea RGAs**

PCR amplification of cowpea DNA prepared from the *Striga*-resistant line Suvita-2 using degenerate oligonucleotide primers recognizing conserved sequences corresponding to the NBS and hydrophobic domains of NBS-LRR type R-genes yielded a heterogeneous mixture of fragments migrating on agarose gels as a single band approximately 500 bp in length. The PCR products were recovered from the gel, cloned into pBluescript plasmids, and the nucleotide sequence of 50 independently derived clones determined. Based upon their nucleotide and predicted amino acid sequences, the various clones were categorized into eight major groups of RGAs. The predicted protein sequence of a representative member of each RGA group is shown in Figure 1. Pairwise comparisons of the nucleotide and predicted protein sequences of the various RGAs showed that they have between 35.6–98.2% and 20.4–97.0% identity, respectively (Table 1). Comparison of the cowpea RGAs to sequences available in the various databases available through NCBI, revealed that at the amino acid levels, the cowpea RGAs were most similar to R-genes and RGAs from other leguminous species (e.g., wild cowpea, alfalfa, soybean) (Table 2). The greatest degree of identity was found with RGAs isolated from *Vigna vexillata* (wild cowpea) and *Vigna unguiculata* variety IT94K-2053 (81–86%), followed by RGAs from soybean (39–73%). Comparisons of the cowpea RGAs to those of other leguminous crops, such as *Medicago* spp., chickpea, and pigeon pea, showed significantly higher levels of identity than RGAs isolated from species more evolutionarily diverged (e.g., *Pinus radiata*, *Sorghum bicolor*, *Hordeum vulgare*) (Fig. 2).

The various RGAs from cowpea contain sequences resembling the consensus G–X–X–G–X–G–K–T–T motif (Motif I) present in almost all NBS-LRR type R genes (Meyers et al. 1999; Pan et al. 2000). This motif, referred to as the P-loop or kinase-I domain and is thought to be necessary for properly orienting the nucleotide phosphate group of the bound ATP or GTP (Saraste et al. 1990; Traut 1994; Mago et al. 1999). The presence of two absolutely conserved phenylalanine residues separated by four amino acids (Motif II) and the almost absolutely conserved W–F–G–X–G–S–R kinase-2

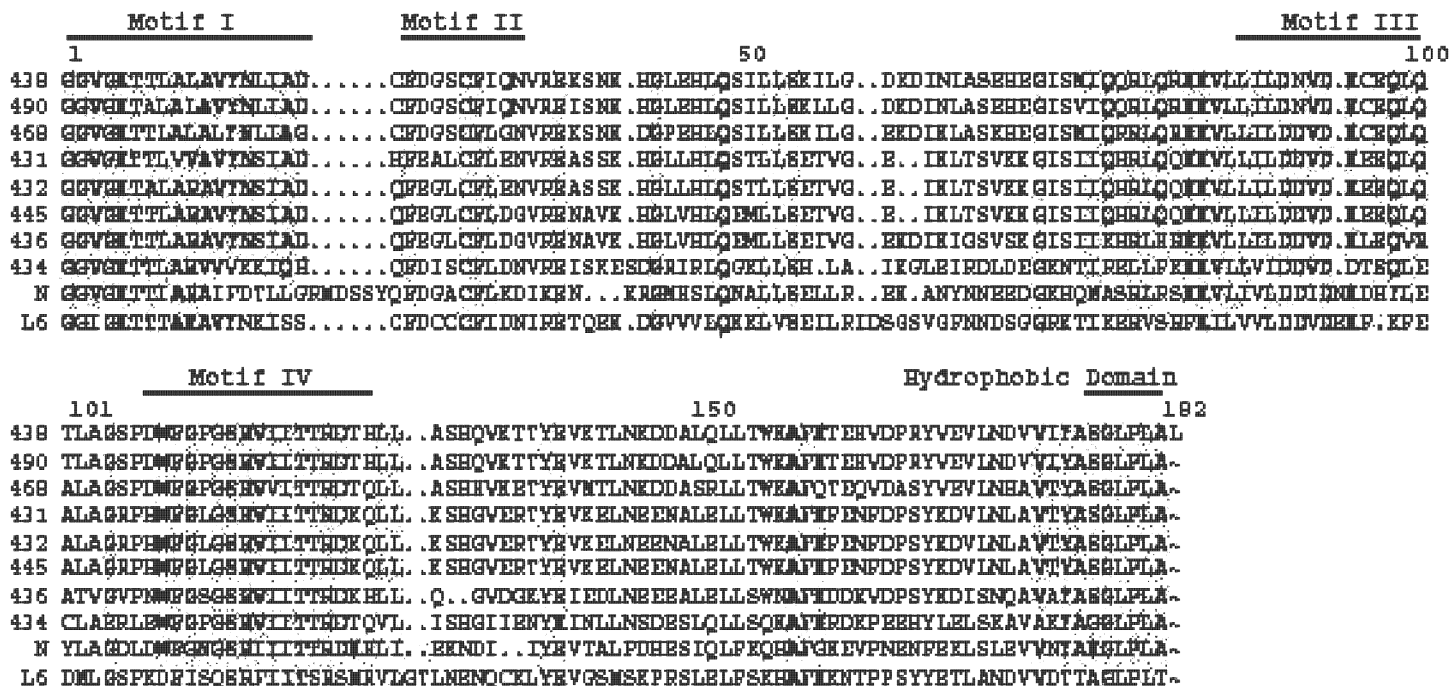


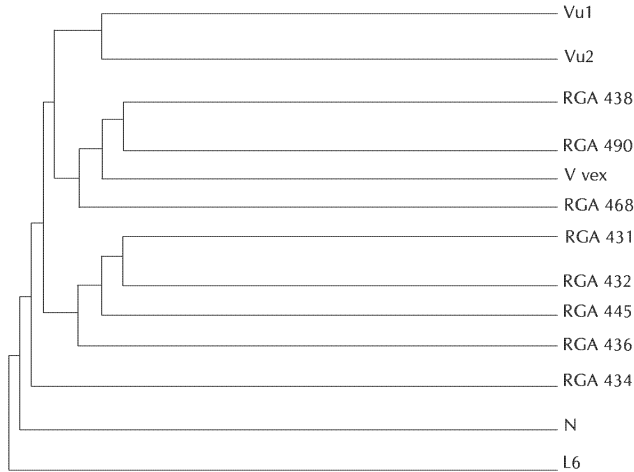
Figure 1. Comparison of the protein sequences for various cowpea RGAs with closely related R-gene products from other plants. Shown is the predicted amino acid sequence of representative cowpea RGAs compared with the relevant portions of the tobacco N (Accession No. 2020282A) and flax L6 (Accession No. T18546) proteins. Amino acid sequence alignment was performed using the PILEUP program. Conserved residues are shaded. The location of various conserved motifs as defined in the text are indicated. The nucleotide sequences for RGAs 431, 432, 434, 436, 438, 445, 468, and 490 reported in this paper appear in Genbank with the Accession numbers AF255460-AF225467, respectively.

**Table 1. Percentage identity among cowpea RGAs in nucleotide and predicted protein sequence.**

| RGA  | 490       | 468       | 445       | 438       | 436       | 434       | 432       | 431     |
|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| (Percentage identity–nucleotide sequence/protein sequence) |           |           |           |           |           |           |           |         |
| 431  | 42.9/25.7 | 42.7/26.3 | 94.4/92.8 | 43.1/26.3 | 60.8/47.9 | 36.0/19.2 | 97.8/97.0 | 100/100 |
| 432  | 42.9/27.5 | 42.5/26.9 | 95.4/94.6 | 42.7/26.9 | 61.8/49.7 | 36.2/20.4 | 100/100   |         |
| 434  | 54.2/47.3 | 52.2/46.2 | 35.6/20.4 | 55.2/47.9 | 43.7/32.3 | 100/100   |           |         |
| 436  | 58.4/47.3 | 60.0/47.9 | 66.0/55.1 | 59.6/49.1 | 100/100   |           |           |         |
| 438  | 98.2/97.0 | 90.2/84.0 | 41.6/25.7 | 100/100   |           |           |           |         |
| 445  | 41.2/25.1 | 41.9/25.7 | 100/100   |           |           |           |           |         |
| 468  | 89.6/82.2 | 100/100   |           |           |           |           |           |         |
| 490  | 100/100   |           |           |           |           |           |           |         |

**Table 2. Distribution of homologs to cowpea RGAs among different plant species.**

| Plant species                  | Common name     | Genebank accession numbers  |
|--------------------------------|-----------------|---|
| <i>Glycine max</i>             | Soybean         | GMU 55803-55811, 2224379A-2224379H, 2224379J, T08819-T08821, T088124, T088131-T088135, T088150  |
| <i>Vigna unguiculata</i>       | Cowpea          | ABO20483, ABO20484, ABO20486, ABO20489, AF1410121, AF1410131  |
| <i>Vigna vexillata</i>         | Wild cowpea     | AF141014, AF141015  |
| <i>Medicago truncata</i>       |                 | AW559555, AW256570  |
| <i>Medicago sativa</i>         | Alfalfa         | AF230816, AF230823, AF230825, AF230827, AF230835, AF230838-AF230840, AF230842, AF230843, AF230848, AF230850, AF230851, AF230853, AF230854, AF230856, AF230857, AF230859, AF230862                     |
| <i>Cicer arietinum</i>         | Chick pea       | AF186624-AF186627, AF186629   |
| <i>Cajanus cajan</i>           | Pigeon pea      | AF186633-AF186639   |
| <i>Lactuca sativa</i>          | Lettuce         | AF017754 1 and AF07177511   |
| <i>Cucumis melo</i>            | Muskmelon       | CME251969 1-CME2518711  |
| <i>Elaeis guinensis</i>        | Oil palm        | AF197921-AF197924   |
| <i>Arabidopsis thaliana</i>    | Mouse-ear cress | AC0080174, AC0080176, AC0080179, AC00801710, AC00801715, AC0071971, T08196, T081916, ATF26013, ATU97217-ATU97219, ATU97221, ATU97224-ATU97226, C71436, E71436, H71436, A71437, B71437, D71437, G71437 |
| <i>Solanum tuberosum</i>       | Potato          | 2303415G, 2303415H, 2303415J-2303415M, T07766, T07767, T07769, T07770, T07772, T07774   |
| <i>Lysopersicon esculentum</i> | Tomato          | 1168481   |
| <i>Capsicum annuum</i>         | Pepper          | AF1214351, AF1214371  |
| <i>Helianthus annuus</i>       | Sunflower       | 966421  |
| <i>Triticum aestivum</i>       | Wheat           | AF087518 21   |
| <i>Hordeum vulgare</i>         | Barley          | AF032679-AF032682, AF03684-AF03687, T04389, T04392-T04394, AF146274   |
| <i>Zea mays</i>                | Maize           | AF0561531, AF0561551  |
| <i>Sorghum bicolor</i>         | Sorghum         | AF186644  |
| <i>Oryza sativa</i>            | Rice            | AF074886-AF074892, AF074894-AF074899, AF1462701, AF146275, AF032688-AF032690, AF032692, AF032693, AF032697, AF032698, AF032700, AF032702, OSH85583, OSH8558121  |



**Figure 2.** Dendrogram showing phylogenetic relationship of cowpea RGAs and related resistance gene products from other plants. A representative sequence from each of the eight cowpea RGAs was compared with corresponding regions from RGAs isolated from *Vigna vexillata* (V vex; Accession No. AF1410151) and *Vigna unguiculata* variety IT94K-2053 Vu1, Accession No. AF1410121 and Vu2 AF1410131, the N protein of tobacco (Accession No. 2020282A), and L6 protein of flax (Accession No. T18546).

domain (Motif IV) (Mago et al. 1999) are characteristic features of NBS-LLR proteins that fall into the Group I category characterized by Pan et al. (2000). Group I NBS-LRR proteins are generally associated with TIR domains at their amino-terminus and are found only in dicotyledonous species. Another notable feature of the cowpea RGA sequences is Motif III, a short stretch of hydrophobic residues followed by two/three aspartate residues which are conserved in almost all of the sequences. This region has been suggested to be involved in stabilizing nucleotide binding with magnesium (Pan et al. 2000). Finally, all of the cowpea RGAs contain a short hydrophobic domain with a consensus amino-acid sequence G-L-P-L adjacent to the NBS.

Interestingly, several of the PCR fragments recovered (clones 447 and 494) contained sequences matching the other RGAs within the regions adjacent to the primer sites, but did not contain complete open reading frames. These fragments showed homology to various retrotransposon-like elements present in the genebank databases. Retrotransposon-like sequences have also been reported in the noncoding regions of the *Xa21* gene from rice (Song et al. 1998). It is possible that clones 447 and 494 represent remnants of R-genes, which have either lost their function due to disruption/rearrangement during evolution as a result of viral insertion.

### Genomic complexity and RFLP mapping of RGAs

In order to determine the relative complexity of the various gene families which encode the RGAs characterized above, gel blot analysis was carried out using DNA isolated from the cowpea lines IT84S-2049 and 527B. IT84S-2049 and 527B are parental lines used to generate the recombinant inbred F8 population used for mapping of the cowpea genome by Menéndez et al. (1997). One representative of each of the eight different classes of

RGA was used as hybridization probes against total genomic DNA digested with either *Eco*RI, *Eco*RV, or *Hind* III. The results of this analysis are shown in Figure 3.

When used as hybridization probes, clones 434 and 436 hybridized to a single fragment from both parental lines indicating that these are likely single copy genes. Clones 431 and 432 hybridized to 2–4 different sized fragments suggesting a small family of related sequences, whereas clones 438, 445, 468, and 490 identified multiple fragments, depending upon the enzymes used in the digest. These clones likely represent members of large multigene families. Clones 438, 468, and 490 identified similar patterns within the genomic digests, although the relative intensities of the hybridization to the individual bands differed. Similarly, identical hybridization patterns were detected with clones 432 and 445. The similar pattern of hybridization observed was consistent with their position on the dendrogram shown in Figure 3. These data suggest that clones 438, 468, and 490

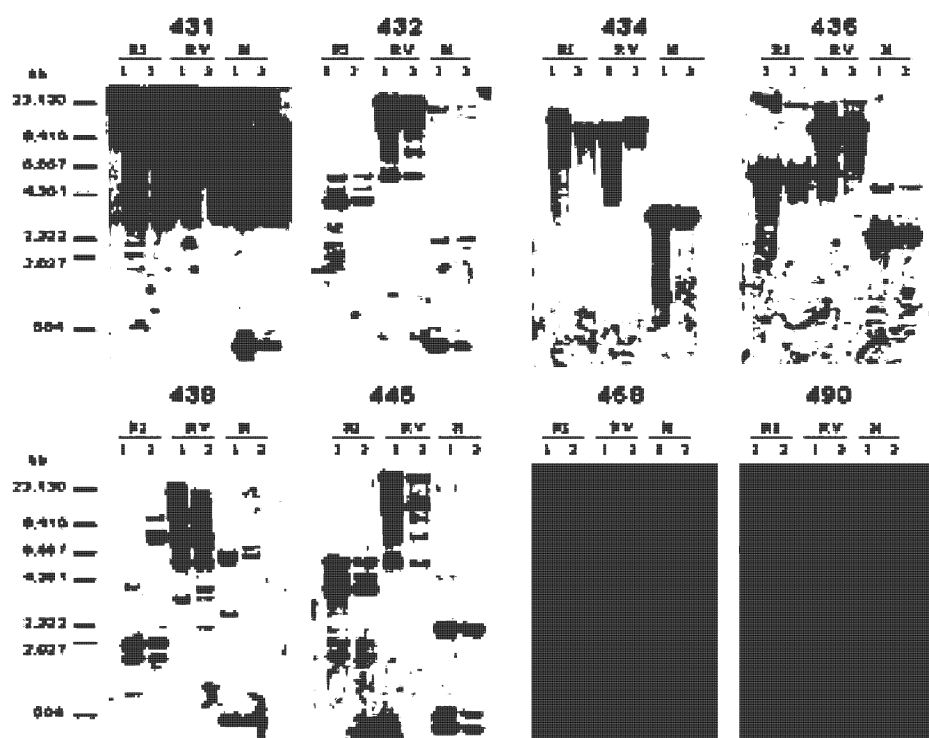


Figure 3. Complexity of the nuclear gene families encoding various cowpea RGAs. Genomic DNA from cowpea cultivars 534B (lane 1) and IT-84S-2049 (lane 2) were digested with *Eco*RI, *Eco*RV or *Hind*III, the restriction digest products separated by agarose gel electrophoresis, blotted to nylon membranes, and hybridized with  $^{32}$ P-labeled probes prepared from the various RGAs (indicated above each blot). The blots were washed under high stringency conditions and visualized by autoradiography. The approximate size of the hybridizing fragments are shown to the left in base pairs (kb).

and clones 432 and 445 likely constitute diverged members of a large family of related R-genes in the cowpea genome.

The various RGAs from each of the eight different classes were also used as hybridization probes in order to map their location onto the existing cowpea genomic map and begin determining whether polymorphisms detected by any of them cosegregate with previously mapped disease or pest resistance genes. RFLPs were detected between the two parental lines, IT84S-2049 and 527B, when gel blots were hybridized with probes prepared from clones 434, 438, 468, and 490. Hybridization probes prepared from clones 438, 468, and 490 detected RFLPs in DNA digested with either *EcoRI*, *EcoRV*, or *Hind III*, whereas hybridization probes prepared from clone 434 only detected RFLP in *EcoRI* and *EcoRV* digested DNA. Segregation analysis of the RFLP markers in the recombinant inbred F8 population derived from crossing IT84S-2049 x 527B was used to map the location of the RGAs within the cowpea genome. RGA 434 mapped to the end of linkage group 2 (LG-2), approximately 8.9 cM from D1289 (Menéndez et al. 1997). RGAs 438, 468, and 490 also mapped to LG-2, but in a different location from RGA 434. These loci clustered near the top of the linkage group between markers M185 (68.3 cM) and OC1 (52.4 cM). The large distances are due in part to the lack of marker data in this area of the genome. Interestingly, loci for cowpea severe mosaic virus (*CPSMV*) and *Fusarium oxysporum* (*FusR*) resistance also map to this general region of LG-2, suggesting that R-genes against a number of different pathogens may be clustered nearby. All the RFLPs segregated in a dominant manner except the *EcoRI* and *EcoRV* fragments of 434. None of the RGAs mapped thus far cosegregate with known disease or pest resistance loci.

## Discussion

We have identified at least eight separate classes of RGAs from cowpea based on the presence of a conserved NBS within their predicted protein coding region. Eight classes of RGAs were also reported to be present in common bean (*Phaseolus vulgaris*) (Rivkin et al. 1999) whereas nine classes of RGAs are found in soybean (*Glycine max*) (Kanazin et al. 1996). At least one class of RGA in soybean, RGA9, contained multiple stop codons and frame-shift mutations, and was thought to represent a pseudogene. Using slightly different experimental conditions and a different set of degenerate oligonucleotide primers from those reported by Kanazin et al. (1996), Yu et al. (1996) amplified 11 different classes of RGAs from soybean. As might be expected, significant similarity was found among the various RGAs in the different classes identified in the two studies.

Although further analysis is necessary, it is clear that the cowpea RGAs characterized in this investigation are homologs of the NBS-LLR type R-genes isolated from other plants including rice (Li and Chen 1999; Mago et al. 1999; Tada 1999), Brassica (Joyeux et al. 1999), maize (Collins et al. 1998), common bean (Rivkin et al. 1999), *Arabidopsis* (Speelman et al. 1998, Aarts et al. 1998), soybean (Kanazin et al. 1996; Yu et al. 1996), potato (Leister et al. 1996, 1998), wheat (Seah et al. 1998), barley (Seah et al. 1998), and lettuce (Shen et al. 1998).

The RGAs identified from cowpea appear to fall into the Group I category of NBS-LLR R-genes characterized by Pan et al. (2000) based upon the nature of conserved residues within various signature motifs within the NBS. This may reflect a bias during the amplification process for a subset of sequences, which could be recognized by the degenerate primers used during PCR. Similar observations were made by other



investigators ( Kanazin et al. 1996; Leister et al. 1996, 1998; Aarts et al. 1998; Meyers et al. 1999; Pan et al. 2000). Thus, the diversity recognized in the present study likely grossly underestimates the number of classes of R-gene sequences present in the cowpea genome. In this regard, work is underway to apply additional RGA sequences using primers corresponding to not only conserved motifs within the NBS, but within the LRR, TIR, and serine/threonine kinase domains of known R-genes. Such a strategy coupled with high resolution polyacrylamide gel electrophoresis has been successfully applied in other plants (Chen et al. 1998). The higher resolution of this system will also assist in the direct identification of polymorphisms between parental lines allowing for a greater ability to map the corresponding RGA directly onto to the existing cowpea map.

The number of classes of RGAs amplified from a plant species not only depends on the type of oligonucleotide primers used, but also depends on the variety/cultivar of a particular plant species that was used as a source of genomic DNA. For example, Speelman et al. (1998) observed that in *Arabidopsis*, RGA sequences obtained with the cultivar Col-0 were all identical and fell into one group, whereas seven different RGA sequence classes were identified when DNA from *Nd-1* was employed. Furthermore, none of the RGAs identified from *Nd-1* were identical to those isolated from Col-0. Aarts et al. (1998) also reported that in *Arabidopsis*, for some RGA fragments, the presence of an R-gene locus and a cosegregating RGA locus is often cultivar dependent.

Gel blot analysis of cowpea genomic DNA revealed that the RGAs isolated in the present study were encoded in gene families that ranged in size from one or two members to large multigene assemblies. RGAs belonging to single, or low copy number gene families have also been reported in rice (Tada 1999). In contrast, RGAs from lettuce, *Arabidopsis*, and wheat all hybridized to multiple fragments of varying intensity (Aarts et al. 1998; Seah et al. 1998; Shen et al. 1998) indicating that they all were members of large families. Interestingly, the size of the gene family recognized by various RGAs (estimated by the number of hybridizing bands) appears to vary depending upon the variety/cultivar of rice and barley used in the analysis (Leister et al. 1998).

In some cases, it has been possible to place RGAs on the respective genomic maps of the plant from which they were derived and to show that a particular RGA was linked to the known disease resistance locus. For example, Collins et al. (1998) reported a perfect segregation between RGA loci and rust resistance loci *rp1* and *rp3* in maize and Seah et al. (1998) showed linkage of RGAs from barley to the loci conferring resistance to cereal cyst nematode and corn leaf aphid. Similarly, various RGAs from rice have been shown to segregate with bacterial blight resistance genes *Xa3*, *Xa4*, *Xa21*, and *Xa10*, blast resistance genes *Pi-1(t)*, *Pi-7(t)*, and *Pi-km*, green leaf hopper resistance locus *Glh*, rice tungro spherical virus resistance locus *RTSV*, and the gall midge resistance gene *Gm2* (Mago et al. 1999; Tada 1999). In soybean, five out of 11 RGA subfamilies mapped by Yu et al. (1996) were found linked to the known soybean genes conferring resistance to potyvirus (*Rsv1* and *Rpv*), *Phytophthora* root rot (*Rps1*, *Rps2*, and *Rps3*) and powdery mildew (*rmd*). RGAs from lettuce were found linked to many downy mildew disease resistance loci (Shen et al. 1998) and Chen et al. (1998) observed a link between the RGA markers and stripe rust resistance genes in wheat. Leister et al. (1996) reported absolute linkage of RGAs to the nematode resistance locus *Gro1* and the *Phytophthora infestans* resistance locus *R7* in potato. RGAs were not only found linked to known single dominant resistance gene loci but also to quantitative

loci (QTL). In pepper, a QTL conferring partial resistance to cucumber mosaic virus (CMV) with an additive effect was found closely linked or allelic to one NBS-type family (Pflieger et al. 1999).

Of the eight different classes of RGAs recognized in cowpea, only four have been placed on the cowpea map. In the cases of the other sequences, no polymorphisms were detected using our present restriction digestion conditions, which would allow us to map them. It is entirely possible that using a different subset of available restriction enzymes, RFLPs may be recognized that allow the remaining RGAs to be mapped as well. Of the RGAs that were mapped, none showed linkage to any of the known R-genes reported in cowpea thus far, including three different loci conferring resistance to race 1 and race 3 of *Striga gesnerioides* (Ouédraogo et al. 2000). At the present time, only a small number of R-genes have been mapped in the cowpea genome. As more information becomes available, it is possible that some of the RGAs identified here will be shown to segregate with known pest and disease resistance traits.

It is also possible that some of the R-genes already mapped do not fall within the NBS-LRR category. Collins et al. (1998) reported that in their studies on maize, none of the cloned RGAs mapped to known disease resistance loci. In addition, different lines or cultivars may have different sets of NBS-LRR genes. In *Arabidopsis*, sequences hybridizing to *RPM1*, an NBS-LRR type R-gene, were totally absent from some lines (Grant et al. 1995). A similar situation was also noted in maize (Collins et al. 1998) where certain RGAs appeared to be absent in some maize lines. It has also been suggested that using a number of different mapping populations may lead to the detection of greater numbers of RGA loci than seen with only one line (Sillito et al. 2000).

The four RGAs placed on the cowpea map were all located to the end of LG-2, suggesting that some clustering of R genes may occur. In soybean, RGA6 mapped close to *Rps1* and *N*, whereas a cluster of RGAs representing five different classes mapped to the same linkage group and encompassed an R-gene cluster that included *Rmd*, *Rps2*, and *Rj2* (Kanazin et al. 1996). Clustering of RGAs has also been reported in rice, soybean, common bean, lettuce, and *Arabidopsis* (Aarts et al. 1998; Shen et al. 1998; Speulman et al. 1998; Yu et al. 1996; Kanazin et al. 1996; Rivkin et al. 1999; and Mago et al. 1999). Many of the well characterized R loci exist either as complex loci containing tandem arrays of closely linked R genes with different specificities (e.g., *M* locus for flax rust resistance [Pryor and Ellis 1993]) or major resistance complexes (MRCs) conferring resistance to different pathogens (Holub 1997). Several mechanisms have been proposed to explain the origin of complex loci and MRCs. These include gene duplication, unequal crossing over, and gene conversion (Pryor 1987; Hammond-Kosack and Jones 1997; Richter and Ronald 2000). However, more detailed study is needed to understand the clustering of RGAs in cowpea.

The studies described here are a first step to a broader understanding of the structure and organization of R-genes in cowpea, and should assist in the eventual cloning and characterization of R-genes against major agronomic pests and diseases of this important food crop.

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## References

- Aarts, M.G.M., B.L. Hekkert, E.B. Holub, J.L. Benyon, W.J. Stiekema, and A. Pereira. 1998. Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interaction* 11: 251–258.
- Aggarwal, V.D. and J.T. Ouédraogo. 1989. Estimation of cowpea yield loss from *S. gesnerioides* infestation. *Tropical Agriculture* 66: 91–92.
- Aggarwal, V.D. 1991. Research on cowpea-*Striga* resistance at IITA. Pages 90–95 in *Proceedings, International Workshop organized by IITA, ICRISAT, and IDRC on Combating Striga in Africa, 22–24 August 1988, Ibadan, Nigeria*, edited by S.K. Kim.
- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402.
- Anderson, P.A., G.J. Lawrence, B.C. Morrish, M.A. Ayliffe, J. Finnegan, and J.E. Ellis. 1997. Inactivation of the flax rust resistance gene M associated with loss of a repeated unit within the leucine-rich repeat coding region. *Plant Cell* 9: 641–651.
- Atokple, I.D.K., B.B. Singh, and A.M. Emechebe. 1995. Genetics of resistance to *Striga* and *Alectra* in cowpea. *Journal of Heredity* 86: 45–49.
- Baker, B., P. Zambriski, B. Staskawicz, and S. P. Dinesh-Kumar. 1997. Signaling in plant-microbe interactions. *Science* 276: 726–733.
- Bashir, M. and R.O. Haptom. 1996. Sources of genetic resistance in cowpea (*Vigna unguiculata* L. Walp.) to cowpea aphid-borne mosaic virus. *European Journal of Plant Pathology* 102: 411–419.
- Bent, A.F., B.N. Kunkel, D. Dhalbeck, K.L. Brown, R. Schmidt, J. Giraudt, J. Leung, and B.J. Staskawicz. 1994. *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. *Science* 265: 1856–1860.
- Botella, M.A., M.J. Coleman, D.E. Hughes, M.T. Nishimura, J.D.G. Jones, and C.R. Somerville. 1997. Map positions of 47 *Arabidopsis* sequences with sequence similarity to disease resistance genes. *Plant Journal* 12: 1197–1211.
- Cai, D., M. Kleine, S. Kifle, H. Hans-Joachim, N.N. Sandal, K.A. Marcker, R.M. Klein-Lankhorst, E.M.J. Salentijn, W. Lange, W.J. Stiekema, U. Wyss, F.M.W. Grundler, and C. Jung. 1997. Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275: 832–834.
- Chen, X.M., R.F. Line, and H. Leung. 1998. Genome scanning for resistance-gene analogs in rice, barley, and wheat by high-resolution electrophoresis. *Theoretical and Applied Genetics* 97: 345–355.
- Collins, N.C., C.A. Webb, S. Seah, J.G. Ellis, S.H. Hulbert, and A. Pryor. 1998. The isolation and mapping of disease resistance gene analogs in maize. *Molecular Plant-Microbe Interaction* 11: 968–978.
- Dixon, M.S., D.A. Jones, J.S. Keddie, C.M. Thomas, K. Harrison, and J.D.G. Jones. 1996. The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. *Cell* 84: 451–459.

- Dixon, M.S., K. Hatzixanthis, D.A. Jones, K. Harrison, and J.D.G. Jones. 1998. The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* 10: 1915–1925.
- Ehlers, J.D. and A.E. Hall. 1997. Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research* 53: 187–204.
- Fery, R.L. 1990. The cowpea: production, utilization, and research in the United States. *Horticultural Reviews* 12: 197–222.
- Gebhardt, C. 1997. Plant genes for pathogen resistance-variation on a theme. *Trends in Plant Science* 2: 243–244.
- Gish, W. and D.J. States. 1993. Identification of protein coding regions by database similarity search. *Nature Genetics* 3: 266–272.
- Gowda, B., M. Sar, X. Mu, J. Cidlowski, and T. Welbourne. 1996. Coordinate modulation of glucocorticoid receptor and glutaminase gene expression in LLC-PK1-F + Cells. *American Journal of Physiology* 270: C825–C831.
- Gowda, B.S., J.L. Riopel, and M.P. Timko. 1999. NRSA-1: a resistance gene homolog expressed in roots of nonhost plants following parasitism by *Striga asiatica* (witchweed). *Plant Journal* 20: 217–230.
- Grant, M.R., L. Godiard, E. Straube, T. Ashfield, J. Lewald, A. Sattler, R.W. Innes, and J.L. Dang. 1995. Structure of the *Arabidopsis* RPM1 gene enabling dual specificity disease resistance. *Science* 269: 843–846.
- Hammond-Kosack, K.E. and J.D.G. Jones. 1997. Plant disease resistance genes. *Annual Review of Plant Physiology, Plant Molecular Biology* 48: 575–607.
- Holub, E.B. 1997. Organization of resistance genes in *Arabidopsis*. Pages 5–26 in *The gene-for-gene relationship in host-parasite interactions*, edited by I.R. Crute, J.J. Burdon, and E.B. Holub, CAB International, Wallingford, UK.
- Jones, D.A., C.M. Thomas, K.E. Hammond-Kosack, P.J. Balint-Kurti, and J.D.G. Jones. 1994. Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* 266: 789–793.
- Joyeux, A., M.G. Fortin, R. Mayerhofer, and A.G. Good. 1999. Genetic mapping of plant disease resistance gene homologs using a minimal *Brassica napus* L. population. *Genome* 42: 735–743.
- Kanazin, V., L.F. Marek, and R.C. Shoemaker. 1996. Resistance gene analogs are conserved and clustered in soybean. *National Academy of Sciences of the United States of America Proceedings* 93: 11746–11750.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Annual Eugenics* 12: 172–175.
- Lagudah, E.S., O. Moullet, and R. Apples. 1997. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region at the *Cre3* nematode resistance locus of wheat. *Genome* 40: 659–665.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M. Daly, S.E. Lincoln, and L. Newberg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174–181.
- Lawrence, G.J., J.E. Finnegan, M.A. Ayliffe, and J.G. Ellis. 1995. The L6 gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene RPS2 and the tobacco viral resistance gene N. *Plant Cell* 7: 1195–1206.
- Leister, D., A. Ballvora, F. Salamini, and C. Gebhardt. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nature Genetics* 14: 421–428.
- Leister, D., J. Kurth, D.A. Laurie, M. Yano, T. Sasaki, K. Devos, A. Graner, and P. Schulze-Lefert. 1998. Rapid reorganization of resistance gene homologs in cereal genomes. *National Academy of Sciences of the United States of America Proceedings* 95: 370–375.

- Li, Z. Y. and S.Y. Chen. 1999. Molecular cloning, chromosomal mapping and expression analysis of disease resistance gene homologs in rice (*Oryza sativa* L.). Chinese Science Bulletin. 44: 1202–1207.
- Mago, R., S. Nair, and M. Mohan. 1999. Resistance gene analogues from rice: cloning, sequencing and mapping. Theoretical and Applied Genetics 99: 50–57.
- Marchuk, D., M. Drumm, A. Saulino, and F.S. Collins. 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. Nucleic Acids Research 19: 1154.
- Martin, G.B., S.H. Brommonschenkel, J. Chunwongse, A. Frary, M.W. Ganal, R. Spivey, T. Wu, E.D. Earle, and S.D. Tanksley. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262: 1432–1436.
- McDowell, J.M., M. Dhandaydham, T.A. Long, M.G.M. Aarts, S. Goff, E.G. Holub, and J.L. Dangl. 1998. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of *Arabidopsis*. Plant Cell 10: 1861–1874.
- Menéndez, C.M., A.E. Hall, and P. Gepts. 1997. A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred domesticated lines. Theoretical and Applied Genetics 95: 1210–1217.
- Meyers, B.C., K.A. Shen, P. Rohani, B.S. Gaut, and R.W. Michelmore. 1998. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 10: 1833–1846.
- Meyers, B.C., A.W. Dickerman, R.W. Michelmore, S. Sivaramakrishnan, B.W. Sobral, and N.D. Young. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide binding family. Plant Journal 20: 317–332.
- Milligan, S.B., J. Bodeau, J. Yaghoobi, I. Kaloshian, P. Zabel, and V.M. Williamson. 1998. The root-knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10: 1307–1319.
- Mindrinos, M., F. Katagiri, G.L. Yu, and F.M. Ausubel. 1994. The *A. thaliana* disease resistance gene RPS2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78: 1089–1099.
- Morel, J. and J.L. Dangl. 1997. The hypersensitive response and the induction of cell death in plants. Cell Death and Differentiation 4: 671–683.
- Muleba, N., J.T. Ouédraogo, and I. Drabo. 1996. Yield stability in relation to *Striga* resistance in cowpea production in West and Central Africa. African Crop Science Journal 4: 29–40.
- Muleba, N., J.T. Ouédraogo, and J.B. Tignegre. 1997. Cowpea yield losses attributed to *Striga* infestations. Journal of Agricultural Science (Cambridge) 129: 43–48.
- Ori, N., Y. Eshed, I. Paran, G. Presting, D. Aviv, S. Tanksley, D. Zamir, and R. Fluhr. 1997. The I2C1 family from the wilt disease resistance locus I2 belong to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9: 521–532.
- Ouédraogo, J.T., V. Maheshwari, D.K. Berner, C.-A. St-Pierre, F. Belzile, and M.P. Timko. 2000. Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. Theoretical and Applied Genetics. In press.
- Pan, Q., J. Wendel, and R. Fluhr. 2000. Divergent evolution of plant NBS-LRR resistance gene homologs in dicot and cereal genomes. Journal of Molecular Evolution 50: 203–213.
- Parker, J.E. and M.J. Coleman. 1997. Molecular intimacy between proteins specifying plant-pathogen recognition. Trends in Biochemical Sciences 22: 291–296.
- Parker, J.E., M.J. Coleman, V. Szabo, L.N. Frost, R. Schmidt, E.A. van der Biezen, T. Moores, C. Dean, M.J. Daniels, and J.D.G. Jones. 1997. The *Arabidopsis* downy mildew resistance

- gene RPP5 shares similarity to the toll and interleukin-1 receptor with N and L6. *Plant Cell* 9: 879–894.
- Pflieger, S., V. Lefebvre, C. Caranta, A. Blattes, B. Goffinet, and A. Palloix. 1999. Disease resistance gene analogs as candidates for QTLs involved in pepper-pathogen interactions. *Genome* 42: 1100–1110.
- Pryor, T. 1987. The origin and structure of fungal disease resistance genes in plants. *Trends in Genetics* 3: 157–161.
- Pryor, T. and J. Ellis. 1993. The genetic complexity of fungal resistance genes in plants. *Advances in Plant Pathology* 10: 281–305.
- Richter, T.E. and P.C. Ronald. 2000. The evolution of disease resistance genes. *Plant Molecular Biology* 42: 195–204.
- Rivkin, M.I., C.E. Vellajos, and P.E. McClean. 1999. Disease-resistance related sequences in common bean. *Genome* 42: 41–47.
- Salmeron, J.M., G.E.D. Oldroyd, C.M.T. Rommens, S.R. Scofield, H.-S. Kim, D.T. Lavelle, D. Dhalbeck, and B.J. Staskawicz. 1996. Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. *Cell* 86: 123–133.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning—a laboratory manual*. Second edition. Cold Spring Harbour Lab Press, New York, USA.
- Saraste, M., P.R. Sibbald, and A. Wittinghofer. 1990. The P-loop: A common motif in ATP- and GTP-binding proteins. *Trends in Biochemical Sciences* 15: 430–434.
- Seah, S., K. Sivasithamparan, A. Karakousis, and E.S. Lagudah. 1998. Cloning and characterization of a family of disease resistance gene analogs from wheat and barley. *Theoretical and Applied Genetics* 97: 937–945.
- Shen, K.A., B.C. Meyers, M. Nurul Islam-Faridi, D.B. Chin, D.M. Stelly, and R.W. Michelmore. 1998. Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. *Molecular Plant-Microbe Interaction* 11: 815–823.
- Sillito, D., I.A.P. Parkin, R. Mayerhofer, D.J. Lydiate, and A.G. Good. 2000. *Arabidopsis thaliana*: A source of candidate disease resistance genes for *Brassica napus*. *Genome* 43: 452–460.
- Singh, B.B., D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai (editors). 1997. *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Singh, B.B. and A.M. Emechebe. 1997. *Advances in research on cowpea, Striga and Alectra*. Pages 215–224 in *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, Ibadan, Nigeria.
- Song, W.-Y., G.-L. Wang, L.-L. Chen, H.-S. Kim, L.-Y. Pi, T. Holsten, J. Gardner, B. Wang, W.-X. Zhai, L.-H. Zhu, C. Fauquet, and P. C. Ronald. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270: 1804–1806.
- Song, W.-Y., L.-Y. Pi, T.E. Bureau, and P.C. Ronald. 1998. Identification and characterization of 14 transposon-like elements in the noncoding regions of members of the *Xa21* family of disease resistance genes in rice. *Molecular and General Genetics* 258: 449–456.
- Speulman, E., D. Bouchez, E.B. Holub, and J.L. Benyon. 1998. Disease resistance gene homologs correlate with disease resistance loci of *Arabidopsis thaliana*. *Plant Journal* 14: 467–474.
- Tada, Y. 1999. PCR-amplified resistance gene analogs link to resistance loci in rice. *Breeding Science* 49: 267–273.
- Thomas, C.M., D.A. Jones, M. Parniske, K. Harrison, P.J. Balint-Kurti, K. Hatzixanthis, and J.D.G. Jones. 1997. Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium*

- fulvum* identifies sequences that determine recognitional specificity in *Cf-4* and *Cf-9*. *Plant Cell* 9: 2209–2224.
- Touré, M., A. Olivier, B.R. Ntare, J.A. Lane, and C.-A. St-Pierre. 1997. Inheritance of resistance to *Striga gesnerioides* biotypes from Mali and Niger in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica* 94: 273–278.
- Touré, M., A. Olivier, B.R. Ntare, J.A. Lane, and C.-A. St-Pierre. 1998. Reaction of cowpea (*Vigna unguiculata*) cultivars to *Striga gesnerioides* races from Mali and Niger. *Canadian Journal of Plant Science* 78: 477–480.
- Traut, T.W. 1994. The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide-binding sites. *European Journal of Biochemistry* 222: 9–19.
- Van der Biezen, E.A. and J.D.G. Jones. 1998. Plant disease-resistance proteins and the gene-for-gene concept. *Trends in Biochemical Sciences* 23: 454–456.
- Varadarajan, G.S. and C.S. Prakash. 1991. A rapid and efficient method for the extraction of total DNA from the sweetpotato and its related species. *Plant Molecular Biology Reporters* 9: 6–12.
- Warren, R.F., A. Henk, P. Mowery, E. Holub, and R.W. Innes. 1998. A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell* 10: 1439–1452.
- Whitham, S., S.P. Dinesh-Kumar, D. Choi, R. Hehl, C. Corr, and B. Baker. 1994. The product of the tobacco mosaic virus resistance gene N: Similarity to toll and the interleukin-1 receptor. *Cell* 78: 1101–1115.
- Yoshimura, S., U. Yamanouchi, Y. Katayose, S. Toki, Z.X. Wang, I. Kono, N. Kurata, M. Yano, N. Iwata, and T. Sasaki. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *National Academy of Science of the USA Proceedings* 95: 1663–1668.
- Yu, Y.G., G.R. Buss, and M.A. Sahai-Maroo. 1996. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. *National Academy of Science of the USA Proceedings* 93: 11751–11756.

## 3.2

# Regeneration and genetic transformation in cowpea

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### Abstract

Over the last three decades, sporadic efforts have been made to develop regeneration and transformation systems in cowpea (*Vigna unguiculata* L. Walp). This paper reviews the progress made to date, including highlights of culture media and explants used for regeneration and chimeric gene constructs employed in transformations. Progress has been slow, mainly due to limited resources, since very few laboratories have been involved. There is an urgent need for more focused and consistent efforts to develop genotype, and tissue-culture dependent and independent approaches for obtaining stable genetic transformation in cowpea.

### Introduction

Cowpea faces several biotic and abiotic stresses for which conventional breeding alone may not provide ultimate solutions. For example, grain yield losses are mainly due to damage caused by insect pests and diseases, as well as abiotic stresses such as heat and drought (Singh et al. 1997). Plant molecular biology and genetic engineering approaches offer alternative ways of overcoming these stresses. In addition to direct transfer of genes of agronomic interest, genetic transformation techniques can be used to answer many basic questions pertaining to cowpea biology such as understanding of gene function and regulation of physiological and developmental processes (Gelvin 1998). These benefits require the development of reliable, efficient, and reproducible methods for cowpea transformation and regeneration.

Although legumes are considered “recalcitrant” to regeneration and transformation, routine protocols for obtaining stable transformants are now available for the major grain legumes such as the common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), pea (*Pisum sativum*), peanut (*Arachis hypogea*), and alfalfa (*Medicago sativa*), as well as the model legume, barrel medic (*Medicago truncata*) (Christou 1992; Puonti-Kaerlas et al. 1990; Russell et al. 1993). In contrast, development of tractable gene transfer systems in cowpea has been impeded by several constraints. Cowpea is not of major economic importance to the most technologically advanced countries in North America and Europe. This crop is mainly grown in tropical Africa, Asia, and Latin America where technical expertise and infrastructure for biotechnology research are either lacking or poor. Therefore, comparatively little work has been done to develop and optimize regeneration and transformation procedures, relative to temperate crops that are of economic importance in the North, including recalcitrant cereals (Komari et al. 1998). This paper reviews

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previous work on cowpea cell and tissue culture and transformation. It also highlights future research directions that could hold promise for the establishment of reliable gene transfer systems for a crop that has tremendous potential as a rich source of dietary protein for millions of people in Africa and Asia.

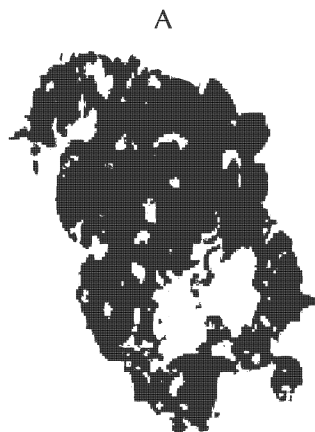
## **Cell and tissue culture**

The two methods commonly used for regeneration of plants from cell cultures are somatic embryogenesis and organogenesis. Both methods are controlled by plant hormones and other factors added to the culture medium. As the name suggests, somatic embryogenesis involves the generation of embryos from somatic tissues, such as roots, cotyledons, leaves, stems, and reproductive organs. The proliferating somatic embryos are either induced in liquid culture or on solid medium. Since embryogenic tissues are very prolific and usually originate from single cells, the embryos are considered excellent targets for transformation (Hansen and Wright 1999). This is why somatic embryogenesis is the method of choice for most genetic transformation protocols for recalcitrant legumes and monocots such as soybean, maize, and rice, respectively (Komari et al. 1998; Puonti-Kaerlas 1993; Trick et al. 1997). In cowpea, induction of somatic embryos has been reported to occur in suspension cultures of calli derived from seedling leaf explants (Ganapathi and Anand 1998). Embryogenic calli were induced on solid Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 1.5 mg/liter (mg/L) of 2-, 4-dichlorophenoxy-acetic acid (2-, 4-D). The maximum frequency of somatic embryos was obtained when callus was transferred to liquid MS medium supplemented with 0.5 mg/L 2-, 4-D. This work is repeated in other laboratories, including characterization of the stages and processes of somatic embryo development. Additionally, other explant sources other than young leaves should also be investigated for their ability to produce somatic embryos in solid and liquid suspension cultures. The basal medium developed for embryo development by Pellegrineschi et al. (1997) could form a starting point for formulating media for growth of somatic embryos *in vitro*. Growth medium supplements that enhanced embryo development included addition of sucrose, casein hydrolysate, and any one of three commonly used cytokins, namely zeatin, benzyl amino purine (BAP), and kinetin, for enhancing embryo maturation.

The establishment and maintenance of embryogenic cultures as well as recovery of plants can be an extremely labor intensive and lengthy process that has the added risk of encountering morphological abnormalities and sterility among regenerants. In contrast, multiple shoot formation via organogenesis is simpler once a suitable explant has been identified. Various laboratories have independently reported successful regeneration of cowpea by direct organogenesis from a variety of explants. These include roots, stem pieces, intact immature cotyledons or protoplasts derived from them, leaves, stem apices, stimulated shoot bud formation following gamma irradiation, or germination of mature seeds in the presence of the herbicide thidiazuron (Kartha et al. 1981; Subramaniam et al. 1968). Shoot regeneration has also been reported using axenic cowpea hypocotyls and cotyledons excised from green immature pods of advanced breeding lines and varieties developed at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, (Pellegrineschi 1997). The apical parts of the embryos were removed and the hypocotyls were transferred to regeneration

media modified from a formulation that was previously employed for embryo rescue (Pellegrineschi et al. 1997). Fertile cowpea plants have been regenerated successfully using nodal thin cell layer (TCL) explants. The TCL, approximately eight cells thick, was obtained by cutting twice over each cotyledonary node, followed by regeneration on MS media containing either 1.1 mg/L zeatin and 0.05 mg/L indole butyric acid (IBA) or 1.1 mg/L BAP and 0.05 mg/L IBA.

Among these explants, direct organogenesis from cotyledons, cotyledonary nodes, epicotyls, and primary leaves cultured on MS containing optimal levels of either N<sup>6</sup>-benzyladenine (BA) or BAP appear to be reproducible and hold promise for use in transformation (Brar et al. 1999; Muthukumar et al. 1995; Obembe et al. 2000a; Pellegrineschi 1997). At IITA, organogenesis has been obtained in several genotypes such as 90K-277, 89D-288, 83D-442, 86D-1010, 93K-624, Vita 3, and Ife Brown (Fig. 1a). Shoot meristem regeneration on MS media supplemented with either the herbicide thidiazuron or BAP has been successfully demonstrated in various genotypes, including CB5, TARS 36, SUV-2, 283, 1137, 275, TN88-63, B301, Tvu 9062, Vita 3, Vita 4, and 58-57 (Kononowicz et al. 1997; Monti et al. 1997). Brar et al. (1999) have recently reported a regeneration system that was applicable to 17 US commercial cowpea cultivars and breeding lines. Cotyledons were initiated on 1/3 MS medium containing 15–35 mg/L of BA followed by shoot regeneration on MS containing 1.0 mg/L of BA. Depending on the genotype, regeneration percentages ranged from 1 to 11, with 4–12 multiple shoots produced per explant. For rooting of cowpea plantlets, the report of Brar et al. (1999) and our results show that hormone-free MS medium works well. However, addition of 1.0 mg/L of indole-3-acetic acid (IAA) or 0.05 mg/L of naphthalene acetic acid (NAA) significantly enhances rooting and survival of plantlets in soil during the hardening and acclimatization phase following transfer from tissue culture conditions (Obembe et al. 2000a). A procedure for protoplast isolation from leaf mesophyll cells and regeneration leading to production of microcalli has also been described. However, plant regeneration from protoplast-derived calli was not possible, rendering the system inapplicable for heritable gene transfer.



**Figure 1a.** In vitro cowpea regeneration from cotyledonary nodes cultured on 0.5 mg/l of benzyl amino purine (BAP).

## Transformation systems

Currently used methods for genetic transformation have been classified into natural and non-natural or in vitro methods (Gelvin 1998). The latter include DNA microinjection (Neuhaus and Spangenberg 1990), direct DNA uptake into protoplasts with or without the use of electroporation (Shillito 1999), use of silicon carbide whiskers (Kaeppeler et al. 1990) and biolistic bombardment (Hadi et al. 1996; McCabe et al. 1998; Shillito 1999). Natural methods involve the use of viral vectors that will result in transient but not stable transformation (Choi et al. 2000; Masuta et al. 2000) and *Agrobacterium tumefaciens* T-DNA-mediated transformation (Zupan et al. 2000).

There are two major causes for the delay in the development of methods for the genetic transformation of legumes, in comparison to other dicotyledonous species. First, is the problem of recalcitrancy to regeneration by somatic embryogenesis and organogenesis, as already discussed. Secondly, transformation mediated by the soil bacterium *A. tumefaciens* was not, initially, readily applicable to legumes. Therefore, attempts at gene transfer initially focused on direct DNA delivery, especially by microprojectile (particle) bombardment which is still a popular technique since it is species- and genotype-independent (Christou 1992; McCabe et al. 1998). It has now been demonstrated that *A. tumefaciens* can efficiently transform legumes such as soybean (Trick et al. 1997). In the following section, we will review the methods and results of previous work that has been done on genetic transformation in cowpea.

## *Agrobacterium*-mediated transformation

The earliest report on *Agrobacterium*-mediated cowpea transformation was based on the tobacco leaf disc transformation method (Horsch et al. 1985). Cowpea leaf discs were punched from primary leaves obtained from 6-day old seedlings and co-cultivated with *A. tumefaciens* strains harboring tumor inducing (*Ti*)-derived vectors containing two copies of a chimeric kanamycin resistance gene (Garcia et al. 1986a, 1986b). *A. tumefaciens* strain C58CI harboring the non-oncogenic *Ti* plasmid pGV3850::1103*neo*, or its derivatives, strain LBA 1010 containing the octopine type *Ti* plasmid pTIB6 and strain LBA 958 containing a nopaline type *Ti* plasmid were all infective on cowpea leaves and stems. For selection of transformed tissues, G418 (50 mg/L) was initially incorporated into the culture media, but tissues were transferred and selected on kanamycin (100 mg/L) during later subcultures. This procedure resulted in stable transformation of callus, but no transgenic plants were regenerated. The full length cDNA of cowpea mosaic virus (*CPMV*) gene under the control of either the cauliflower mosaic virus (*CaMV* 35S) or nopaline synthase (*nos*) promoter was stably transferred and expressed in cowpea calli (Garcia et al. 1986b). The *CaMV* 35S was also more than ten times stronger than the *nos* promoter. Moreover, this work showed that 7-day old cowpea plants (stems) are susceptible to *Agrobacterium* infection, since both oncogenic *Agrobacterium* strains LBA 1010 and LBA 958 induced crown galls at wounded stem sites. An earlier study by Saedi et al. (1979) showed that cowpea seedlings fail to develop tumors after being inoculated with *A. tumefaciens* if, at times earlier than one day later, they were inoculated on the primary leaves with a cowpea mosaic virus that systemically infects them. Inoculation with buffer or with a virus that is restricted to a localized infection, or to which the cowpea is immune, did not interfere with the subsequent development of tumors. These observations indicated that systemic virus infection may induce in cowpeas a translocated substance that prevents tumor induction

by *A. tumefaciens*. Therefore, the pathology of cowpea tissues may be an important factor to consider during *Agrobacterium*-mediated transformation. We have found LBA 4404 (carrying octopine type plasmid pTiA6) to be least virulent on cowpea tissues cultured in vitro, compared to AGL1, a disarmed, hypervirulent strain harboring mannopine-type *Ti* plasmid pTiBo542. PGV3850, another disarmed, wide host range hypervirulent strain harboring a nopaline-type *Ti* plasmid pTiC58, is also very virulent on cowpea (Obembe et al. 2000b).

Only a few other reports have appeared in scientific literature concerning *Agrobacterium*-mediated transformation of cowpea since the excellent early work of Garcia et al. (1986a, 1986b). Perkins et al. (1987) and Filippone (1990) were able to show stable transformation of callus by co-cultivation of mature embryos, cotyledonary node buds, epicotyls, and apical meristems with *A. tumefaciens*. Cowpea accessions used in Filippone's work were IT81D-994, Tvu 9062, and cv VITA4. Transformations utilized the hypervirulent *A. tumefaciens* strain 6044 containing plasmid pGA472 carrying the neomycin phosphotransferase (*NPTII*) gene. Selection of transformed calli was carried out on 100 mg/L kanamycin or 50 mg/L geneticin. When cowpea embryos were used, the parts most amenable to transformation were the collar and epicotyls (Filippone 1990). Penza et al. (1991) reported the production of chimeric beta-glucuronidase (*gus*) (Jefferson 1989) in transgenic cowpea plants from mature embryos co-cultivated with *A. tumefaciens*. Using excised, ungerminated embryos was seen as a way of bypassing problems associated with regeneration from callus and differentiated tissues. Co-cultivation of embryos with the disarmed *A. tumefaciens* strain C58 (pGV2260/p35SGUSINT) carrying a *gus* intron resulted in chimeric, transformed shoots derived from axillary buds. Transformed cells were mostly located in subepidermal regions of the plant stems where the L2 meristematic layer is positioned (Fletcher and Meyerowitz 2000). Since the L2 layer potentially can contribute to flower buds, it still remains unclear why the transgenes were not transmitted through the germline, despite extensive plant propagation through nodal culture (Penza et al. 1991). The ability to regenerate cowpea *in planta* (Machuka 2000) as well as the use of positive selection systems (Joersbo et al. 1998) may provide avenues for recovery of stable transformed plants. If successful, the mature embryo co-cultivation method would be simple and easy to use for large-seeded legumes such as cowpea. Using excised leaf, epicotyl, and hypocotyl explants, stable callus transformation was obtained after co-cultivation of the explants with LBA 4404 carrying the *gus*-intron plasmid p35SGUSINT. Through co-cultivation of these explants with *A. rhizogenes*, the same workers demonstrated production of transgenic hairy roots following in vitro selection on kanamycin. Hairy root transformation was also reported earlier (Suzuki et al. 1993). These workers used a soybean cell wall protein gene (*SbPRPI*) promoter-GUS construct to show localization of *SbPRPI* in actively growing roots (apical and elongating regions) during cowpea seedling growth.

Publications on stable *Agrobacterium*-mediated transformation incorporating southern analysis of primary transformants are available (Muthukumar et al. 1996; Kononowicz et al. 1997; Monti et al. 1997). Muthukumar and co-workers used mature de-embryonated cotyledons excised from 2–3-day old seedlings. The cotyledons were co-cultivated with *A. tumefaciens* and transformed tissues selected on 25 mg/L hygromycin. Our preliminary work on the effect of hygromycin on in vitro regeneration and rooting of untransformed cowpea has established significant inhibition levels at  $\geq 20$  mg/L (Obembe et al. 2000b).

Although Muthukumar et al. (1996) reported that 15–19% of explants produced shoots on hygromycin selection medium, 13 out of 17 putative transformants died. Unfortunately, seeds from the four remaining plants failed to germinate, thus leaving us without reproducible evidence of stable transformation. Research teams at Purdue University (USA) and the University of Naples (Italy) obtained transformed T0 plants using the *gus* reporter gene as well as two useful genes. However, results from further analysis to establish proof of stable transformation and reliability of the protocols have not been forthcoming. Despite this, the work was useful in many respects. For example, tests pertaining to the virulence of *Agrobacterium* strains revealed that A281, a hypervirulent oncogenic strain, was most infective, followed by EHA 101, whereas LBA 4404 had the lowest virulence (Kononowicz et al. 1997; Monti et al. 1997). For many plant species, *Agrobacterium*-mediated transformation is relatively efficient, and a low copy number of intact, nonrearranged transgenes are frequently integrated into the plant genome (Zupan 2000). These observations and the foregoing discussion indicate that *Agrobacterium*-mediated transformation in cowpea is feasible and may yet be the preferred choice for laboratories that work or plan to begin work on genetic transformation in cowpea.

## Transformation with naked DNA

Microprojectile bombardment can be performed with any tissue of most species; however, the process is relatively inefficient because few cells are stably transformed. When DNA is delivered by this method, the conversion rate from transient expression to stable integration is estimated to be <1 to 9% (Hansen and Wright 1999; Finer et al. 2000). This method of transformation has been used on cowpea cotyledon segments, immature embryos, and shoot meristems (Ikea 1998; Kononowicz et al. 1997; Monti et al. 1997). However, convincing molecular evidence of transformation in T1 and subsequent progeny was not provided. In the work of Kononowicz et al. (1997) and Monti et al. (1997), some chimeric gene constructs used in transformations contained the phosphinothricin (*bar*) resistance, *gus* and *NPTII* genes, driven by *CaMV* 35S or *nos* promoters. Other constructs contained sequences encoding the common bean  $\alpha$ -amylase inhibitor or Bex (2S albumin) protein from Brazil nut, under control of phaseolin (seed-specific) or *CaMV* 35S (constitutive) promoters. Putative transformed tissues were selected on 50 mg/L kanamycin, which is probably not stringent enough to prevent escapes.

Plant transformation using protoplasts is laborious and requires a lot of finesse. Once isolated mechanically or using enzymes, the protoplasts can be transformed either by *Agrobacterium* or by direct DNA uptake methods, facilitated by polyethylene glycol (PEG) treatment, electroporation, or liposomes (Shillito 1999). The method has the advantage that single cells can be targeted for transformation, provided the protoplasts can regenerate into whole plants. Using cowpea leaf mesophyll protoplasts, stable, PEG-mediated protoplast co-transformation of two plasmids (pGL2 and pMONGUS) carrying the hygromycin resistance and *gus* genes were obtained. Stable transgenic microcalli were obtained that could not be regenerated into plants.

Electroporation of cells or tissues in the presence of DNA is used for the introduction of transgenes either stably or transiently into bacterial, fungal, animal, and plant cells (Lurquin 1997; Joersbo and Brunstedt 1991). The method is not often used in plant transformation because of its low reproducibility. However, owing to difficulties encountered in regenerating transformed cowpea cells and tissues in vitro, electroporation of intact

tissues and organs has been resorted to with promising results. Early work using cowpea seed-derived embryos showed that chimeric transgenes could be expressed in cowpea protoplasts and seedlings after passive or electroporation-mediated naked DNA transfer (Akella and Lurquin 1993; Penza et al. 1992). Electroporation-mediated DNA delivery into seedling tissues was also demonstrated by Dillen et al. (1995), not only in cowpea but also in other grain legumes such as the common bean, pea, and soybean. Linearization of plasmid DNA markedly increased transient DNA expression levels in intact hypocotyls and epicotyls. It is not clear what is the conversion rate from transient expression to stable integration in the plant genome using electro-transformation, but it is likely to be low (Lurquin 1997; Joersbo and Brunstedt 1990).

Chowrira et al. (1995) at Washington State University, Pullman, provided evidence of both transient and stable expression of the *gus* gene after electroporation of axillary nodal meristems *in planta*. The branches that grew out of the nodal meristems were chimeric and expressed the introduced gene up to 20 days after electroporation (Chowrira et al. 1996). Transgenic T1 pea, lentil, and cowpea plants were recovered from seeds originating on these chimeric branches as shown by Southern blot hybridization and *gus* expression. Although transgenic T2 soybean and lentil plants were also obtained, no transgenic T2 cowpeas were reported. Segregation ratios in these populations showed a strong bias against transgene presence or expression. This *in vivo* transformation approach has at least two advantages. First, electroporation equipment is cheap and the protocols are easy to optimize (Lurquin 1997). Secondly, seeds can be obtained without need for *in vitro* steps, thereby speeding up the process of generating transgenic plants. The occurrence of chimeras may be reduced if selection systems can be developed for cowpea, such as phosphinothricin (Fig. 1b) and kanamycin painting and chlorophyll fluorescence for phosphinothricin and kanamycin resistance, respectively (Eu et al. 1998; Rasco-Gaunt et al. 1999).

## Other promising transformation methodologies

The recent development of simple and routine *de novo* floral and seedling dipping and/or infiltration procedures for *Agrobacterium*-transformation in *Arabidopsis* and *M. truncata* (Clough and Bent 1998; Trieu et al. 2000) has sparked new optimism to develop similar techniques for other crops. In comparison with these model plants, cowpea has few flowers that would be the key target for transformation. Furthermore, comparatively few seeds are set. Since electroporation of cowpea nodal tissue has already been reported (Chowrira et al. 1996), work is in progress at IITA to maximize the number of vegetative and floral buds produced at every node or at the shoot apex through hormonal applications (Machuka 2000). This procedure has potential for coupling to *in planta* transformation techniques, notably electroporation and dipping of hormone-induced organs in *Agrobacterium* suspensions (Fig. 1c). Transient *gus* expression assays indicate that use of Silwet-L77 in conjunction with acetosyringone enhances expression following vacuum infiltration of excised mature cowpea embryos (Fig. 2). Experiments utilizing these additives in *Agrobacterium* seedlings and floral dipping and infiltration solutions are in progress at IITA. Selection of transformed tissue is likely to be the key obstacle for reliable adoption and exploitation of a *de novo* cowpea regeneration-based transformation system. Natural plant transformation technologies that include the use of viral vectors for transient transformation should also be explored for cowpea (Choi et al. 2000; Masuta et al. 2000). It is already known that full-length cDNA copies of cowpea mosaic virus RNA cloned downstream of the *CaMV*

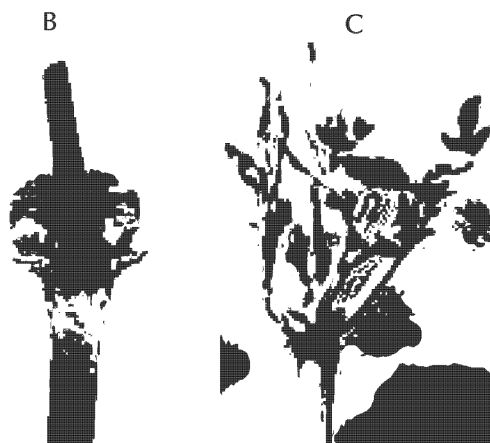


Figure 1 B, C. *In planta* cowpea regeneration of decapitated seedlings (B) and three-week old plants (C) treated with 10 mg/L BAP.

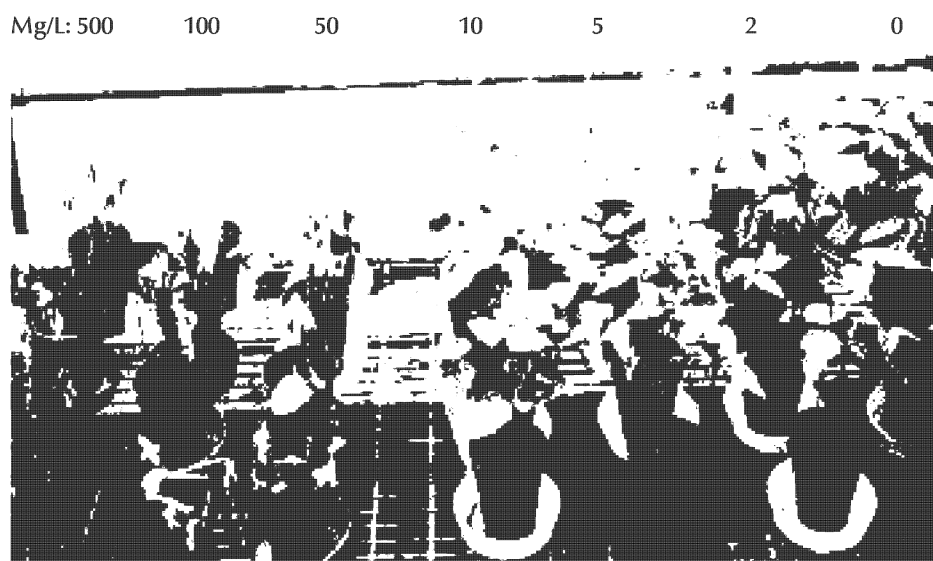


Figure 2. Phosphinothricin (PPT, Duchefa Bichemie, Haarism, Holland) painting of cowpea plants. Numbers represent PPT concentrations. As seen in this photo, survival of seedlings was nil 7 days after spraying with PPT concentrations exceeding 50 mg/L.

35S promoter give rise to cowpea mosaic virus-like symptoms when inoculated onto cowpea plants (Dessens and Lomonossoff 1993). More recently, the clover yellow vein virus has been developed as an efficient vector system for stable foreign gene expression in legumes *in planta* (Masuta et al. 2000).

Techniques for DNA delivery using silicone carbide whiskers (potential carcinogens), microinjection, and laser microbeams (Hansen and Wright 1999) require much finesse and may not be easily adapted for use in African and Asian countries which are likely to

benefit most from genetic modification in cowpea breeding. However, groups working on cowpea transformation need to experiment with techniques that combine the best attributes of *Agrobacterium*-mediated transformation (high efficiency, low copy number, and intact transgenes) with particle technologies (Gelvin 1998). For example, a novel strategy termed “Agrolistic” transformation could be used on cowpea tissues that are susceptible to transformation by particle bombardment (Ikea 1998; Kononowicz et al. 1997; Monti et al. 1997). This technique has the potential to integrate a low copy number of transgenes without integration of plasmid vector sequences (Hansen and Chilton 1996).

## Conclusions

The powerful combination of conventional and genetic modification breeding has the potential of greatly enhancing the productivity of cowpeas by increasing resistance to pests, diseases, *Striga*, and abiotic stress, as well as seed quality and other traits that impact on cowpea utilization for fodder and grain. To be of value, genetically modified plants must faithfully transmit their transgenes. From the works surveyed in this review, it is apparent that this has not been achieved in cowpea. Recalcitrance to plant regeneration of transformed tissues, epidermal transformation, and transgene instability are likely causes of failure to achieve stable transformation and transgene transmission. Improvements in existing cell and tissue culture systems to allow regeneration of stable transformed cowpea plants is urgently needed. With so many available advances and new breakthroughs in plant transformation technologies, it is hoped that cowpea’s stubborn resistance to genetic engineering will soon be overcome.

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## References

- Akella, V. and P.F. Lurquin. 1993. Expression in cowpea seedlings of chimeric transgenes after electroporation into seed-derived embryos. *Plant Cell Reports* 12: 110–117.
- Brar, M.S., J.M. Al-Khayri, T.E. Morelock, and E.J. Anderson. 1999. Genotypic response of cowpea *Vigna unguiculata* (L.) to *in vitro* regeneration from cotyledon explants. *In Vitro Cellular Developmental Biology* 35: 8–12.
- Choi, I-R., D.C. Stenger, T.J. Morris, and R. French. 2000. A plant virus vector system for expression of foreign genes in cereals. *The Plant Journal* 23: 547–556.
- Chowrira, G., V. Akella, and P.F. Lurquin. 1993. Transformation of peas and lentils by *in vivo* electroporation of nodal meristems. *Western Society of Crop Science Abstracts*. 2p.
- Chowrira, G., V. Akella, and P.F. Lurquin. 1995. Electroporation-mediated gene transfer into intact nodal meristems *in planta*: Generating transgenic plants without *in vitro* tissue culture. *Molecular Biotechnology* 3: 17–23.
- Chowrira, G., V. Akella, P.E. Fuerst, and P.F. Lurquin. 1996. Transgenic grain legumes obtained by *in planta* electroporation-mediated gene transfer. *Molecular Biotechnology* 5: 85–96.
- Christou, P. 1992. Genetic engineering and *in vitro* culture of crop legumes. Technomic Publishing, Pennsylvania, USA. 307 p.
- Clough, S.J. and A.F. Bent. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* 16: 735–743.



- Dessens, J.T. and G.P. Lomonosoff. 1993. Cauliflower mosaic virus 35S promoter-controlled DNA copies of cowpea mosaic virus RNAs are infectious on plants. *Journal of General Virology* 74: 889–892.
- Dillen, W., G. Engler, M.C. Van Montagu, and G. Angenon. 1995. Electroporation-mediated DNA delivery to seedling tissues of *Phaseolus vulgaris* L. (common bean). *Plant Cell Reports* 15: 119–124.
- Eu, Y.J., M.H. Lee, H.S. Chang, T.H. Rhew, H.Y. Lee, and C.H. Lee. 1998. Chlorophyll fluorescence assay for kanamycin resistance screening in transgenic plants. *Plant Cell Reports* 17: 189–194.
- Filippone, E. 1990. Genetic transformation of pea and cowpea by co-cultivation of tissues with *Agrobacterium tumefaciens* carrying binary vectors. Pages 175–181 in *Cowpea genetic resources, contributions in cowpea exploration, evaluation and research from Italy and IITA*, edited by N.Q. Ng and L.M. Monti. IITA, Ibadan, Nigeria.
- Filippone, E. and R. Penza. 1992. *Agrobacterium tumefaciens*-mediated gene transfer. Pages 197–202 in *Biotechnology: enhancing research on tropical crops in Africa*, edited by G. Thottapilly, L.M. Monti, D.R. Mohan Raj, and A.W. Moore. CTA/IITA copublication. IITA, Ibadan, Nigeria.
- Finer, J.J., K.R. Finer, and T. Ponappa. 2000. Particle bombardment-mediated transformation in Current topics in microbiology and immunology plant biotechnology: new products and applications, edited by J. Hammond, P.B. McGarvey, and V. Yusibov. Springer-Verlag Vol. 240.
- Fletcher, C. and E.M. Meyerowitz. 2000. Cell signaling within the shoot meristem. *Current Opinion in Plant Biology* 3: 23–30.
- Ganapathi, A. and P. Anand. 1998. Somatic embryogenesis from young leaves of cowpea (*Vigna unguiculata* (L.) Walp. (Abstracts) in *Plant Biotechnology and in vitro biology for the 21st Century*. IX International Congress on Plant Tissue and Cell Culture, 14–19 June 1998, Jerusalem, Israel,
- Garcia, J.A., J. Hillie, and R. Goldbach. 1986a. Transformation of cowpea *Vigna unguiculata* cells with an antibiotic resistance gene using a Ti-plasmid-derived vector. *Plant Science* 44: 37–46.
- Garcia, J.A., J. Hillie, and R. Goldbach. 1986b. Transformation of cowpea *Vigna unguiculata* cells with a full length DNA copy of cowpea mosaic virus m-RNA. *Plant Science* 44: 89–98.
- Gelvin, S.B. 1998. The introduction and expression of transgenes in plants. *Current Opinion in Biotechnology* 9: 227–232.
- Hadi, M.Z., M.D. McMuller, and J.J. Finer. 1996. Transformation of 12 different plasmids into soybeans via particle bombardment. *Plant Cell Reports* 15: 500–505.
- Hansen, G. and M.D. Chilton. 1996. “Agrolistic” transformation of plant cells: integration of T-strands generated *in planta*. *Proceedings National Academic Science USA* 93: 14978–14983.
- Hansen, G. and S.M. Wright. 1999. Recent advances in the transformation of plants. *Trends in Plant Science* 4: 226–231.
- Horsch, R.B., J.E. Fry, N. Hoffman, D. Eichlitz, S.G. Rogers, and R.T. Fraley. 1985. A simple and general method of transferring genes into plants. *Science* 227: 1229–1231.
- Ikea, J. 1998. Transformability of cowpea (*Vigna unguiculata* L. Walp.) by particle bombardment. PhD Thesis, The University of Ibadan, Ibadan, Nigeria.
- Jefferson, R.A. 1989. The gus reporter gene system. *Nature* 342: 837–838.
- Joersbo, M. and J. Brunstedt. 1990. Quantitative relationship between parameters of electroporation. *Journal of Plant Physiology* 137: 169–174.
- Joersbo, M. and J. Brunstedt. 1991. Electroporation: Mechanism and transient expression, stable transformation and biological effects in plant protoplasts. *Physiologia Plantarum* 81: 256–264.
- Joersbo, M., I. Donaldson, J. Kreiberg, S.G. Petersen, J. Brunstedt, and F.T. Okkels. 1998. Analysis of mannose selection used for transformation of sugar beet. *Molecular Breeding* 4: 111–117.
- Kaepllar, H.F., W. Gu, D.A. Somers, H.W. Rines, and A.F. Cockburn. 1990. Silicon carbide fibre-mediated DNA delivery into plant cell. *Plant Cell Reports* 8: 415–418.

- Kartha, K.K., K. Pahl, N.L. Leung, and L.A. Mroginski. 1981. Plant regeneration from meristems of grain legumes: soybean, cowpea, peanut, chickpea, and bean. *Canadian Journal of Botany* 59: 1672–1574.
- Komari, T., Y. Hiei, Y. Ishida, T. Kumashiro, and T. Kubo. 1998. Advances in cereal gene transfer. *Current Opinion in Plant Biology* 1: 161–165.
- Kononowicz, A.K., K.T. Cheah, M.L. Narasimhan, L.L. Murdock, R.E. Shade, M.J. Chrispeels, E. Filippone, L.M. Monti, R.A. Bressan, and P.M. Hasegawa. 1997. Developing a transformation system for cowpea (*Vigna unguiculata* L.Walp.). Pages 361–371 in *Advances in Cowpea Research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Lurquin, P.F. 1997. Gene transfer by electroporation. *Molecular Biotechnology* 7: 15–35.
- Machuka, J. 2000. Cowpea regeneration *in planta*: can it be coupled to transformation? Page 20 in *Abstracts, World Cowpea Research Conference III*, 4–7 September 2000, Ibadan, Nigeria.
- Masuta, C., T. Yamana, Y. Tacahashi, I. Uyeda, M. Sato, and T. Matsumura. 2000. Development of clover yellow vein virus as an efficient stable gene expression system for legume species. *The Plant Journal* 23: 547–556.
- McCabe, D.E., W.F. Swain, B.J. Martinell, and P. Christou. 1998. Stable transformation of soybean (*Glycine max*) by particle acceleration. *Biotechnology* 6: 923–926.
- Monti, L.M., L.L. Murdock, and G. Thottappilly. 1997. Opportunities for biotechnology in cowpea. Pages 341–351 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays and with tobacco tissue culture. *Physiologia Plantarum* 15: 473–497.
- Muthukumar, B., M. Mariamma, and A. Gnanam. 1995. Regeneration of plants from primary leaves of cowpea. *Plant Cell Tissue and Organ Culture* 42: 153–155.
- Muthukumar, B., M. Mariamma, K. Valuthambi, and A. Gnanam. 1996. Genetic transformation of cotyledon explants of cowpea (*Vigna unguiculata* L. Walp.) using *Agrobacterium tumefaciens*. *Plant Cell Reports* 15: 980–985.
- Neuhaus, G. and G. Spangenberg. 1990. Plant transformation by microinjection techniques. *Physiologia Plantarum*. 79: 213–217.
- Neuhaus, G., G. Spangenberg, O. Mittelsten-Scheid, and H.G. Schweizer. 1987. Transgenic rape seed plants obtained by the microinjection of DNA into microspore-derived embryoids. *Theoretical and Applied Genetics* 75: 30–36.
- Obembe, O.O., M. Kadiri, and J. Machuka. 2000a. Induction of multiple shoots and regeneration from cotyledonary nodes and epicotyls. Page 20 in *Abstracts, World Cowpea Research Conference III*, 4–7 September 2000, Ibadan, Nigeria. 32 p.
- Obembe, O.O., A. Adesoye, M. Kadiri, and J. Machuka. 2000b. The role of antibiotics in the control of *Agrobacterium* and as potential selective agents in *Agrobacterium*-mediated transformation of cowpea. Page 19 in *Abstracts, World Cowpea Research Conference III*, 4–7 September 2000, Ibadan, Nigeria. 32 p.
- Pellegrineschi, A. 1997. *In vitro* plant regeneration via organogenesis of cowpea [*Vigna unguiculata* (L.) Walp.]. *Plant Cell Reports* 17: 89–95.
- Pellegrineschi, A., C.A. Fatokun, G. Thottappilly, and A.A. Adepoju. 1997. Cowpea embryo rescue. Influence of culture media composition on plant recovery from isolated immature embryos. *Plant Cell Reports* 17: 133–138.
- Penza, R., V. Akella, and P.F. Lurquin. 1992. Transient expression and histological localization of a *gus* chimeric gene after direct transfer to mature cowpea embryos. *Biotechniques* 13: 576–580.

- Penza, R., P.F. Lurquin, and E. Filippone. 1991. Gene transfer by co-cultivation of mature embryos with *Agrobacterium tumefaciens*: application to cowpea (*Vigna unguiculata* L. Walp.). *Journal of Plant Physiology* 138: 39–43.
- Perkins, E.J., C.M. Stiff, and P.F. Lurquin. 1987. Use of *Alcaligenes eutropus* as a source of genes for 2-, 4-D resistance in plants. *Weed Science* 35: 12–18.
- Puonti-Kaerlas, J. 1993. Methods in grain legume transformation. *Grain Legumes* 2: 14–15.
- Puonti-Kaerlas, J., T. Eriksson, and P. Engstrom. 1990. Production of transgenic pea (*Pisum sativum* L.) plants by *Agrobacterium tumefaciens*-mediated gene transfer. *Theoretical and Applied Genetics* 84: 443–450.
- Rasco-Gaunt, S., A. Riley, P. Lazzeri, and P. Barcelo. 1999. A facile method for phosphinothricin (PPT)-resistant wheats. *Molecular Breeding* 5: 255–262.
- Russell, D.R., K.M. Wallace, J.H. Bathe, B.J. Martinell, and D.E. McCabe. 1993. Stable transformation of *Phaseolus vulgaris* via electric discharge-mediated acceleration. *Plant Cell Reports* 12: 165–169.
- Saedi, D., G. Bruening, C.I. Kado, and J.C. Dutra. 1979. Tumor induction by *Agrobacterium tumefaciens* prevented in *Vigna sinensis* seedlings systemically infected by ribonucleic acid viruses. *Infection and Immunology* 23: 298–304.
- Shillito, R. 1999. Methods of genetic transformations: electroporation and polyethylene glycol treatment. Pages 9–20 in *Molecular improvement of cereal crop*, edited by I. Vasil. Kluwer, Dordrecht, The Netherlands.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–50 in *Advances in Cowpea Research* edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), Ibadan, Nigeria.
- Subramaniam, M.K., S. Subramaniam, P.M. Gopinath, K.C. Gupta, and S. Vasantha. 1968. Histogenesis, organogenesis and morphogenesis in callus cultures of *Trigonella foenum-graecum* and *Vigna unguiculata* L. *Current Science* 37: 398–399.
- Suzuki, H., T. Fowler, and M. Tierney. 1993. Deletion analysis and localization of SbPRP1, a soybean cell wall protein gene, in roots of transgenic tobacco and cowpea. *Plant Molecular Biology* 21: 109–119.
- Trick, N.U., R.D. Dinkins, E.R. Santarem, R.D.V. Samoylo, C.A. Meurer, D.R. Walker, W.A. Parrot, J.J. Finer, and G.B. Collins. 1997. Recent advances in soybean transformation. *Plant Tissue Culture and Biotechnology* 3(1): 9–26.
- Trieu, A.T., S. H. Burleigh, I.E. Kardailsky, W.K. Maldonado-Mendoza, L.A. Versaw, H. Shin, A. Blaylock, T. Chion, H. Katagi, G.R. Dewbare, D. Weigel, and M.J. Harrison. 2000. Transformation of *Medicago truncata* via infiltration of seedlings or flowering plants with *Agrobacterium*. *The Plant Journal* 22 (6): 531–541.
- Zupan, J., T.R. Muth, O. Draper, and P. Zambryski. 2000. The transfer of DNA from *Agrobacterium* into plants: a feat of fundamental insights. *The Plant Journal* 23: 11–28.

### 3.3

## Molecular cloning in cowpea: perspectives on the status of genome characterization and gene isolation for crop improvement

M.P. Timko<sup>1</sup>

### Abstract

Cowpea (*Vigna unguiculata* L.) is a grain legume of significant economic importance worldwide and for many people in the semiarid areas of West and Central Africa, it is the major source of dietary protein necessary for human nutrition. As a result of its widespread use and economic importance, numerous programs aimed at the improvement of various agronomic and nutritional quality traits are underway. Included among these initiatives are selective breeding programs aimed at identifying new sources of disease and pest resistance from wild species for introgression into cultivated varieties, gene isolation, and characterization studies aimed at understanding the factors controlling plant growth and development, as well as plant cell culture, and genetic transformation programs aimed at the direct manipulation of traits through genetic engineering. Recent progress on genome organization and evolution, and gene characterization in cowpea is reviewed and prospects for future improvement of cowpea through biotechnological applications are discussed.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is a food legume of significant economic importance worldwide. It is grown in North and South America, Africa, Europe, and Asia, primarily in the semiarid and humid tropical regions lying between 35 °N and 30 °S of the equator. It is estimated that cowpea is now cultivated on at least 12.5 million hectares with an annual production of over 3 million tonnes of grains worldwide (Singh et al. 1997). Currently, Central and West Africa account for more than 64% of the total area under cowpea cultivation, followed by South America, Asia, East, and South Africa (Fery 1985; Singh et al. 1997). In the United States, cowpea is a crop of minor significance, grown on just over 80 000 hectares (Fery 1985; 1990).

Part of the popularity of cowpea as a food staple for people in the semiarid and humid tropical regions of Africa stems from the fact that it is relatively drought-tolerant, performing well under conditions where most other food legumes do not. Its unique ability to fix nitrogen even in very poor soils with low organic matter also contributes to its widespread use among farmers (Singh et al. 1997). Like most crop plants, cowpea production is limited by numerous biotic and abiotic factors. Both severe heat and drought limit cowpea productivity (Marfo and Hall 1992). Cowpea is also attacked by a wide range of insect pests, microbial and fungal diseases, nematodes, and two different parasitic angiosperms (Bashir and Haptom 1996; Ehlers and Hall 1997; Fery and Singh 1997; Singh and Emechebe 1997).

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As a result of its widespread use, numerous programs directed at the improvement of agronomic and nutritional quality traits are underway, including breeding programs aimed at screening wild and cultivated germplasm for sources of disease and pest resistance genes as well as plant cell culture and genetic transformation programs aimed at direct manipulation of traits through genetic engineering. With few exceptions, the application of biotechnology to cowpea improvement offers the promise of increased productivity by speeding the development of varieties that yield more, are more resistant to biotic and abiotic stresses, and are more economical and efficient to produce. This paper reviews some recent advances in genome characterization, gene isolation, and genetic manipulation of cowpea, and offers perspectives on emerging areas of research. Discussions on other related aspects of cowpea research, including genetics, breeding, and cell culture are also in this paper.

## Phylogeny and genome organization

Cowpea (*Vigna unguiculata*) is one of several important cultivated species which constitute the genus *Vigna*. Other members include mung bean (*V. radiata*), azuki bean (*V. angularis*), blackgram (*V. mungo*), and the bambara groundnut (*V. subterranea*). The genus was initially divided into several subgenera by Marechal et al. (1978) based upon morphological characteristics, extent of genetic hybridization/reproductive isolation, and geographic distribution of species. The major groupings consist of the African subgenera *Vigna* and *Haydonia*, the Asian subgenus *Ceratotropis*, and the American subgenera *Sigmoidotropis* and *Lasiopron*. Under the scheme proposed by Marechal and his colleagues, cultivated cowpea was placed in the subgenus *Vigna*, whereas mung bean and blackgram were placed in the Asian subgenus.

The development and use of biochemical-based analytical techniques and molecular marker technologies, such as analysis of restriction fragment length polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs) (Williams et al. 1990), amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995), minisatellites (Sonnante et al. 1994), and simple sequence repeats (SSRs) (Akkaya et al. 1992, 1995), have greatly facilitated the analysis of the structure of plant genomes and their evolution and have contributed significantly to our understanding of cowpea genome organization. Using RFLP analysis, Fatokun et al. (1993a) analyzed 18 *Vigna* species including five of the subgenus *Ceratotropis* to determine the taxonomic relationship between the subgenus *Ceratotropis* and other subgenera. These investigators showed that a high level of genetic variation exists within the genus, with a remarkably higher amount of variation associated with *Vigna* species from Africa relative to those from Asia. Their data supported the taxonomic separation of the Asian and African genera as proposed by Marechal et al. (1978) and underscored the previously held viewpoint that Africa is likely to be the center of diversity for *Vigna*. Generally, the placement of species and subspecies based upon molecular taxonomic procedures by Fatokun et al. (1993a) substantiated prior classifications based on classical taxonomic criteria, such as morphological and reproductive traits.

Genetic variation in the subgenus *Ceratotropis* was subsequently reinvestigated by Kaga et al. (1996a) using RAPD analysis. Examining 23 accessions of five species within the subgenus *Ceratotropis* for polymorphisms, these investigators identified approximately 404 amplified fragments capable of providing comparative information. Based

on the degree of polymorphism at these informative loci, these investigators were able to separate the accessions into two main groups differing by approximately 70% at the molecular level. Within each of the main groups, the accessions could be further divided into five subgroups which composition were in complete agreement with their taxonomic species classifications.

Sonnante et al. (1996) examined isozyme variation between *V. unguiculata* and other species in the subgenus *Vigna* and showed that *V. unguiculata* was more closely related to *V. vexillata*, a member of the subgenus *Plectotropis*, than to any other species belonging to the section *Vigna*. This is not surprising since *V. vexillata* is thought to be the intermediate species between African and Asian *Vigna* species. Vaillancourt and Weeden (1996) reached a similar conclusion. Based on their analysis of variation in chloroplast DNA structure (Vaillancourt and Weeden 1992), and isozyme polymorphisms (Vaillancourt et al. 1993), these investigators suggested that *V. vexillata* and *V. reticulata* were the closest relatives of *V. unguiculata*. While the close relationship between *V. unguiculata* and *V. vexillata* proposed by Vaillancourt and Weeden (1996) is consistent with previous observations (Marechal et al. 1978), *V. reticulata* was placed in a different cluster based upon RFLP analysis (Fatokun et al. 1993a).

Polymorphisms in 21 different enzyme systems were used by Pasquet (1999) to evaluate the relationship among 199 accessions of wild and cultivated cowpea differing in breeding system and growth characteristic (i.e., annual versus perennial growth habit). Based on these allozyme data, perennial subspecies of cowpea (spp. *unguiculata* var. *unguiculata*) were shown to form a coherent group closely related to annual forms (ssp. *unguiculata* var. *spontanea*). Among the 10 subspecies studied, *V. unguiculata* var. *spontanea* and ssp. *pubescens* were the closest taxa to be cultivated into cowpea. Most recently, Ajibade et al. (2000) used inter simple sequence repeat (ISSR) DNA polymorphism analysis to study the genetic relationships among 18 *Vigna* species. The study showed that closely related species within each subgenus clustered together (e.g., *V. umbellata* and *V. angularis* (subgenus *Ceratotropis*), *V. adenantha* and *V. caracalla* (subgenus *Sigmoidotropis*), and *V. luteola* and *V. ambacensis* (subgenus *Vigna*). Cultivated cowpea was grouped closely with the wild subspecies of *V. unguiculata*, and the entire species was separated from its most closely allied species *V. triphylla* and *V. reticulata*. ISSR polymorphism analysis split *Vigna* into groupings that differed in their composition from previous classifications. For example, the subgenus *Vigna* was split into three lineages, with *V. unguiculata*/*reticulata*/*friesorum* forming one group, *V. luteola*/*ambacensis* forming a second, and *V. subterranea* being far from the other two. *Ceratotropis* was split into two sections, with three species (*V. radiata*, *V. mungo*, and *V. aconitifolia*) in one section and two species (*V. angularis* and *V. umbellata*) in a second section. While such groupings had been suggested previously (Marechal et al. 1978; Fatokun et al. 1993a; Vaillancourt and Weeden 1996), it should be noted that ISSR analysis was not as effective at resolving genetic distance relationships at the subgeneric level as it was at resolving relationships at the species level and below. Therefore, the authors note that their conclusions regarding subgeneric classifications should be taken with caution. Thus, there is still considerable need to develop appropriate strategies and molecular techniques to resolve exact taxonomic relationships among members of this important genus.

Repetitive DNA sequences have been shown to represent a substantial fraction of the nuclear genome of all higher plant species and to account for much of the variation in

genomic DNA content observed among species (Flavell et al. 1994). Many of the repeat sequences found in plant genomes appear to have originated through the activity of transposable elements (transposons), that move either by first forming an RNA intermediate (i.e., retrotransposons [Boeke et al. 1985]) or by direct DNA transposition intermediates (i.e., transposons [Federoff 1989]). To gain insight into the genomic organization and evolution of species within *Vigna*, Galasso et al. (1997) examined the genomic organization and distribution of Ty1-*copia* type retrotransposons in seven different species and subspecies of *Vigna* and several related leguminous plants. Gel blot analysis of genomic DNA from *V. unguiculata*, *V. luteola*, *V. oblongifolia*, *V. ambacensis*, and *V. vexillata* probed with radioactively-labeled probes to the reverse transcriptase gene amplified from *V. unguiculata* subsp. *unguiculata*, *V. unguiculata* subsp. *dekintiana*, *V. luteola*, and *V. vexillata*, showed variable hybridization patterns and intensities generally correlating with their previously defined taxonomic position. Fluorescent *in situ* hybridization analysis of the distribution of the Ty1-*copia* type sequences showed that these elements represented a major fraction of the cowpea genome and were dispersed relatively uniformly over all the chromosomes. Little or no hybridization was found associated with centromeric, subtelomeric, and nucleolar organizing regions of the chromosomes, indicating that these portions of the genome may not be suitable sites for transposition. Comparisons of retrotransposon structural similarity between *Vigna* and other genera of legumes generally supported the subdivision of the tribes *Phaseoleae* and *Vicieae*, with greater homology seen between members of the *Cicereae* and *Phaseoleae* than *Cicer* species and those from the *Vicieae* (Galasso et al. 1997).

In addition to providing insight into phylogenetic relationships, molecular marker technologies have also been used in the construction of genetic maps for most of the important crop species, including cowpea. The first attempt to generate a comprehensive linkage map for cowpea was by Fatokun et al. (1993b) who used polymorphisms detected by 87 random genomic DNA fragments, five cDNAs, and RAPDs to generate a map consisting of ten linkage groups spanning 680 cM. Improvement on this initial map was made by Menéndez et al. (1997) who were able to develop a linkage map for *V. unguiculata* consisting of 181 loci falling into 12 linkage groups. The resolution of the map was to approximately 6.4 cM between loci. Similarly, Menancio-Hautea et al. (1993a, b) used RFLP analysis to construct a genome map of mung bean (*V. radiata*). The map consisted of 172 markers placed into 11 linkage groups and provided 1570 cM coverage with an average distance of 9 cM between loci. It is worth noting that even at these early stages of genome comparison, significant colinearity was recognized between the cowpea and mung bean genomes (Menacio-Hautea et al. 1993b). A total of 132 markers (108 RAPDs, 19 RFLPs, and five morphological markers) have been mapped in azuki bean using an interspecific population generated from a cross of *V. angularis* × *V. nakashimae* (Kaga et al. 1996b). Comparison of the linkage map of azuki bean with those of mung bean and cowpea using 20 RFLP markers indicated that, as might be expected, the three genomes have many linkage blocks in common.

Among the most recent developments in understanding cowpea genome organization is the report by Li et al. (1999) who used DNA amplification fingerprinting (DAF) and AFLP analysis to identify additional molecular markers segregating in the F<sub>8</sub> recombinant inbred population derived from a cross between IT84S-2049 and 524B (Menéndez et al. 1997). These researchers screened 400 randomly generated DAF decamers and 128

AFLP primer combinations, and were able to place 57 DAF and 90 AFLP markers to the existing cowpea genetic map. Studies are underway to further saturate the map with additional markers to increase its utility for future map-based cloning activities in cowpea. Additionally, a map of the wild relative of cowpea *V. vexillata* has also been generated (Ogundiwin et al. 2000) adding even greater breadth to our understanding of genomic relationships in *Vigna*.

The considerable progress made in recent years on the development of genomic maps for cowpea and related species is reflected in the ever increasing number of growth, yield, and resistance trait loci that have now been located within the various genomes (Fatokun et al. 1992, 1997; Myers et al. 1996; Roberts et al. 1996; Menéndez et al. 1997; Ouédraogo et al. 2001; Gowda et al. 2002). Table 1 lists the various agronomic and disease resistance trait loci that have now been placed on the cowpea genetic map.

**Table 1. Agronomic, growth habit, and disease and pest resistance trait loci currently placed on the cowpea genetic map<sup>1</sup>.**

| Linkage group | Locus designation              | Character or function                                  |
|---------------|--------------------------------|--|
| LG1           | PodL                           | Pod length   |
|               | SW                             | Seed weight (100 seed)                                 |
|               | C                              | General flower color factor                            |
|               | P                              | Pod pigmentation                                       |
|               | Er                             | Pod attachment (erect pod)                             |
|               | <i>Rac1 (Rac2)</i>             | Resistance to <i>Aphis craccivora</i> (aphid)          |
| LG2           | Rsg1, Rsg2, Rsg4               | Race-specific resistance to <i>Striga gesnerioides</i> |
|               | PodN                           | Pod number per plant                                   |
|               | (NTF)                          | Nodes to 1st flower (D1301a)                           |
|               | CPSMV ( <i>ims</i> )           | Cowpea severe mosaic virus resistance                  |
|               | <i>FusR</i>                    | Resistance to <i>Fusarium oxysporum</i>                |
|               | CPMV                           | Cowpea mosaic virus                                    |
|               | RGA-434                        | Resistance gene analogs (pathogen unknown)             |
|               | RGA-438,468,490                | Resistance gene analogs (pathogen unknown)             |
| LG3           | SBMV( <i>sbv-1, 2</i> )        | Resistance to southern bean mosaic virus               |
| LG5           | PodN                           | Pod number per plant                                   |
|               | SW                             | Seed weight (100 seed; OB6a)                           |
| LG6           | SW                             | Seed weight (100 seed)                                 |
|               | Rk ( <i>Nem</i> <sup>®</sup> ) | Root-knot nematode ( <i>Meloidogyne incognita</i> )    |
| Resistance    | BECMV                          | Resistance to blackeye cowpea mosaic virus             |
| LG7           | Maturity                       | Maturity   |
|               | 50% FL                         | 50% flowering  |
|               | SW                             | Seed weight  |
|               | Dehydrin                       | Dehydrin protein                                       |
| LG8           | Height                         | Plant height   |
| LG9           | PodN                           | Pod number per plant                                   |
| LG12          | GluC                           |  |

<sup>1</sup>Data taken from Fatokun et al. (1992, 1993b, 1997), Myers et al. (1996); Roberts et al. (1996); Menéndez et al. (1997); Ouédraogo et al. (2001); Gowda et al. (2002).



## Gene isolation and characterization

Developing innovative biotechnologies for cowpea improvement requires not only an understanding of genome organization and complexity, but also of gene structure and function. In the genus *Vigna*, only limited progress has been made in basic gene discovery and only a modest number of studies have appeared in the literature examining differential gene regulation during growth and development or in response to biotic or abiotic stress. Table 2 summarizes the number of nucleotide and translated protein sequences currently available from cowpea and related species in the genus *Vigna* in comparison to other legumes and major crops. Mung bean (*V. radiata*) and cowpea (*V. unguiculata*) lead among *Vigna* species in the number of gene sequences available to researchers. Basic information available for these species is between 2- and 5-fold lower than that available for pea (*Pisum sativum*), French bean (*Phaseolus vulgaris*), and alfalfa (*Medicago sativum*), and over 500-fold lower than currently available for soybean (*Glycine max*). A large proportion of the nucleic acid sequences present in the databases for cowpea and mung bean are either ribosomal RNA coding and spacer regions or nuclear genomic sequences of unidentified function developed for RFLP mapping, further accentuating

**Table 2. Number of nucleotide and deduced amino acid sequences available for various *Vigna* species and related plants<sup>†</sup>.**

| <i>Vigna</i> species                                |  |
|---|--|
| <i>Vigna aconitifolia</i> - 11 (23)                 | <i>Vigna oblongifolia</i> - 2 (0)                    |
| <i>Vigna adenantha</i> - 3 (0)                      | <i>Vigna parviflora</i> - 1 (0)                      |
| <i>Vigna angularis</i> (azuki bean) - 39 (29)       | <i>Vigna parvifolia</i> - 1 (0)                      |
| <i>Vigna caracalla</i> - 1 (0)                      | <i>Vigna peduncularis</i> - 2 (0)                    |
| <i>Vigna gentryi</i> - 1 (0)                        | <i>Vigna populnea</i> - 1 (0)                        |
| <i>Vigna glabrescens</i> - 2 (0)                    | <i>Vigna racemosa</i> - 2 (0)                        |
| <i>Vigna hosei</i> - 2 (0)                          | <i>Vigna radiata</i> (mung bean) - 192 (167)         |
| <i>Vigna kirkii</i> - 2 (0)                         | <i>Vigna radiata</i> subsp. <i>sublobata</i> - 2 (0) |
| <i>Vigna lasiocarpa</i> - 2 (0)                     | <i>Vigna reticulata</i> - 2 (0)                      |
| <i>Vigna linearis</i> - 1 (0)                       | <i>Vigna sinensis</i> (cowpea) - 5 (0)               |
| <i>Vigna lobatifolia</i> - 2 (0)                    | <i>Vigna speciosa</i> - 3 (0)                        |
| <i>Vigna longifolia</i> - 2 (0)                     | <i>Vigna subterranea</i> (ground-bean) - 1 (1)       |
| <i>Vigna luteola</i> - 2 (0)                        | <i>Vigna trilobata</i> - 2 (0)                       |
| <i>Vigna membranacea</i> - 2 (0)                    | <i>Vigna triphylla</i> - 2 (0)                       |
| <i>Vigna minima</i> - 2 (0)                         | <i>Vigna umbellata</i> - 3 (1)                       |
| <i>Vigna multinervis</i> - 2 (0)                    | <i>Vigna unguiculata</i> (cowpea) - 148 (128)        |
| <i>Vigna mungo</i> (blackgram) - 17 (20)            | <i>Vigna vexillata</i> - 8 (1)                       |
| <i>Vigna mungo</i> subsp. <i>sylvestris</i> - 2 (0) |  |
| <b>Other plant species</b>                          |  |
| <i>Arabidopsis thaliana</i> - 180,056 (40,933)      | <i>Lens culinaris</i> (lentil) - 20 (34)             |
| <i>Arachis hypogaea</i> (peanut) - 46 (121)         | <i>Medicago sativa</i> (alfalfa) - 1024 (604)        |
| <i>Cajanus cajan</i> (pigeon pea) - 9 (11)          | <i>Phaseolus vulgaris</i> (French bean) - 462 (490)  |
| <i>Canavalia gladiata</i> (sword bean) - 10 (16)    | <i>Pisum sativum</i> (pea) - 1081 (1662)             |
| <i>Cicer arietinum</i> (chick pea) - 229 (218)      | <i>Vicia faba</i> (fava bean) - 247 (380)            |
| <i>Glycine max</i> (soybean) - 123,492 (1758)       | <i>Zea mays</i> - 77,295 (3337)                      |
| <i>Lathyrus sativus</i> (chickling vetch) - 1 (4)   |  |

<sup>†</sup>Data taken from genebank sequence database, National Center for Biotechnology Information, 1 September 2000.

the paucity of genetic information. Thus, there is a need for more research in basic gene discovery for cowpea.

The gene products characterized from cowpea thus far fall into one of several categories based on their confirmed or predicted function (Table 3). Among the largest number of nucleotide sequences available are those encoding rRNA (either of nuclear or plastid origin) and their associated intergenic spacer regions, randomly generated nuclear fragments used for RFLP analysis, and cDNAs generated from differential display analysis of root-hair mRNAs collected 24 hours after inoculation with *Rhizobium* sp. NGR234. These are followed by sequences encoded by genes turned on in response to pathogen attack (e.g., acidic and basic chitinase, pathogenesis-related proteins, and various resistance gene analogs) or in response to abiotic stress such as drought and low temperature (e.g., dehydrin, acid phosphatases, and phospholipases, seed associated proteins (trypsin inhibitors,  $\alpha$ -amylase), and general metabolic enzymes.

**Table 3. Summary of gene products characterized from cowpea<sup>†</sup>.**

| Gene designation                | Accession number(s) | Identity or function  |
|---------------------------------|---------------------|---|
| <b>Seed associated proteins</b> |                     |   |
| $\alpha$ -amylase               | (AJ225087)          | $\alpha$ -amylase   |
| Asp-protease                    | (U61396)            | Aspartic proteinase   |
| Arcelin 9                       | (AF147793)          | Homolog of phaseolus arcelin gene   |
| cpi                             | (Z21954)            | Cysteine proteinase inhibitor   |
| tpi-f IV                        | (X51617)            | Bowman-Birk type trypsin inhibitor (f IV)   |
| tpi-f IV                        | (X51618)            | Bowman-Birk type trypsin inhibitor (f IV)   |
| <b>Stress response</b>          |                     |   |
| CpABA1                          | (AB030295)          | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD8                           | (D83970)            | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD12                          | (D88121)            | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD14                          | (D83971)            | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD22                          | (D83972)            | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD46                          | (D88122)            | Water stress-inducible gene for neoxan thin cleavage enzyme involved in abscisic-acid biosynthesis under water stress |
| CPRD65                          | (AB030293)          | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| CPRD86                          | (AB030294)          | Water stress-inducible genes in the highly drought-tolerant cowpea  |
| <i>Dhn1</i>                     | (AF159804)          | Chilling tolerance induced dehydrin   |
| <i>papB</i>                     | (AF171230)          | Phosphatidic acid phosphatase $\beta$   |
| <i>papA</i>                     | (AF165891)          | Phosphatidic acid phosphatase $\beta$   |
| <i>PplC</i>                     | (U85250)            | Phosphoinositide-specific phospholipase C   |
| <i>PplD</i>                     | (U92656)            | Water stress-induced phospholipase D  |

...continued

(Table 3 continued)

| Gene designation   | Accession number(s)                                | Identity or function   |
|--|--|--|
| <b>Nodulation-nitrogen fixation</b>                        |  |  |
| AKCS9  | (X79604)   | Nodulation associated lipid transfer protein.  |
| <i>flbr</i>  | (AF181096)   | Ferric leghemoglobin reductase   |
| <i>LbI</i>   | (U33206)   | Leghemoglobin I  |
| <i>LbII</i>  | (U33207)   | Leghemoglobin II   |
| <i>LbII</i>  | (2033205)  | Leghemoglobin II   |
| <i>sod</i>   | (AF077224)   | Iron-superoxide dismutase precursor from root nodules  |
| EST1-27  | (AI759142-<br>AI759148)<br>(AI755286-<br>AI755305) | cDNA sequences from display analysis of mRNA collected 24 hr following inoculation with <i>Rhizobium</i> sp. NGR234          |
| <b>Agronomic traits</b>                                    |  |  |
| Vu-Yld   | (AB028025)   | Regulatory protein for wall yield threshold (yielding)   |
| <b>Resistance gene products and pathogen-induced genes</b> |  |  |
| <i>chi1</i>  | (X88800)   | Chitinase class 1  |
| <i>chi3</i>  | (X88802)   | Acidic chitinase class 3   |
| <i>chi3B</i>   | (X88801)   | Basic chitinase class 3  |
| <i>chi4</i>  | (X88803)   | Chitinase class  |
| 4CHS   | (X74821)   | Chalcone synthase  |
| CRGA1-8  | (AB020483-<br>AB020490)                            | Nucleotide-binding site sequence containing resistance gene analogs; CRGA5 is linked to Cry1 (CMV strain Y) resistance locus |
| KIND11,12  | (AF141011,<br>AF141012)                            | Resistance gene protein homolog  |
| KINE12   | (AF141013)   | Resistance gene protein homolog  |
| loc431-490   | (AF255460-<br>AF255467)                            | Nucleotide-binding site sequence containing resistance gene analogs  |
| PAL  | (AF165998)   | Phenylalanine ammonia-lyase  |
| POX  | (U61379)   | Ascorbate peroxidase   |
| PR3  | (AB027154)   | Pathogenesis-related protein PR3   |
| PR4.2  | (X98608)   | Pathogenesis-related protein PR4.2   |
| S1-1   | (AB038691)   | Cucumber mosaic virus infection induced mRNA   |
| S1-3   | (AB038692)   | Cucumber mosaic virus infection induced mRNA   |
| <b>Mitochondrial/plastid localized functions</b>           |  |  |
| <i>atpA</i>  | (AF141143)   | Chloroplast ATP synthase $\beta$ subunit   |
| <i>cox2</i>  | (AF211254)   | Mitochondrial cytochrome c oxidase subunit 2   |
| <i>cpF2</i>  | (AF052058)   | Chloroplast associated ferritin subunit precursor, nuclear gene 2  |
| <i>cpF3</i>  | (AF052057)   | Chloroplast associated ferritin subunit precursor, nuclear gene 3  |

...continued

(Table 3 continued)

| Gene designation                                | Accession number(s) | Identity or function   |
|---|---------------------|--|
| <i>psbA</i>                                     | (X80932)            | Photosystem II D1 protein  |
| <i>rbcl</i>                                     | (Z95543)            | Large subunit, ribulose biphosphate carboxylase                      |
| <i>rpl16, rpl14</i>                             | (M80799)            | Chloroplast ribosomal proteins L16 and L14                           |
| <b>General cellular and metabolic functions</b> |                     |  |
| <i>A3</i>                                       | (X90487)            | Unknown protein (A3 gene)  |
| <i>ARF</i>                                      | (AF022389)          | ADP-ribosylation factor (ARF)  |
| <i>cdc2</i>                                     | (X89400)            | Protein kinase ( <i>cdc2</i> ) homolog                               |
| <i>cp-wap11</i>                                 | (AF005278)          | Type IIIa Golgi-associated membrane protein                          |
| <i>cp-wap13</i>                                 | (AF005279)          | Type IIIa Golgi-associated membrane protein                          |
| <i>ext127</i>                                   | (X86028)            | Extensin-like protein127   |
| <i>ext3</i>                                     | (X86029)            | Root-hair-specific extensin-like protein                             |
| <i>ext26</i>                                    | (X86030)            | Root-hair-specific extensin-like protein                             |
| <i>ext26G</i>                                   | (X91836)            | Extensin 26G gene  |
| <i>GRP</i>                                      | (X87948)            | Glycin-rich protein  |
| <i>pfe1, pfe2, pfe5</i>                         | (X67754-X67757)     | Ferritin gene exons 1 and 2  |
| <i>pur1</i>                                     | (AF071862)          | Phosphoribosylpyrophosphate amido-transferase                        |
| <i>pur2</i>                                     | (U30896)            | Glycinamide ribonucleotide (GAR) synthetase                          |
| <i>pur3</i>                                     | (AF160196)          | Glycinamide ribonucleotide transformylase                            |
| <i>Vupur3</i>                                   | (U30875)            | Glycinamide ribonucleotide transformylase                            |
| <i>pur5</i>                                     | (U30895)            | Aminoimidazole ribonucleotide (AIRS) synthetase                      |
| <i>SSI11</i>                                    | (AJ225088)          | Starch synthase isoform III; ADP-glucose-starch glucosyl transferase |
| <i>SSV</i>                                      | (AJ006752)          | Starch synthase isoform V; ADP-glucose-starch glucosyl transferase   |
| <i>Ted2</i>                                     | (Y088624)           | Ted2 protein homolog to marker gene for differentiation              |
| <i>AG81-1</i>                                   | (AF062782)          | Microsatellite AG81-1 repeat region                                  |
| Centromeric DNA                                 | (Z49817)            | Satellite DNA (centromeric region)                                   |
| <i>Ty1-copia</i> -like                          | (Y12763, Y12764)    | <i>Ty1-copia</i> -like retrotransposable element repeat region       |
| Unknown   | (AZ254216-AZ254227) | RFLP sequences of cowpea <i>Vigna unguiculata</i> genomic DNA        |

<sup>1</sup>Data taken from genebank sequence database, National Center for Biotechnology Information, 1 September 2000.

## Plant cell transformation and cell culture

Progress in cowpea improvement over the past several decades relied largely upon traditional selection and breeding strategies for the introduction of new traits into existing cultivars. Excellent discussions of previous and current cowpea breeding activities can be found elsewhere in this volume. With the advent of molecular techniques for gene isolation and gene transfer among species, plant breeders now have at their disposal the ability to rapidly move single gene characteristics among agronomically preferred cultivars. More importantly, the ability to introduce genes into plant cells from distant genera and even other kingdom (e.g., genes of nonplant origin) allow researchers to bypass interspecific barriers which often stymied efforts to introduce desirable traits from wild species into preferred cultivars. Transgenic approaches essentially expand the genepool to include all available genetic information, whether naturally occurring or synthetically created.

In order to take full advantage of transgenic approaches for crop improvement, it is necessary to ensure efficient and reproducible methods for gene transfer (i.e., plant cell transformation) and the identification and recovery of transgenic plants. Attempts to establish procedures for plant transformation in cowpea have met with mixed success. Garcia et al. (1986, 1987) reported obtaining transgenic calli and chimeric plantlets following *Agrobacterium*-mediated leaf-disc transformation of *V. unguiculata*. Similar findings were reported by Penza et al. (1991) following *Agrobacterium*-mediated transformation of axillary buds and embryonic tissues. However, the ability to produce mature transgenic plants with these procedures was never confirmed. Several groups (Finer et al. 1992; Penza et al. 1992; Kononowicz et al. 1997) have attempted to introduce foreign DNA into cowpea leaf tissues and embryos by microprojectile bombardment (biolistics). These researchers obtained high levels of transient expression of the  $\beta$ -glucuronidase (*gus*) transgene, but were unable to regenerate plantlets from the transformed cells. Similarly, Akella and Lurquin (1993) described the expression of  $\beta$ -glucuronidase (GUS) activity in a variety of tissues following electroporation of embryos with plasmid DNA. Unfortunately, it was not possible to produce mature transgenic plants that stably inherited the transgene. In contrast, Muthukumar et al. (1996) reported the successful transformation of mature de-embryonated cowpea cotyledons by *Agrobacterium*-mediated transformation. Cotyledon explants inoculated with *A. tumefaciens* pUCD2614 carrying plasmid pUCD2340 containing a hygromycin phosphotransferase (*hpt*) transgene conferring hygromycin-B resistance were cultured on shooting medium and approximately 15–19% of the explants produced shoots which could be rooted in the presence of antibiotics. The presence of the *hpt* gene in the transgenic plants was confirmed by genomic DNA gel blot hybridization analysis. It should be noted, however, that no information is available on whether the antibiotic resistance trait was transferred to subsequent generations. Among the more recent reports of attempts to overcome the limitations to cowpea transformation, Brar et al. (1999) showed that there were genotype effects on the performance of various *V. unguiculata* cultivars during cell culture. They also found that endogenous ethylene levels influenced in vitro regeneration rates. Machuka and colleagues at the International Institute of Tropical Agriculture (IITA) are attempting to optimize parameters for cowpea transformation through the establishment of antibiotic thresholds for selection of transformed cowpea tissues and development of shoot elongation and rooting media. Details of these studies can be found elsewhere in this volume.

The development of successful genetic transformation protocols for cowpea is essential to realize the potentials of transgenic approaches for germplasm improvement of cowpea. At the present time, a number of candidate genes that could have substantial impact on yield are available for introduction into cowpea. These include a range of genes whose products (e.g., lectins, serine and thiol-protease inhibitors,  $\alpha$ -amylase inhibitors, trypsin inhibitors, cysteine proteases, chitinases, and *Bacillus thuringiensis* toxin) have been shown to be effective in the control of many of the major insect pests that diminish seed yield and quality, including bruchid beetles (*Callosobruchus maculatus*), pod-sucking bugs (*Clavigralla tomentosicollis*), and pod borers (*Maruca vitrata*). Enhanced resistance of cowpea to a wide spectrum of disease pathogens can also be achieved through transgenic manipulation by both the introduction of single or multiple gene resistance traits from other species or through metabolic engineering (Hilder and Boulter 1999). The effectiveness and durability of disease and pest resistance are likely to be greater in engineered transgenic plants in which multiple resistance genes are introduced (so-called "resistance gene pyramiding"). Such pyramiding is time consuming and often difficult to achieve through traditional breeding approaches due to interspecific barriers, but readily achievable through transgenic approaches.

Beyond disease and pest resistance, the ability to transform cowpea opens up the potential for manipulation of numerous other plant characteristics including seed protein composition and nutritional quality (e.g., protein content, amino acid balance, etc. [Chopra et al. 1999]) and abiotic stress tolerances (e.g., drought, heat, and salinity tolerance [Vandemark 1999]). Each of these characteristics is being successfully manipulated in other crop species (Hilder and Boulter 1999; Mazur et al. 1999; Somerville and Somerville 1999) where well established protocols for transformation and regeneration already exist.

## Perspective and future directions

Much of the foundation for the future successful manipulation of cowpea by genetic engineering is now in place. A genetic map of the cowpea genome which provides a reasonable degree of coverage to rapidly locate loci of interest has already been established and studies are underway in a number of laboratories to further saturate the map with additional markers in order to improve its utility. Genetic linkage maps are also available for a number of related species, including *V. radiata*, *V. angularis*, and *V. vexillata*. Given the high degree of colinearity and conservation in genome organization between species that have been studied, progress made on the mapping of genes in one species should be useful in all species.

Numerous single gene and quantitative trait loci have already been placed on the cowpea map. As the use of molecular-marker analysis for gene mapping becomes more widespread in the cowpea community, the number and variety of traits placed on the map will increase. A large number of populations segregating for disease and pest resistance, drought tolerance, growth and yield parameters, and other characteristics which can be used in mapping activities have already been developed through the effort of breeders. Coordination of efforts between laboratories to exploit these resources is important to ensure rapid future progress.

In addition to efforts aimed at refining the genetic map, the development of physical maps linking genetically defined markers with DNA fragments is essential for the future map-based cloning of genes in cowpea. Techniques are now available for the construction

of ordered libraries of large DNA fragments using either Yeast Artificial Chromosomes (YACs) (Burke et al. 1987; Coulson et al. 1988) or Bacterial Artificial Chromosomes (BACs) (Shizuya et al. 1992; Woo et al. 1994), with the latter being the method of choice in recent years due to higher cloning efficiency, ease of handling, and greater stability of the recombinant clones. The generation of large insert DNA libraries for cowpea and the establishment of a physical map based on an assembly of overlapping contigs should be one of the priorities over the next few years.

To complement work on the physical and genetic mapping of the cowpea genome, there should be increased research activity centered on basic gene discovery and studies on gene regulation. The past several years have seen major advances in DNA chip technologies for the rapid measurement of differential gene expression in plants (Lemieux et al. 1998). The use of oligonucleotide and cDNA microarray technologies (Lockhart et al. 1996; DeRisi et al. 1997; Heller et al. 1997) offer researchers an efficient, inexpensive, and rapid means to measure transcript levels for thousands of genes simultaneously, allowing the identification of genes participating in common metabolic activities or activated/repressed in response to changes in any number of selected internal or external cues (e.g., changes in developmental age, challenge by disease or pests, and alterations in physical environment). Information provided from such analysis in combination with data on inheritance of desirable agronomic (growth, yield, and resistance) traits should give researchers the ability to pinpoint changes necessary to achieve rapid improvements in germplasm through marker-assisted breeding or genetic engineering (Somerville and Somerville 1999).

Finally, it is essential that open and rapid exchange of information occurs between researchers working within various disciplines whether at the molecular, genetic, cell culture, or agricultural extension level. Working groups and fora established by electronic communication have greatly facilitated progress on other crop plants and efforts to enhance and extend current activities should be a priority within the cowpea community. Integrated with a clear understanding of the needs of producers and desires of consumers, current technologies and new biotechnology-based strategies under development should have significant impact on expanding the economic importance of cowpea in the coming decades.

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## References

- Ajibade, S.R., N.F. Weeden, and S.M. Chite. 2000. Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Euphytica* 111: 47–55.
- Akella, V. and P.F. Lurquin. 1993. Expression in cowpea seedlings of chimeric transgenes after electroporation into seed-derived embryos. *Plant Cell Reports* 12: 110–117.
- Akkaya, M.S., A.A. Bhagwat, and P.B. Cregan. 1992. Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics* 132: 1131–1139.
- Akkaya, M.S., R.C. Shoemaker, J.E. Specht, A.A. Bhagwat, and P.B. Cregan. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Science* 35: 1439–1445.
- Bashir, M. and R.O. Haptom. 1996. Sources of genetic resistance in cowpea (*Vigna unguiculata* L. Walp.) to cowpea aphid-borne mosaic virus. *European Journal of Plant Pathology* 102: 411–419.

- Boeke, J.D., D.J. Garfinkel, C.A. Styles, and G.R. Fink. 1985. Ty elements transpose through an RNA intermediate. *Cell* 40: 491–500.
- Brar, M.S., J.M. Al-Khayri, T.E. Morelock, and J.E. Anderson. 1999. Genotypic response of cowpea *Vigna unguiculata* (L.) to in vitro regeneration from cotyledon explants. *Cellular and Developmental Biology* 35: 8–12.
- Burke, D.T., G.F. Carle, and M.V. Olsen. 1987. Cloning of large fragments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 236: 806–812.
- Chopra, V.L., V.S. Malik, and S.R. Bhat (editors). 1999. Applied plant biotechnology. Science Publishers Inc., Enfield, New Hampshire, USA. 384 p.
- Coulson, A., R. Waterson, J. Riff, J. Sulston, and Y. Kohara. 1988. Genome linking with yeast artificial chromosomes. *Nature* 335: 184–186.
- DeRisi, J.L., V.R. Iyer, and P.O. Brown. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278: 680–686.
- Ehlers, J.D. and A.E. Hall. 1997. Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research* 53: 187–204.
- Fatokun, C.A., N.D. Young, and G.O. Myers. 1997. Molecular markers and genome mapping in cowpea. Pages 352–360 in *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, Ibadan, Nigeria.
- Fatokun, C.A., D.I. Menancio-Hautea, D. Danesh, and N.D. Young. 1992. Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics* 132: 841–846.
- Fatokun, C.A., D. Danesh, N.D. Young, and E.L. Stewart. 1993a. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. *Theoretical and Applied Genetics* 86: 97–104.
- Fatokun, C.A., D. Danesh, D.I. Menancio-Hautea, and N.D. Young. 1993b. A linkage map for cowpea (*Vigna unguiculata* [L.] Walp.) based on DNA markers. Pages 6256–6258 in *A compilation of linkage and restriction maps of genetically studied organisms*. Genetic maps 1992, edited by J.S. O'Brien. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
- Federoff, N.V. 1989. About maize transposable elements and development. *Cell* 56: 181–191.
- Fery, R.L. 1985. The genetics of cowpeas: a review of the world literature. Pages 25–62 in *Cowpea research, production and utilization*, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Fery, R.L. 1990. The cowpea: production, utilization, and research in the United States. *Horticulture Review* 12: 197–222.
- Fery, R.L. and B.B. Singh. 1997. Cowpea genetics: a review of the recent literature. Pages 13–29 in *Advances in cowpea research*, Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, Ibadan, Nigeria.
- Finer, J.J., P. Vain, M.W. Jones, and M.D. McMullen. 1992. Development of the particle inflow gun for DNA delivery to plant cells. *Plant Cell Reports* 11: 323–328.
- Flavell, A.J., S. Pearce, and A. Kumar. 1994. Plant transposable elements and the genome. *Current Opinions in Genetics and Development* 4: 838–844.
- Galasso, I., G.E. Harrison, D. Pignone, A. Brandes, and J.S. Heslop-Harrison. 1997. The distribution and organization of Ty1-copia-like retrotransposable elements in the genome of *Vigna unguiculata* (L.) Walp. (cowpea) and its relatives. *Analytical Botany* 80: 327–333.



- Garcia, J.A., J. Hille, and R. Godbach. 1986. Transformation of cowpea *Vigna unguiculata* cells with an antibiotic resistance gene using a Ti-plasmid-derived vector. *Plant Science* 44: 37–46.
- Garcia, J.A., J. Hille, P. Vos, and R. Godbach. 1987. Transformation of cowpea *Vigna unguiculata* with a full-length DNA copy of cowpea mosaic virus m-RNA. *Plant Science* 48: 89–98.
- Gowda, B.S., J.L. Miller, S.S. Rubin, D.R. Sharma, and M.P. Timko. 2002. Isolation, sequencing and linkage mapping of resistance-gene analogs in cowpea (*Vigna unguiculata* L.). *Euphytica*. 126:(3) 365–377.
- Heller, R.A., M. Schena, A. Chai, D. Shalon, T. Bedilion, J. Gilmore, D.E. Wooley, and R.W. Davis. 1997. Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. *Proceedings of National Academy of Science USA* 94: 2150–2155.
- Hilder, V.A. and D. Boulter. 1999. Genetic engineering of crop plants for insect resistance—a critical review. *Crop Protection* 18: 177–191.
- Kaga, A., N. Tomooka, Y. Egawa, K. Hosaka, and O. Kamijima. 1996a. Species relationships in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. *Euphytica* 88: 17–24.
- Kaga, A., M. Ohnishi, T. Ishii, and O. Kamijima. 1996b. A genetic linkage map of azuki bean constructed with molecular and morphological markers using an interspecific population (*Vigna angularis* x *V. nakashimae*). *Theoretical and Applied Genetics* 93: 658–663.
- Kononowicz, A.K., K.T. Cheah, M.L. Narasimhan, L.I. Murdock, R.E. Shade, M.J. Chrispeels, E. Filippone, L.M. Monti, R.A. Bressan, and P.M. Hasegawa. 1997. Developing a transformation system for cowpea (*Vigna unguiculata* [L.] Walp.). Pages 361–371 in *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, Ibadan, Nigeria.
- Lemieux, B., A. Aharoni, and M. Schena. 1998. Overview of DNA chip technology. *Molecular Breeding* 4: 277–289.
- Li, J., G. He, P. Gepts, and C.S. Prakash. 1999. Development of a genetic map for cowpea (*Vigna unguiculata*) using DNA markers. Page 237 in *Abstracts, Plant & Animal Genome VII Conference*, 17–21 January 1999. San Diego, California, USA.
- Lockhart, D.J., H. Dong, M.C. Byrne, M.T. Follettie, M.V. Gallo, M.S. Chee, M. Mittmann, C. Wang, M. Kobayashi, H. Horton, and E.L. Brown. 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnology* 14: 1675–1680.
- Marechal, R., J.M. Mascherpa, and F. Stainer. 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques traitées par l'analyse informatique. *Boissiera* 28: 1–273.
- Marfo, K.O. and A.E. Hall. 1992. Inheritance of heat tolerance during pod set in cowpea. *Crop Science* 32: 912–918.
- Mazur, B., E. Krebbers, and S. Tingy. 1999. Gene discovery and product development for grain quality traits. *Science* 285: 372–375.
- Menancio-Hautea, D., L. Kumar, D. Danesh, and N.D. Young. 1993a. A genome map for mung bean (*Vigna radiata* [L.] Wilczek) based on DNA genetic markers (2N = 2x = 22). Pages 6259–6261 in *A compilation of linkage and restriction maps of genetically studied organisms. Genetic maps 1992*, edited by J. S. O'Brien. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
- Menancio-Hautea, D., C.A. Fatokun, L. Kumar, D. Danesh, and N.D. Young. 1993b. Comparative genome analysis of mung bean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walp.) using RFLP mapping data. *Theoretical and Applied Genetics* 86: 797–810.

- Menéndez, C.M., A.E. Hall, and P. Gepts. 1997. A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred domesticated lines. *Theoretical and Applied Genetics* 95: 1210–1217.
- Muthukumar, B., M. Mariamma, K. Veluthambi, and A. Gnanam. 1996. Genetic transformation of cotyledon explants of cowpea (*Vigna unguiculata* [L.] Walp.) using *Agrobacterium tumefaciens*. *Plant Cell Reports* 15: 980–985.
- Myers, G.O., C.A. Fatokun, and N.D. Young. 1996. RFLP mapping of an aphid resistance gene in cowpea (*Vigna unguiculata* [L.] Walp.). *Euphytica* 91: 181–187.
- Ogundiwin, E.A., C.A. Fatokun, G. Thottappilly, M.E. Aken'Ova, and M. Pillay. 2000. Genetic linkage map of *Vigna vexillata* based on DNA markers and its potential usefulness in cowpea improvement. Page 19 in *Abstracts, World cowpea research conference III*, 4–7 September 2000, Ibadan, Nigeria.
- Ouédraogo, J.T., V. Maheshwari, D.K. Berner, C.-A. St-Pierre, F. Belzile, and M.P. Timko. 2001. Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. *Theoretical and Applied Genetics* 102: 1029–1036.
- Pasquet, R.S. 1999. Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. Based on allozyme variation. *Theoretical and Applied Genetics* 98: 1104–1119.
- Penza, R., P.F. Lurquin, and E. Filippone. 1991. Gene transfer by co-cultivation of mature embryos with *Agrobacterium tumefaciens*. Application to cowpea (*Vigna unguiculata* L. Walp.). *Journal of Plant Physiology* 138: 39–43.
- Penza, R., V. Akella, and P.F. Lurquin. 1992. Transient expression and histological localization of a gus chimeric gene after direct transfer to mature cowpea embryos. *Bio Techniques* 13: 576–579.
- Roberts, P.A., W.C. Matthews, and J.D. Ehlers. 1996. New resistance to virulent root-knot nematodes linked to the Rk locus of cowpea. *Crop Science* 36: 889–894.
- Shizuya, H., B. Birren, U.J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proceedings of National Academy of Science USA* 89: 8794–8797.
- Singh, B.B. and A.M. Emechebe. 1997. Advances in research on cowpea *Striga* and *Alectra*. Pages 215–224 in *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. IITA, Ibadan, Nigeria.
- Singh, B.B., D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai (editors). 1997. *Advances in cowpea research*. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Somerville, C. and S. Somerville. 1999. Plant functional genomics. *Science* 285: 380–383.
- Sonnante, G., T. Stockton, R.O. Nodari, V.I. Becerra Velásquez, and P. Gepts. 1994. Evolution of genetic diversity during the domestication of common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 89: 629–635.
- Sonnante, G., A.R. Piergiovanni, Q.N. Ng, and P. Perrino. 1996. Relationships of *Vigna unguiculata* (L.) Walp., *V. vexillata* (L.) A. Rich., and species of section *Vigna* based on isozyme variation. *Genetic Resources and Crop Evolution* 43: 157–165.
- Vaillancourt, R.E. and N.F. Weeden. 1992. Chloroplast DNA polymorphism suggests a Nigerian center of domestication for the cowpea, *Vigna unguiculata* (Leguminosae). *American Journal of Botany* 79: 1194–1199.
- Vaillancourt, R.E., N.F. Weeden, and J.D. Barnard. 1993. Isozyme diversity in the cowpea species complex. *Crop Science* 33: 606–613.

- Vaillancourt, R.E. and N.F. Weeden. 1996. *Vigna unguiculata* and its position within the genus *Vigna*. Pages 89–93 in *Advances in legume systematics 8: legumes of economic importance*, edited by B. Pickersgill and J.M. Lock. Royal Botanic Gardens, Kew, UK.
- Vandemark, G.J. 1999. Transgenic plants for the improvement of field characteristics limiting crop production. Pages 219–273 in *Molecular biotechnology for plant food production*, edited by O. Paredes-López. Technomic Publishing Co. Inc. Lancaster, UK.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Fritjers, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.
- Williams, J.G.K., A.R. Kubelik, J.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.
- Woo, S.S., J. Jiang, B.S. Gill, A.H. Paterson, and R.A. Wing. 1994. Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acids Research* 22: 4922–4931.

## Potential role of transgenic approaches in the control of cowpea insect pests

J. Machuka<sup>1</sup>

### Abstract

Crops' incompatibility makes conventional breeding approaches untenable in transferring available insect resistance from wild *Vigna* sp. into cowpea. The alternative recourse is to isolate and transfer alien resistance genes using genetic transformation. This has the added advantage of using useful genes from distantly related organisms to control cowpea pests. Artificial diet bioassays carried out on the *Maruca* pod borer, pod sucking bugs, and cowpea weevils indicate that these insects can be controlled by *Bacillus thuringiensis* crystal proteins, plant lectins, protease,  $\alpha$ -amylase inhibitors, chitinases, and/or ribosome-inactivating proteins. The challenge now is to express the genes encoding these proteins in transgenic cowpea and hope that what happens in artificial diets will, at least in some cases, be replicated in transgenics. Other candidate genes include enzymes encoding biochemical pathways in secondary metabolism. It can be anticipated that useful information emerging from current global genomics efforts in crop species, including model legumes, will have a bearing on cowpea improvement through genetic engineering. What cowpea researchers need to do now is develop a comprehensive pest resistance management strategy. Such a strategy must take into account criteria such as transformation of elite cowpea lines that are adapted to each of the major agroecological zones, gene flow between cultivated and wild cowpea, and strategies for dissemination and adoption of biotechnologically improved cowpea lines. This paper reviews previous work on candidate genes, presents some recent results, and makes projections on how research on cowpea breeding through genetic modification for insect resistance may move from the laboratory into farmers' fields, especially in sub-Saharan Africa.

### Introduction

Grain yield losses in cowpea (*Vigna unguiculata*) are mainly due to biotic stresses, especially insect pests, including aphids, thrips, Maruca pod-borer (MPB [*Maruca vitrata*]), bruchids, and pod-sucking bugs (PSB). Although modest levels of host plant resistance are available in cowpea germplasm, there is nearly none to MPB. Insect resistance genes are present in wild cowpea relatives (*Vigna* spp.) as well as other non-*Vigna* legumes that are infested by MBP such as African yam bean (AYB [*Sphenostylis stenocarpa*]). However, breeding barriers make conventional breeding approaches untenable in transferring resistance from wild *Vigna* and other legumes into cowpea. The alternative recourse is to isolate and transfer alien resistance genes using genetic transformation. This has the added advantage of using useful genes from distantly related organisms to control cowpea pests. Artificial diet bioassays carried out on MPB, PSB, and cowpea weevils indicate that these insects can be controlled by *Bacillus thuringiensis* (*Bt*) crystal proteins, plant

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lectins, protease and  $\alpha$ -amylase inhibitors, chitinases and/or ribosome-inactivating proteins. The challenge now is to express the genes encoding these proteins in transgenic cowpeas and hope that what happens in artificial diets will, at least in some cases, be replicated in transgenics. Other candidate genes include enzymes encoding biochemical pathways in secondary metabolism.

This paper reviews research related to identification of candidate insect resistance genes and makes projections on how cowpea genetic modification breeding for insect resistance may move from the laboratory into farmers' fields, especially in sub-Saharan Africa.

## Methods for isolation of insect resistance factors

The first step in generating insect resistant transgenic crops is to identify insecticidal proteins or compounds that are active against the target pest. The most common way of doing this involves the use of artificial diets or seeds that contain proteins, secondary metabolites, or other compounds that are suspected or known to have anti-insect properties (Duck and Evola 1997). *Bacillus thuringiensis* crystal proteins were the first to be used to generate transgenic insect resistant crops (reviewed by Krattiger 1997). Proteins from other microorganisms as well as plants have also been used for direct screening for insecticidal activities (Schulera et al. 1998). The common higher plant defense proteins tested to date include lectins, protease, and  $\alpha$ -amylase inhibitors (Duck and Evola 1997). In addition to screening known factors, random screening without bias regarding origin or source of protein, chemical, or extract may be performed. The compounds or proteins may even be purchased from commercial sources. For example, *Streptomyces* cholesterol oxidase, a potent insecticidal enzyme against the cotton boll weevil, was isolated by screening culture filtrates from over 10 000 microbial fermentations (Purcell 1997). Callus-based insect bioassays from susceptible and resistant crop lines have also been used to investigate insect resistance (Williams et al. 1987). Map-based cloning using techniques such as chromosome walking which utilize molecular probes that map near resistance loci is another approach to isolate genes for deployment in genetic engineering for insect resistance (Gibson and Somerville 1993). Although some work has been done to transfer insect resistance genes from mammals and insects into crops (Schulera et al. 1998), the following discussion focuses mainly on microbial and plant genes that have potential for deployment in transgenic insect resistance in cowpea.

## Resistance genes from microorganisms

*Bacillus thuringiensis* is a spore-forming soil bacterium that produces insecticidal protein crystals, also called *Bt* toxins, endotoxins, or crystal (Cry) proteins, within its cells during sporulation. Spores and purified protein crystals of several *Bt* strains have been used as microbial insecticides since the 1950s and now have an established role in some integrated pest management systems (Fietelson et al. 1992). Different strains of *Bt* produce different crystal proteins, coded for by Cry genes that are highly toxic to specific insects, nematodes, and other invertebrates. *Bt* toxins tend to be specific in their activities either to Lepidoptera, Coleoptera or other insects. Their mechanism of action is not quite clear, but it is believed that the proteins damage the membrane of the insect's midgut epithelial cells, causing massive water uptake (Gatehouse et al. 1992). This may in turn lead to the disruption of the electrical K<sup>+</sup> and pH gradients by creating pores, resulting in irreversible damage to the midgut wall.

To date, several genes encoding different Bt toxins have been engineered into crop plants (Schulera et al. 1998). Research at IITA has shown that Cry IAb, Cry IC, and CryIIA proteins are toxic to MPB (Jackai, unpublished). For control of cowpea pests, it is imperative that other different *Bt* toxins be tested in artificial diets or seeds for their efficacy against MPB, bruchids, and PSB for which assay systems are available (Jakai and Raulston 1988; Shade et al. 1986). Moreover, artificial insect resistance assays need to be developed for other problematic pests, particularly thrips, to allow screening of Cry proteins against these pests.

*Bacillus thuringiensis* also produces vegetative insecticidal proteins (ViPs) when it is not sporulating (Estruch et al. 1996; Warren 1997). The ViPs are unrelated to crystal proteins and appear to be active against lepidopteran pests such as fall armyworm, beet armyworm, corn rootworm, and tobacco budworm (Estruch et al. 1997). Other candidate genes for insect protection include *Streptomyces* cholesterol oxidase (Purcell 1997), fungal chitinases (Kramer et al. 1997), the isopentenyl-transferase gene (*ipt*) from *Agrobacterium tumefaciens* (Smigocki et al. 1997) and genes encoding insect viral RNAs (Hanzlik and Gordon 1997). Additionally, the bacterium *Photorrhhabdus luminescens*, which lives in entomophagous nematodes has recently been shown to produce insecticidal toxins that may be useful for transgenic insect control (Bowen et al. 1998).

## Insect resistance genes from higher plants

It is important to discover new genes that can be pyramided with *Bt* genes to enhance resistance levels. Other limitations of *Bt* genes include possibilities of resistance breakdown, limited scope of pests covered by Cry proteins, and public perception issues (Stewart 1999). To overcome some of these limitations, plant-derived genes have been cloned and transferred into several crop species (Schulera et al. 1997, Snow and Palma 1997).

## Genes for bruchid resistance

Coleopteran insects in the family *Bruchidae* cause serious cowpea grain losses in storage. *Callosobruchus maculatus* is key among these pests. Through conventional breeding efforts at IITA and elsewhere, modest levels of resistance to *C. maculatus* have been attained (Singh and Jackai 1985). To enhance these modest resistance levels, efforts have also been underway to identify plant genes that affect *C. maculatus* development. The majority of artificial seed bioassays have involved the use of plant lectins (Gatehouse et al. 1984, 1991; Heusing et al. 1991a; Machuka et al. 1999a, 1999b, 2000; Murdock et al. 1990; Omitogun et al. 1999; Pratt et al. 1990). Vicilins (7S seed storage proteins) and protease and  $\alpha$ -amylase inhibitors and  $\alpha$ -amylase inhibitor-like proteins (AIL), are also insecticidal to bruchids (Hilder et al. 1987; Ishimoto et al. 1999; Pittendrigh et al. 1997; Sales et al. 1996; Yunes et al. 1998; Huesing et al. 1991c). Table 1 summarizes the toxicity mechanism of these proteins. Transgenic pea and azuki seeds containing the bean  $\alpha$ -amylase inhibitor are resistant to bruchid beetles (Ishimoto et al. 1996, Shade et al. 1994). Plans are underway to introduce this gene into modestly bruchid resistant IITA cowpea lines once the transformation system becomes routine. Various compounds are toxic to cowpea beetles. For example, leaf, fruit, seed, and oil extracts from some African shrubs possess larvicidal and ovicidal activities against *C. maculatus* (Leonard et al. 1993, Seck et al. 1993). However, these toxins are more applicable in biocontrol than transgenic insect control strategies, at least in the short term.

**Table 1. Candidate genes for transgenic resistance to bruchids.**

| Protein   | Possible mechanism(s) of action   |
|---|---|
| Cowpea vicilins<br>$\alpha$ -amylase inhibitors<br>Cowpea protease inhibitors e.g. cystein, Bowman-Birk, trypsin, and chymotrypsin inhibitors | Bind insect chitin<br>Inhibition in insect $\alpha$ -amylases<br><ul style="list-style-type: none"> <li>• Depletion of essential amino acids resulting from hypersecretion of digestive enzymes</li> <li>• Inhibition of insect digestive proteases</li> <li>• Carbohydrate binding to insect midgut epithelium or peritrophic matrix/membrane</li> </ul> |
| Lectins   | <ul style="list-style-type: none"> <li>• Resistance to proteolysis</li> </ul>   |

### Genes for resistance to the *Maruca* pod borer

Unlike studies focusing on cowpea weevils, only two studies have been reported that pertain to the biological effects of plant lectins on growth, development, and fecundity of MPB in artificial diet bioassays (Machuka et al. 1999b, 2000). Table 2 shows the list of plant lectins so far tested for their effects against MPB. At least 26 lectins from 15 plant families and representing seven carbohydrate-binding specificity groups have been tested. Results from this screening work indicated that mannose-specific lectins from twayblade (*Listera ovata*) and snowdrop (*Galanthus nivalis*) have detrimental effects on MPB larval development at all stages of development. Others, such as wheat germ and jackfruit agglutinins possess latent effects that only manifest at (a) subsequent unique stage(s). A type 1 ribosome-inactivating protein (RIP) from *Iris* and bean (*Phaseolus vulgaris*)  $\alpha$ -mylase inhibitor are not toxic to MPB larvae although the latter mildly affects pupal development and adult emergence (Machuka et al. 1999b). The galactose-specific seed lectin from Nigerian-grown African yam bean (*Sphenostylis stenocarpa*) does not affect MPB larval development, although it inhibits *C. maculatus* development (Machuka et al. 2000). Generally, relatively few lectins are toxic to lepidopteran insects, even when they have been found stable to proteolysis by enzymes in the insect gut (Czapla and Lang 1990; Czapla 1997; Gatehouse et al. 1995).

Apart from lectins, plant proteinaceous inhibitors (PIs) of insect proteinases (serine, cysteine, aspartic, and metallo proteinases) are considered potential candidates for gene transfer for insect resistance (Ryan 1990). Serine proteases are the dominant class in lepidopteran insects larvae such as MPB, whereas coleopteran species have a wider range of dominant gut proteinases (Gerald et al. 1997). Since serine and cysteine PIs mainly inhibit the growth and development of lepidopteran (and coleopteran) species, it would be useful to screen a wide range of these PIs against MPB in artificial diets. To date, more than 14 different plant PI genes have been introduced into crop plants, with efforts concentrated on serine PIs from the plant families Fabaceae, Solanaceae, and Poaceae (Koiwa et al. 1997, Schulera et al. 1998). So far, the most active PI identified is the cowpea trypsin inhibitor (CpTI), isolated from an IITA bruchid resistant line, TVnu 2027 (Hilder et al. 1987). Serine PI-like proteins have been identified from seeds of Nigerian-grown velvetbeans (*Mucuna* spp.) (Machuka 2000a). These proteins, as well as affinity purified trypsin and chymotrypsin inhibitors from two wild *Vigna* species (*V. vexillata* and *V. oblongifolia*) and AYB, are not toxic to MPB (Machuka unpublished). The advantage of

**Table 2. Plant lectins tested against the *Maruca* pod borer in artificial diets.**

| Lectin <sup>†</sup>  | Plant family    | Lectin specificity group                 |
|--|-----------------|--|
| ASA, <i>Allium sativum</i> (garlic) agglutinin                                   | Alliaceae       | Mannose                                  |
| AUA, <i>Allium ursinum</i> (ramson) lectin                                       | Alliaceae       |  |
| *GNA, <i>Galanthus nivalis</i> (snowdrop) agglutinin                             | Amaryllidaceae  |  |
| *LOA, <i>Listera ovata</i> (twayblade) agglutinin                                | Orchidaceae     |  |
| *NPA, <i>Narcissus pseudonarcissus</i> (daffodil) agglutinin                     | Orchidaceae     | Mannose/maltose                          |
| *CSA, <i>Calystegia sepium</i> (hedge bindweed) agglutinin                       | Convolvulacea   |  |
| PSL, <i>Pisum sativum</i> (garden pea) lectin                                    | Fabaceae        | Mannose/glucose                          |
| SSA, <i>Sphenostylis stenocarpa</i> (African yam bean) agglutin                  | Apiaceae        |  |
| APA, <i>Aegopodium podagraria</i> (ground elder) lectin                          | Curcubitaceae   | Galactose/<br>N-acetyl-<br>galactosamine |
| BDA, <i>Bryonia dioica</i> agglutinin (white bryony)                             |                 |  |
| *BPA, <i>Bauhinia purpurea</i> agglutinin (carmel's foot tree)                   | Moraceae        |  |
| DBA, <i>Dolichos biflorus</i> agglutinin (horse gram)                            | Fabaceae        |  |
| *IRA, <i>Iris hybrid</i> agglutinin (Dutch iris)                                 | Iridaceae       | N-acetyl-<br>glucosamine                 |
| JCA, <i>Artocarpus integrifolia</i> lectin (jackfruit)                           | Caesalpiniaceae |  |
| SBA, <i>Glycine max</i> agglutinin (soybean)                                     |                 |  |
| *SNA-II, <i>Sambucus nigra</i> agglutinin (elderberry)                           | Fabaceae        |  |
|  | Sambucaseae     | N-acetyl-<br>glucosamine                 |
| DSL, <i>Datura stramonium</i> lectin (jimson weed)                               | Solanaceae      |  |
| *UDA, <i>Urtica dioica</i> agglutinin (stinging nettle)                          | Urticaceae      |  |
| WGA, <i>Triticum aestivum</i> (wheat germ) (Wheat) agglutinin                    | Gramineae       | Sialic acid                              |
| MAA, <i>Maackia amurensis</i> (Maackia) agglutinin                               | Fabaceae        |  |
| SNA-I, <i>Sambucus nigra</i> (elderberry) agglutinin                             | Sambucaceae     |  |
| CAA, <i>Colchicum autumnale</i> (meadow saffron) agglutinin                      | Liliaceae       | Complex glycan                           |
| PHA-E, <i>Phaseolus vulgaris</i> (red kidney bean) phytohemagglutinin isoform E  | Fabaceae        |  |
| *PHA-L, <i>Phaseolus vulgaris</i> (red kidney bean) phytohemagglutinin isoform L | Fabaceae        |  |
| TLC-I, <i>Tulipa hybrid</i> (tulip) agglutinin                                   | Liliaceae       |  |
| RPA, <i>Robinia pseudoacacia</i> (false/black acacia) agglutinin                 | Fabaceae        |  |

<sup>†</sup>Candidate lectins for transgenic resistance to *Maruca* pod borer. Detailed references of names and classification of lectins and pod borer bioassays can be found in Van Damme et al. (1998a, b) and Machuka et al. (1999b, 2000).

using PIs and other genes from plants, especially edible ones, for enhanced insect resistance is that the nutritional penalty after gene transfer is absent or minimal and there are fewer public perception problems. This has been demonstrated through mammalian toxicity tests, for example, in the case of the cowpea trypsin inhibitor gene (Pusztai et al. 1992).

Recently, it has been shown that expression of plant proteases rather than protease inhibitors may be a novel insect defence mechanism in plants (Pechan et al. 2000). Based on the use of Arginine Sepharose B chromatography for isolation of animal serine proteases, novel insecticidal proteins against MPB larvae have been isolated from *Mucuna* seeds (Machuka 2000b). Although protein database searches revealed that the N-terminus of these proteins is similar to a novel human synovial membrane fluid protein, it is not clear exactly what these proteins are and what their role is in plants. Other candidate genes



that may be implicated in MPB resistance may include chitinases and lectin-like proteins (Colucci et al. 1999, Machuka and Okeola 2000).

## Genes for resistance to pod-sucking bugs

PSBs are probably the next most serious pests of cowpea for which conventional breeding approaches have been inadequate. Omitogun et al. (1999) were the first to demonstrate that crude lectin-enriched extracts from AYB affect development of the cowpea coreid bug (*Clavigralla tomentosicollis* [Stal]) (Hemiptera: Coreidae). Subsequently, the purified seed lectin (SSA) from AYB has been shown to be toxic to *C. tomentosicollis* in an artificial cowpea seed system (Machuka et al. 1999a, Okeola et al. 2000). Wheat germ agglutinin, the nonprotein amino acid (para-aminophenylalanine, PAPA) from *V. vexillata*, and a cysteine protease inhibitor (E-64) also inhibit development of *C. tomentosicollis* nymphs (Jackai, Shade, and Murdock, unpublished). More studies are needed to identify other candidate proteins for resistance to PSB.

## Some ecological issues related to projected transgenic cowpea release

It is clear from the above survey that candidate genes for transgenic insect control in cowpea are available. In order to realise the potential of this approach it is imperative to establish a stable genetic transformation system for this crop. At the same time, it is also crucial for cowpea scientists to begin to discuss the ecological issues associated with release of transgenic cowpeas, particularly in Africa.

Although it is true that pest resistance genes identified in wild and cultivated *Vigna* germplasm have been incorporated into cultivated varieties by farmers and breeders for several years (Singh et al. 1990; Fatokun 1991; Jackai et al. 1996) the use of genetic engineering raises questions related to the transfer of transgenes to compatible wild or weedy *Vigna* species related to cowpea (Krattiger 1997; Snow and Palma 1997; Stewart 1999). Some of the issues to consider at this point include the possibility that introduced pest resistance may confer added fitness to cowpea, resulting in enhancement of weedy characteristics due to its increased ability to survive and spread outside of cultivation. Secondly, would transgenic cowpeas transfer pest resistance (or other traits) by natural hybridization to produce hybrid progeny that are more aggressive or more difficult to control? Although gene flow to related species is likely to be limited to *V. unguiculata* subspecies such as *V. unguiculata* ssp. *dekindtiana*, it is important to carry out field trials to determine rates of gene flow. Such a study is underway at IITA (Fatokun, personal communication). Obviously, information will be required from many disciplines such as weed science, agronomy, population biology and genetics, entomology, plant breeding, ecology, plant pathology, molecular biology, and from farmers.

## Conclusion

Reliable and efficient bioassay systems need to be continuously developed and refined to aid the discovery of insecticidal proteins for control of key cowpea pests. It can be anticipated that useful information emerging from current global genomics efforts in crop species, including model legumes, will have a bearing on cowpea improvement through genetic engineering. What cowpea researchers need to do now is develop a comprehensive pest resistance management strategy that incorporates transgenic approaches. Such

a strategy must take into account criteria such as transformation of elite cowpea lines that are adapted to each of the major agroecological zones, gene flow between cultivated and wild cowpeas, and strategies for dissemination and adoption of biotechnologically-improved cowpea lines.

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## References

- Bowen, D., T.A. Rocheleau, M. Blackburn, O. Andreev, E. Golubeva, R. Bhartia, and R.H. French-Constant. 1998. Insecticidal toxins from bacterium *Photorhabdus luminescens*. *Science* 280: 2129–2132.
- Colucci, G., J. Machuka, and M.J. Chrispeels. 1999. cDNA cloning of a class III acid chitinase from the African yam bean (*Sphenostylis stenocarpa*) (Accession no. AF137070). *Plant Physiology* 120: 633.
- Czapla, T.H. 1997. Plant lectins as insect control agents in transgenic plant. Pages 123–138 in *Advances in insect control: the role of transgenic plants*, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Czapla, T.H. and B.A. Lang. 1990. Effect of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and southern corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 83: 2480–2485.
- D'Silva, I., G.G. Poirier, and M.C. Heath. 1998. Activation of cysteine proteases in cowpea plants during the hypersensitive response—a form of programmed cell death. *Experimental and Cell Research* 245: 2389–2399.
- Duck, N.B. and S. Evola. 1997. Use of transgenes to increase host plant resistance to insects: opportunities and challenges. Pages 1–18 in *Advances in insect control: the role of transgenic plants*, edited by N. Carozzi and M.G. Koziel. Taylor and Francis, London, UK.
- Estruch, J.J., N.B. Carozzi, N.B. Duck, G.W. Warren, and M.G. Koziel. 1997. Transgenic plants: an emerging approach to pest control. *Nature Biotechnology* 15: 137–141.
- Estruch, J.J., G.W. Warren, M.A. Mullins, G.J. Nye, J.A. Craig, and M.G. Koziel. 1996. ViP3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings National Academy of Sciences of the USA* 93: 5389–5394.
- Fatokun, C.A. 1991. Wide hybridization in cowpea: problems and prospects. *Euphytica* 54: 137–140.
- Fietelson J., J. Payne, and L. Kim. 1992. *Bacillus thuringiensis*: insects and beyond. *Bio/Technology* 10: 271–275.
- Gatehouse, A.M.R., F.M. Dewey, J. Dove, and K.A. Fenton. 1984. Effect of seed lectins from *Phaseolus vulgaris* on the development of larvae of *Callosobruchus maculatus*, mechanism of toxicity. *Journal of the Science of Food and Agriculture* 35: 373–380.
- Gatehouse, A.M.R., D.S. Howe, J.E. Flemming, V.A. Hilder, and J.A. Gatehouse. 1991. Biochemical basis of insect resistance in winged bean (*Psophocarpus tetragonolobus*) seeds. *Journal of Science Food and Agriculture* 55: 63–74.
- Gatehouse A.M.R., V.A. Hilder, and D. Boulter. 1992. *Plant genetic manipulation for crop protection*. CAB International, Oxford, UK.
- Gatehouse, A.M.R., K.S. Powell, W.J. Peumans, E.J.M. Van Damme, and J.A. Gatehouse. 1995. Insecticidal properties of lectins; their potential in plant protection. Pages 35–58 in *Lectins: biomedical perspectives*, edited by A.J. Pusztai and S. Bardocz. Taylor and Francis, London, UK.

- Gerald, R.R., K.J. Kramer, J.E. Baker, J.F. Kanost, and C.A. Behke. 1997. Proteinase inhibitors and resistance of transgenic plants to insects. Pages 157–183 in *Advances in insect control: the role of transgenic plants*, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Gibson S. and C. Somerville. 1993. Isolating plant genes. *Trends in Biotechnology* 11: 306–312.
- Hanzlik T.N. and K.H.J. Gordon. 1997. The Tetraviridae. *Advances in Virus Research* 48: 101–168.
- Hilder V. A., A.M.R. Gatehouse, S.E. Sheerman, R.F. Barker, and D. Boulter. 1987. A novel mechanism of insect resistance engineered into tobacco. *Nature* 300: 160–163.
- Huesing, J.E., L.L. Murdock, and R.E. Shade. 1991a. Rice and stinging nettle lectins: insecticidal activity similar to wheat germ agglutinin. *Phytochemistry* 30: 3565–3568.
- Huesing, J.E., L.L. Murdock, and R.E. Shade. 1991b. Effect of wheat germ isolectins on development of cowpea weevil. *Phytochemistry* 30: 785–788.
- Huesing J.E., R.E. Shade, M.J. Chrispeels, and L.L. Murdock. 1991c.  $\alpha$ -amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. *Plant Physiology* 96: 993–996.
- Ishimoto M., T. Yamada, and A. Kaga. 1999. Insecticidal activity of an  $\alpha$ -amylase inhibitor-like protein resembling a putative precursor of  $\alpha$ -amylase inhibitor in the common bean, *Phaseolus vulgaris* L. *Biochimica et Biophysica Acta* 1432: 104–112.
- Ishimoto, M., T. Sato, M.J. Chrispeels, and K. Kitamura. 1996. Bruchid resistance of transgenic azuki bean expressing the seed  $\alpha$ -amylase inhibitor of common bean. *Entomologia Experimentalis et Applicata* 79: 309–315.
- Jackai, L.E.N. and J.R. Raulston. 1988. Rearing of legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae) on artificial diet. *Tropical Pest Management* 34: 168–172.
- Jackai, L.E.N., S. Padulosi, and Q. Ng. 1996. Resistance to the legume pod borer, *Maruca vitrata* Fabricius, and the probable modalities involved in wild *Vigna*. *Crop Protection* 15: 753–761.
- Koiwa, H., R.A. Bressan, and P.M. Hasegawa. 1997. Regulation of protease inhibitors and plant defense. *Trends in Plant Sciences* 2: 379–383.
- Kramer, K.L., S. Muthukrishnan, L. Johnson, and F. White. 1997. Pages 185–193 in *Advances in insect control: the role of transgenic plants*, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Krattiger, A. F. 1997. Insect resistance in crops: a case study of *Bacillus thuringiensis* (Bt) and its transfer to developing countries. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, USA.
- Leonard, V., D. Seck, G. Lognay, C. Gaspqr, and M. Severin. 1993. Biological activity of *Cassia occidentalis* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 25: 311–318.
- Machuka, J. 2000a. Characterization of the seed proteins of velvetbean (*Mucuna pruriens*) from Nigeria. *Food Chemistry* 68: 421–427.
- Machuka, J. 2000b. Insecticidal effects of velvetbean seed proteins against *Maruca* pod-borer larvae, in Abstracts, Proceedings of World Cowpea Conference III, 4–7 September 2000. IITA, Ibadan, Nigeria.
- Machuka J. and O.G. Okeola. 2000. One and two dimensional gel-electrophoresis identification of African yam bean seed proteins. *Journal of Agricultural and Food Chemistry* 48: 2296–2299.
- Machuka, J.S., O.G. Okeola, E.J.M. Van Damme, M.J. Chrispeels, F.V. Leuven, and W.J. Peumans. 1999a. Isolation and partial characterization of galactose-specific lectins from African yam beans, *Sphenostylis stenocarpa* Harms. *Phytochemistry* 51: 721–728.
- Machuka, J.S., E.J.M. Van Damme, W.J. Peumans, and L.E.N. Jackai. 1999b. Effect of plant lectins on larval development of the legume pod borer, *Maruca vitrata*. *Entomologia Experimentalis et Applicata* 93: 179–187.

- Machuka, J.S., O.G. Okeola, M.J. Chrispeels, and L.E.N. Jackai. 2000. African yam bean seed lectins affect the development of the cowpea weevil but do not affect the development of larvae of legume pod borer. *Phytochemistry* 53: 667–674.
- Murdock, L.L., J.E. Huesing, S.S. Nielsen, R.C. Pratt, and R.E. Shade. 1990. Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* 29: 85–89.
- Okeola, O.G., J. Machuka, and I.O. Fasidi. 2000. Insecticidal activities of the African yam bean seed lectin on the development of bruchid beetle and pod sucking bugs, *in* Abstracts, Proceedings of World Cowpea Conference III, 14–17 September 2000. IITA, Ibadan, Nigeria.
- Omitogun, O.G., L.E.N. Jackai, and G. Thottappilly. 1999. Isolation of insecticidal lectin-enriched extracts from African yam bean, *Sphenostylis stenocarpa* Harms, and other legume species. *Entomologia Experimentalis et Applicata* 90: 301–311.
- Pechan, T., L. Ye, Y. Chang, A. Mitra, L. Lin, F.M. Davis, W.P. Williams, and Dawn S. Luthe. 2000. A unique 33-kd cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other Lepidoptera. *Plant Cell* 12: 1031–1040.
- Pittendrigh, B.R., J.E. Huesing, R.E. Shade, and L.L. Murdock. 1997. Effects of Cry1A/Cry1B Bt endotoxins, PAPA, protease and  $\alpha$ -amylase inhibitors, on the development of the rice weevil, *Sitophilus oryzae*, using an artificial seed bioassay. *Entomologia Experimentalis et Applicata* 82: 201–211.
- Pratt, R.C., N.K. Singh, R.E. Shade, L.L. Murdock, and R.A. Bressan. 1990. Isolation and partial characterization of a seed lectin from Tepary Bean that delays bruchid beetle development. *Plant Physiology* 93: 1453–1459.
- Purcell, L. 1997. Cholesterol oxidase for the control of boll weevil. Pages 95–108 *in* Advances in insect control: the role of transgenic plants, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Pusztai A, G. Grant, D.J. Brown, J.C. Stewart, S. Bardocz, S.W. Ewen, A.M.R. Gatehouse, and V. Hilder. 1992. Nutritional evaluation of the trypsin (EC 3.4.21.4) inhibitor from cowpea (*Vigna unguiculata* Walp.). *British Journal of Nutrition* 68: 783–791.
- Ryan, C.A. 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology* 28: 425–449.
- Shade R.E., H.E. Schroeder, J.J. Pueyo, L.M. Tabe, L.L. Murdock, T.J.V. Higgins, and M.J. Chrispeels. 1994. Transgenic pea seeds expressing the alpha-amylase inhibitor of the common bean are resistant to bruchid beetles. *Bio/Technology* 12: 793–796.
- Sales M.P., V.M. Gomes, K.V. Fernandes, and J. Xavier-Filho. 1996. Chitin-binding proteins from cowpea (*Vigna unguiculata*) seeds. *Brazilian Journal of Medical and Biological Research* 29: 319–326.
- Shade, R.E., L.L. Murdock, D.E. Foard, and M.A. Pomeroy. 1986. Artificial seed system for bioassay of cowpea weevil (Coleoptera: Bruchidae) growth and development. *Environmental Entomology* 15: 1286–1291.
- Shade, R.E., H.E. Schroeder, J.J. Pueyo, L.M. Tabe, L.L. Murdock, T.J.V. Higgins, and M.J. Chrispeels. 1994. Transgenic pea seeds expressing the  $\alpha$ -amylase inhibitor of the common bean are resistant to bruchid beetles. *Biotechnology* 12: 793–796.
- Schulera, T.H., G. Poppya, B.R. Kerrya, and I. Denholmb. 1998. Insect-resistant transgenic plants. *Trends in Biotechnology* 16: 168–175.
- Seck, D., G. Lognay, E. Haubruge, J-P. Wathelet, M. Marler, C. Gaspqr, and M. Severin. 1993. Biological activity of the shrub *Boscia senegalensis* (Pers.) Lam. Ex Poir. (Capparaceae) on stored grain pests. *Journal of Chemical Ecology* 19: 377–389.
- Singh, S.R. and L.E.N. Jackai. 1985. Insect pests of cowpea in Africa: their life cycle, economic importance, and potential for control. Pages 217–231 *in* Cowpea research, production, and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.

- Singh S.R., L.E.N. Jackai, J.H.R. Dos Santos, and C.B. Adalla. 1990. Insect pests of cowpea. Pages 43–91 *in* Insect pests of tropical food legumes. John Wiley and Sons, Chichester, UK.
- Smigocki, A., S. Heu, I. Mccanna, C. Wozniak, and G. Buta. 1997. Insecticidal compounds induced by regulated overproduction of cytokinins in transgenic plants. Pages 225–2363 *in* Advances in insect control: the role of transgenic plants, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Snow, A.A. and P.M. Palma. 1997. Commercialization of transgenic plants: potential ecological risks. *BioScience* 47: 86–96.
- Stewart, C.N. 1999. Insecticidal transgenes into nature: gene flow, ecological effects, relevancy, and monitoring. Pages 179–190 *in* Gene flow and agriculture, relevance for transgenic crops. Proceedings of a symposium held at the University of Keele, 12–14 April 1999, edited by P.J.W. Lutman. British Crop Protection Council, Surrey, UK.
- Van Damme, E.J.M., W.J. Peumans, A. Pusztai, and S. Bardocz. 1998a. A handbook of plant lectins: properties and biomedical applications. John Wiley and Sons, Chichester, UK.
- Van Damme, E.J.M., W.J. Peumans, A. Barre, and P. Rougé. 1998b. Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Critical Reviews in Plant Sciences* 17: 575–692.
- Warren, G.W. 1997. Vegetative insecticidal proteins: novel proteins for control of corn pests. Pages 109–121 *in* Advances in insect control: the role of transgenic plants, edited by N. Carozzi and M. Koziel. Taylor and Francis, London, UK.
- Williams, W.P., P.M. Buckley, and F. Davis. 1987. Tissue culture and its use in investigations of resistance of maize. *Agriculture, Ecosystems and Environment* 18: 185–190.
- Yunes, A.N., T.M. de Andrade, P.M. Sales, R.A. Morais, V.S. Fernandez, V.M. Gomes, and J. Xavier-Filho. 1998. Legume seed vicilins interfere with the development of the cowpea weevil (*Callosobruchus maculatus*). *Journal of Agricultural and Food Chemistry* 76: 111–116.

# Insecticidal activities of the African yam bean seed lectin on the development of the cowpea beetle and the pod-sucking bug

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## Abstract

The cowpea beetle, *Callosobruchus maculatus*, and pod-sucking bug, *Clavigralla tomentosicollis*, are two of the major insect pests of cowpea in Africa. A lectin was purified from the seeds of the African yam bean (AYB), *Sphenostylis stenocarpa*, by affinity chromatography on Galactose-Sepharose 4B. The purified AYB lectin (AYBL) was tested on the two insect pests of cowpea. When *C. maculatus* larvae were fed on artificial cowpea seed containing 0.2, 2, and 5% (w/w) of dietary lectin, larval mortality ranged from 30 to 88% and delay in number of days to first emergence from 4–13 days. When AYBL was tested on *C. tomentosicollis*, nymphal mortalities ranged from 76 to 81% at 1% and 87 to 94% at 2%. From 4 to 8%, no nymph survived up to six days after infestation. The results of these insect bioassays provided a scientific basis for isolating a lectin gene from AYB for the transformation of cowpea.

## Introduction

The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and the pod-sucking bug, *Clavigralla tomentosicollis* Stal (Hemiptera: Coreidae) are two major insect pests of cowpea in Africa. *C. maculatus* attacks cowpea during storage (Jackai and Adalla 1997). Farm storage for six months is accompanied by about 30% loss in weight with up to 70% of the seeds infested and unfit for consumption (Singh and Jackai 1985).

*C. tomentosicollis* feeds primarily on the developing pods in the field where it causes extensive damage to pods and seeds (Jackai 1984). These insect infestations cause weight and quality losses that lead to a reduction in commercial value and seed viability.

In recent times, control of crop insect pests has focused mainly on the use of genetic engineering to develop transgenic plants that express insecticidal proteins. For example, the toxic proteins produced by *Bacillus thuringiensis* conferred resistance on cotton against pink bollworm (Wilson et al. 1992) while cowpea inhibitor genes conferred resistance on tobacco against corn earworm (Hoffman et al. 1991). Other control agents are peroxidases, chitinases, and plant lectins (Duck and Evola 1997; Machuka et al. 1999).

Lectins are a large and heterogeneous group of proteins (Van Damme et al. 1998) possessing at least one noncatalytic domain, which binds reversibly to a specific mono- or oligosaccharide (Peumans and Van Damme 1995). In a preliminary investigation, Omitogun et al. (1999) conducted bioassays on *C. maculatus* and *C. tomentosicollis* using

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crude lectin extracts from 20 resistant *Vigna* and non-*Vigna* legumes. The extracts from the African yam bean (AYB), *Sphenostylis stenocarpa* (Harms) was toxic to the insect pests at 5% (w/w) concentration. However, the result of their investigation was inconclusive as it could not be ascertained whether the lectin or other protein contaminants present in the extracts were responsible for the toxicity.

This study was conducted to investigate the effect of purified AYB lectins on *C. maculatus* and *C. tomentosicollis*.

## Materials and methods

### Seed

Seeds of AYB were obtained from the Genetic Resources Unit of International Institute of Tropical Agriculture (IITA), Ibadan. Seeds of three AYB accessions were used in this study; EN953, EN982, and UMUE9832. EN953 was used by Omitogun et al. (1999) while EN982 and UMUE9832 were recent collections. Seeds of Ife Brown, a cultivated cowpea variety, were used as the susceptible control and a wild *Vigna* (*V. vexillata*) accession (TVnu 72), was the resistant control.

### Insects

The insects were obtained from the insect rearing laboratory at IITA, Ibadan. This laboratory is maintained at  $26 \pm 2^\circ\text{C}$  and 70–80% RH.

### Preparation of affinity matrix

Galactose was coupled to Sepharose 4B by the divinyl sulphone coupling method according to Peumans et al. (1995).

### Lectin purification

Five hundred grams of AYB seeds were milled in a Warren blender. The seed meal was then extracted in 2 L of ascorbic acid solution by stirring for at least one hour at room temperature. The homogenate was then centrifuged at 5000 rpm for four to five minutes. The supernatant was saved, adjusted to pH 7.5 with sodium hydroxide, and then filtered on Whatman filter paper (No. 1). The resulting filtrate was applied onto a column (5 × 2 cm) of Galactose-Sepharose 4B that had been equilibrated with 1M  $\text{NH}_4\text{SO}_4$ . Unbound proteins were washed off with 1M  $\text{NH}_4\text{SO}_4$  until  $A_{280}$  fell below 0.3. The lectin was then eluted with 20 mM 1, 3-diamino-propane (DAP), desalted on Sephadex G 25 column and lyophilized.

### Artificial seeds

Artificial cowpea seeds (ACS), as previously described by Shade et al. (1986), were used as the delivery method in these insect bioassays. ACS were prepared by milling decoricated Ife Brown seeds, adding aqueous solutions containing 0.2, 1, 2, 4, 5, 6, and 8% (w/w) lectins to make a paste. The paste was injected into a teflon mold, frozen, and then lyophilized for 24 hours. The resulting pellets were hydrated at  $29^\circ\text{C}$  and  $60 \pm 5\%$  RH and coated with 8% gelatin solution (w/w).

### Insect bioassays

ACS containing 0.2, 2, and 5% (w/w) lectin were tested with *C. maculatus*. Two adult insects (male and female) were placed in a Petri dish containing five artificial or control seeds. The dishes were incubated on a shelf at  $27 \pm 2^\circ\text{C}$  and  $65 \pm 2\%$  RH for 24 hours.

This was to allow the insects to mate and lay eggs, after which they were removed. After seven days, the eggs on the seeds were counted for each sample. After two weeks, the various treatments were examined daily for adult emergence. Emerged adults were counted and removed daily. Observations were terminated two weeks after the first adult emerged.

For *C. tomentosicollis*, we employed a bioassay previously developed in the Insect Rearing Unit of IITA. One ACS of each lectin treatment (1, 2, 4, 6, and 8%) was placed in a separate box (6.5 cm × 6.5 cm × 2.5cm). An inverted lid of a 10-dram vial with a slightly moistened cotton wool swab was placed in the box to provide water for the insects. Five first instar nymphs of *C. tomentosicollis* were placed inside each box and covered. Other treatments with either blank ACS without lectin and intact seeds (Ife Brown and TVnu 72) were included as controls. Seeds and cotton swabs were changed only if mold started to grow on them. The boxes were left undisturbed on laboratory shelves (10:14h; light:dark;  $26 \pm 2^\circ\text{C}$ ; and 70 to 80% RH) until the end of the experiment. Each treatment was replicated five times. The following variables were determined from the bioassays: number of eggs per seeds (for *C. maculatus*), number of emerged adults, mortality (the total number of hatched eggs/first instar nymphs used for infestation for each treatment, minus the total number of emerged adults for each treatment, divided by the number of hatched eggs/first instar nymphs for each treatment, times a hundred), and total development time (TDT).

### Statistical analysis

The data were analyzed using General Linear Model (GLM) procedures and the means were separated by Duncan's Multiple Range Test (DMRT) (SAS 1989). Percentage data were transformed using arcsin transformation prior to analysis.

## Results and discussion

AYB lectin is a tetrameric protein of about 122 kDa. It is composed of four subunits with molecular mass of about 27, 29, 32, and 34 kDa, respectively (Fig. 1). During purification, higher and lower molecular weight-contaminating proteins were successfully removed (Fig. 1). The tetrameric nature of AYB lectin was similar to that of *Glycine max* (soybean) and *Phaseolus vulgaris* (red kidney bean) in that they also have four subunits with molecular weights of 115–140 and 120 kDa, respectively (Sharon 1973). However, not all plant lectins are tetrameric proteins. Some are dimeric, containing only two subunits. For example, the lectin from the greater celandine contains two subunits with molecular weights of 9.5 and 11.5 kDa, respectively (Peumans et al. 1985).

When bruchid beetles were fed on ACS containing 0.2, 2, and 5% (w/w) dietary lectin, larvae mortality of *C. maculatus* ranged from 30 to 88% (Fig. 2) whereas low mortality (5%) was observed for larvae fed on Ife Brown (Fig. 2). In an earlier study, Huesing et al. (1991) observed that larval mortality ranged from 8 to 12% when fed on susceptible cowpea lines. When EN953 and UMUE9832 lectins were increased from 0.2 to 2%, nymph mortality increased from 30 to 33.3% with EN953 and from 29.67 to 33.33% with UMUE9832. However, when the lectin concentration from the two AYB accessions was increased to 5%, the percentage mortality increased between 2-fold and 2.5-fold (Fig. 2).

AYB lectin greatly reduced *C. maculatus* progeny and delayed the TDT, compared to the susceptible control (Fig. 3). Sixteen adults in all emerged from Ife Brown, whereas only 10 adults emerged from ACS containing EN953 and 11 adults from UMUE9832 at 0.2% (w/w) lectin. The toxic effect of AYB lectin was more pronounced at 5% dietary



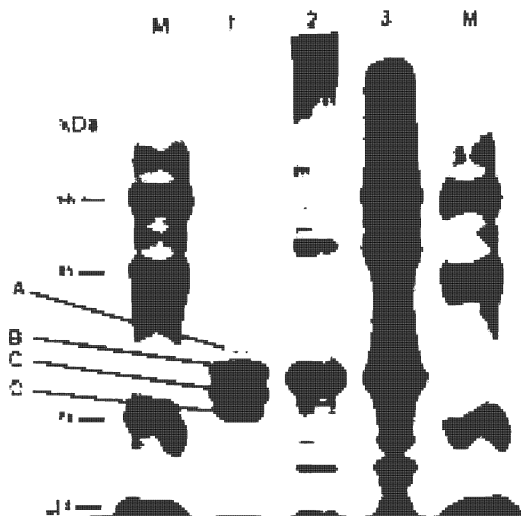


Figure 1. SDS-PAGE (12%) of *Sphenostylis stenocarpa* (EN982) seed proteins.

M = Molecular mass reference proteins

1 = Affinity purified lectin

2 = Non-lectin fraction

3 = Total protein

Identical pattern was visualized for all accessions of AYB.

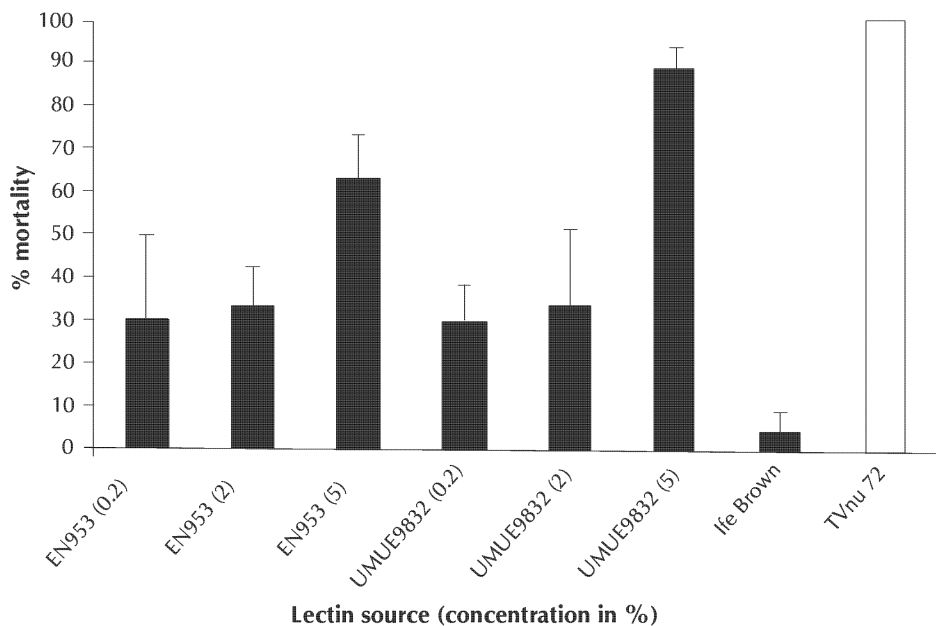


Figure 2. Effect of lectin from *Sphenostylis stenocarpa*, cowpea, and *V. vexillata* on the mortality of *C. maculatus*.

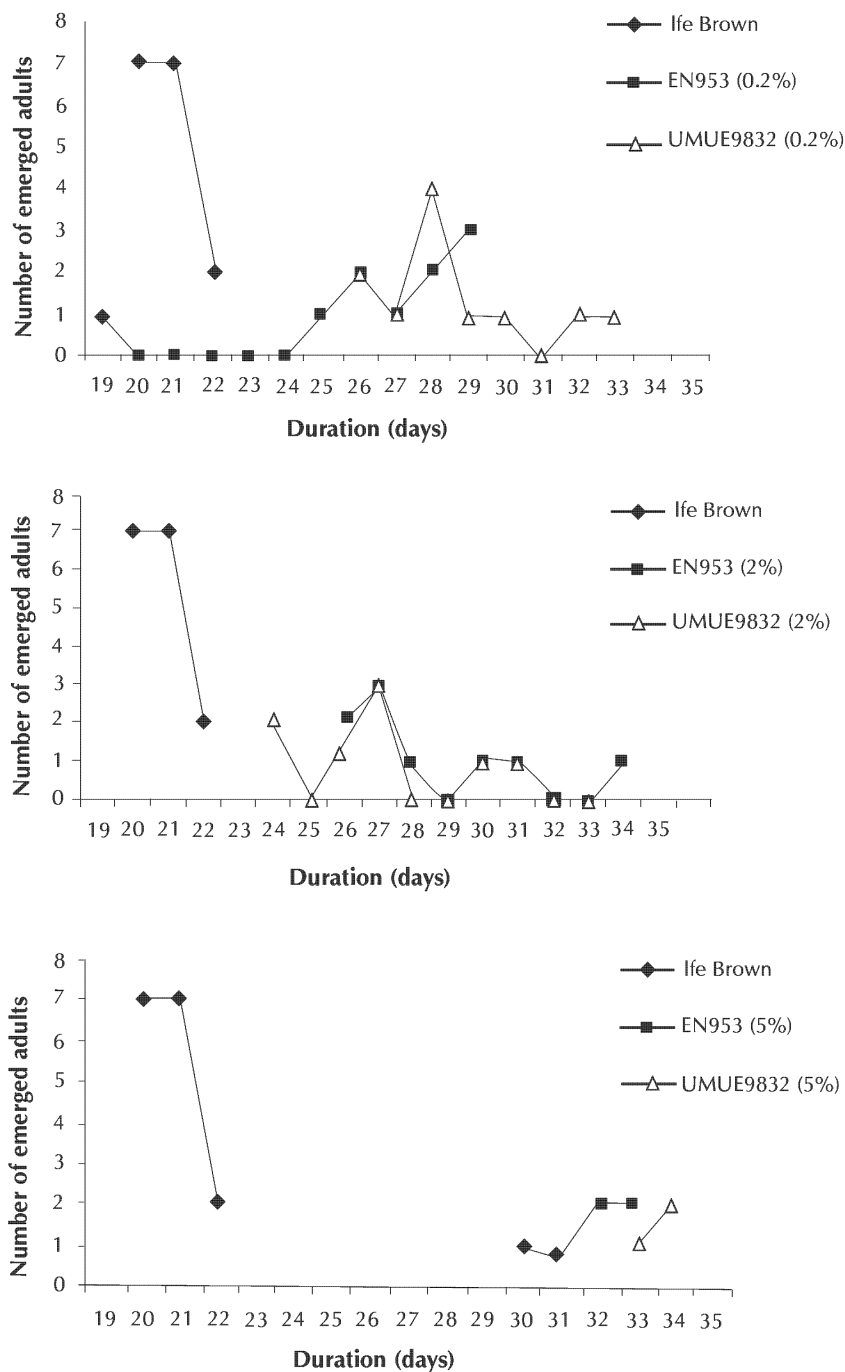


Figure 3. Emergence patterns of *C. maculatus* from artificial cowpea seeds containing different concentrations of *S. stenocarpa* seed lectin.

lectin concentration. Only six adults emerged from ACS containing EN953 lectin and three from ACS containing UMUE9832 lectin (Fig. 3). With the exception of EN953 (0.2%), delay in number of days to first emergence also ranged from 4 to 13 days in all the different lectin concentrations (Fig. 3). Similar effects of plant lectins on *C. maculatus* had been reported by Murdock et al. (1990). Lectins from *Arachis hypogaea*, *Solanum tuberosum*, *Datura stramonium*, *Triticum aestivum*, and *Maclura pomifera* were found to cause a significant delay in *C. maculatus* developmental time at a dietary level of 1%. The presents study shows that AYB lectin is insecticidal to *C. maculatus*.

The deleterious effect of AYB lectins on *C. tomentosicollis* is shown in Figure 4. Nymph mortality ranged from 76 to 81% at 1% and from 87 to 94% at 2% dietary lectin concentrations. From 4 to 8% lectin concentrations, no nymph survived more than six days. *C. tomentosicollis* nymphs survived on Ife Brown and blank ACS. No adult emerged from the resistant control seed and ACS containing 4% lectin (Fig. 4). This lectin was obviously toxic to *C. tomentosicollis*.

Furthermore, there was a significant difference in the TDT obtained when *C. tomentosicollis* nymphs were fed on blank ACS ( $17.65 \pm 0.47$  days) as compared to the intact susceptible seed treatment ( $13.77 \pm 0.29$  days) ( $P < 0.05$ ). This was unexpected. These insects are different in their feeding mode from cowpea bruchids for which the ACS was originally developed. Although ACS has been previously used on *C. tomentosicollis* (Omitogun et al. 1999; Koona 1999), this delivery system has some defects for bioassays on *C. tomentosicollis*. Possibly by reducing the concentration of gelatin used in making the ACS, insect development similar to that observed when insects are fed on intact susceptible seeds could be obtained. Plans are underway to examine the optimum gelatin concentration that will be required for making ACS for bioassays on *C. tomentosicollis*.

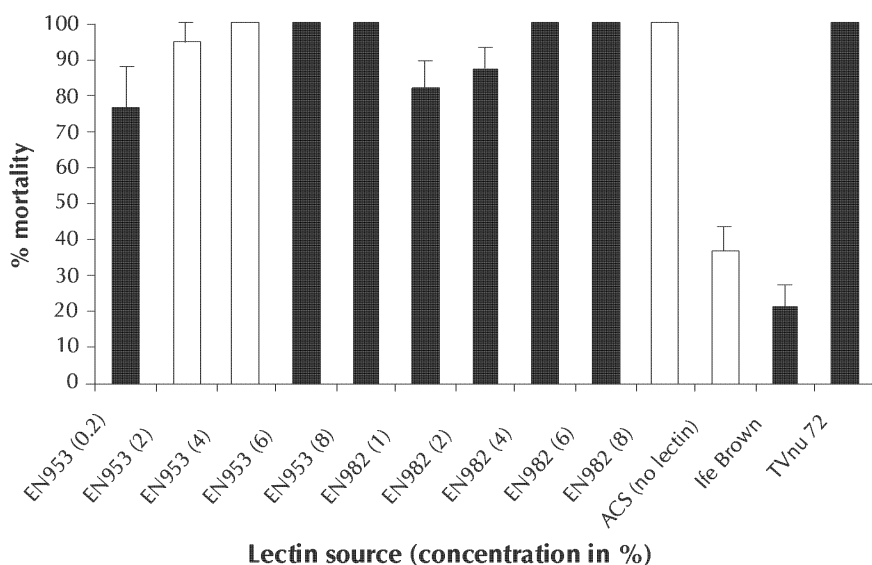


Figure 4. Effect of *Sphenostylis stenocarpa* lectin on the mortality of *C. tomentosicollis*.

Reports on lectin bioassays on *C. tomentosicollis* are not common, as most lectin bioassays have been on *C. maculatus* (Gatehouse et al. 1984; Murdock et al. 1990; Huesing et al. 1991; Zhu et al. 1996). Considering the high larval/nymph mortality rate and delay in TDT observed in these bioassays, AYB lectin seems to be biologically active and a promising candidate for genetic transformation of cowpea against *C. maculatus* and *C. tomentosicollis*.

## Acknowledgements

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## References

- Duck, N. and S. Evola. 1997. Use of transgenes to increase host plant resistance to insects; opportunities and challenges. Pages 1–18 in *Advances in insect control*, edited by N. Carizzi and M. Koziel. Taylor and Francis, London, UK.
- Gatehouse, A.M.R., F.M. Dewey, J. Dove, H.A. Fenton, and A. Putztai. 1984. Effect of seed lectins from *Phaseolus vulgaris* on the development of larvae of *Callosobruchus maculatus*: a mechanism of toxicity. *Journal of Science and Food Agriculture* 35: 373–380.
- Hoffman, M.P., F.G. Zalom, J.M. Smilanick, L.T. Wilson, L.D. Malyj, J. Kiser, V.A. Hilder, and W.M. Barnes. 1991. Field evaluation of transgenic tobacco containing genes encoding *Bacillus thuringiensis* endotoxin or cowpea trypsin inhibitor efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 85: 2516–2522.
- Huesing, J.E., L.L. Murdock, and R.E. Shade. 1991. Rice and stinging nettle lectins: insecticidal activity similar to wheat germ agglutinin. *Phytochemistry* 30: 3565–3568.
- Jackai, L.E.N. 1984. Studies on the feeding behaviors of *Clavigralla tomentosicollis* (Stal.) (Hemiptera: Coreidae) and their potential use in bioassays for host plant resistance. *Journal of Applied Entomology* 98: 344–350.
- Jackai, L.E.N. and C.B. Adalla. 1997. Pest management practices in cowpea. Pages 240–259 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Koona, A.A. 1999. Anatomical and biochemical basis of resistance of wild and cultivated *Vigna* species to the Coreid bug, *Clavigralla tomentosicollis* Stal. PhD thesis, University of Ibadan, Ibadan, Nigeria. 213 pages.
- Machuka, J., E.J.M. Van Damme, W.J. Peumans, and L.E.N. Jackai. 1999. Effect of plant lectins on survival development of the pod borer *Maruca vitrata*. *Entomologia Experimentalis et Applicata* 93: 179–187.
- Murdock, L.L., J.E. Huesing, S.S. Nielsen, R.C. Pratt, and R.E. Shade. 1990. Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* 29: 85–89.
- Omitogun, O.G., L.E.N. Jackai, and G. Thottappilly. 1999. Isolation of insecticidal lectin enriched extracts from African yam bean, *Sphenostylis stenocarpa* (Harms) and other legume species. *Entomologia Experimentalis et Applicata* 90: 301–311.
- Peumans, W.J., M. de Ley, H.M. Stinissen, and W.F. Broekaert. 1985. Isolation and partial characterization of a new lectin from seeds of the greater celandine (*Chelidonium majus*). *Plant Physiology* 78: 379–383.
- Peumans, W.J. and E.J.M. Van Damme. 1995. Lectins as plant defence proteins. *Plant Physiology* 109: 347–352.
- Peumans, W.J., H.C. Winter, V. Berner, F. Van Leuven, I.J. Goldstein, P. Truffa-Bachi, and E.J.M. Van Damme. 1995. Isolation of a novel plant lectin with unusual specificity from *Calsepa sepium*. *Glycoconjugate Journal* 14: 259–265.

- SAS User's Guide. 1989. SAS Institute Inc., Cary, NC, USA.
- Shade, R.E., L.L. Murdock, D.E. Foard, and M.A. Pomeroy. 1986. Artificial seed system for bioassay of cowpea weevil (Coleoptera: Bruchidae) growth and development. *Environmental Entomology* 15: 1286–1291.
- Sharon, N. 1973. Glycoproteins of higher plants. Pages 235–252 *in* Carbohydrate biochemistry, edited by J.B. Pridham. Academic Press, London, UK.
- Singh, R. and L.E.N. Jackai. 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and potential for control. Pages 217–231 *in* Cowpea research, production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons, Chichester, UK.
- Van Damme, E.J.M., W.J. Peumans, A. Putzai, and S. Bardocz. 1998. Handbook of plant lectins: properties and biomedical applications. John Wiley and Sons, Chichester, UK. 452 pages.
- Wilson, F.D., H.M. Flint, W.R. Deton, D.A. Fischhoff, F.J. Perlak, J.A. Armstrong, R.L. Fuchs, S.A. Berberich, N.J. Parks, and V.B.R. Stapp. 1992. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelichiidae) and other insects. *Journal of Economic Entomology* 85: 1516–1521.
- Zhu, K., J.E. Huesing, R.E. Shade, R.A. Bressan, P.M. Hasegawa, and L.L. Murdock. 1996. An insecticidal N-acetylglucosamine-specific lectin gene from *Griffonia simplicifolia* (Leguminosae). *Plant Physiology* 110: 195–202.

## **Section IV**

**Cowpea contributions to farming  
systems/agronomic improvement  
of cowpea production**



## Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa

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### Abstract

Agriculture in the dry savannas is intensifying in response to increasing populations of humans and livestock. As a result, increased productivity demands are placed upon integrated crop–livestock systems and more emphasis is on the roles of legumes such as cowpea. Cowpea has the potential to function as a key integrating factor in intensifying systems through supplying protein in the human diet, and fodder for livestock, and bringing nitrogen into the farming system through nitrogen fixation. This paper describes the development and evaluation of integrated “best-bet” options which maximize the benefits of cowpea and addresses aspects of improved crop varieties, crop and livestock management, nutrient cycling, and soil fertility. The approach used includes a multicenter, multidisciplinary approach to working with farmers which combines complementary strengths of previous component research involving crops and livestock by key international and national research institutions in the region.

### Introduction

Cowpea is an important crop for farmers in much of the West African region, particularly in the dry savannas. Estimates of world hectareage of cowpea is in the range of 12.5 million, with about 8 million in West Africa, the majority of these being in Niger and Nigeria (Singh et al. 1997). Current FAO estimates for 1999 are lower than these figures, although the proportions are similar (FAO 2000). The same database estimates average cowpea grain production in West Africa as 358 kg/ha whereas Singh et al. (1997) estimate 240 kg/ha as an average for northern Nigeria. The apparent popularity of the crop may seem paradoxical if only the relatively low grain yields on farmers’ fields are considered. Perhaps this is related to the fact that cowpea is a legume with the potential for multiple contributions not only to household food production, but also as a cash crop (grain and fodder), livestock feed, and soil ameliorant. In this context, it is a crop that may have a wide role

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in contributing to food security, income generation, and the maintenance of the environment for millions of small-scale farmers who grow it in the region. In order to place such contributions in context, this paper will begin by considering the ongoing evolution of farming systems in West Africa, especially the integration of crop and livestock production, with reference to the particular features of the dry savannas where these scenarios are prominent. The potential role that cowpea can play in addressing the opportunities posed will also be addressed and as part of this, ongoing research which includes the utilization of such multiple benefits of cowpea will be considered.

## **The changing face of agriculture**

In sub-Saharan Africa, the population may reach 1.2 billion by 2025 and be combined with a demographic shift from about 30% of the population (in 1990) in urban areas to at least 50% (Winrock 1992). These changes will mean an increasing demand for crops and livestock and even if production expands at the rate of 3% annually, which would be necessary to meet this demand (Winrock 1992), it is likely that at least 21% of the children, about 39 million, will remain undernourished (Badiane and Delgado 1995). Recent studies have indicated that through both natural accretion and the change in requirements related to urbanization (Ehui et al. 1998), livestock demand in particular is likely to increase dramatically, ranging between an increase of 2.5% for mutton, pork, and poultry, to 4.2% for beef between 1993 and 2020 (Delgado et al. 1999).

Within sub-Saharan Africa, more than 40% of the region's current population is in West Africa (based on FAO estimates for 1999; FAO 2000) meaning that the opportunities and challenges presented by the intensification scenario will be heightened in this region. One of the responses of farming systems to agricultural intensification is the integration of crop and livestock production (McIntire et al. 1992). As crop farmers seek to increase production, their cropping activities spread onto marginal land, fallow periods become reduced or absent, and consequently, the demand for nutrient inputs is raised. In the absence of reliable and cheap supplies of inorganic fertilizers, manure from transhumant livestock becomes more important. At the same time, as livestock keepers enlarge their herds, crop residues from crop farmers increasingly become the major feed resource because there is no longer marginal or fallow land for grazing. Estimates have shown that ignoring crop residues as a feed resource would result in serious feed shortages (Naazie and Smith 1997). In these scenarios, crop farmers may begin to own their own livestock for ready access to manure and simultaneously sell off some of the marginal land to livestock keepers, who settle and begin crop farming, using the manure from their animals (and possibly traction) as an input (Okike et al. 2001). In the dry savannas of West and Central Africa, crop-livestock integration is already a common feature of the farming systems.

## **Dry savannas**

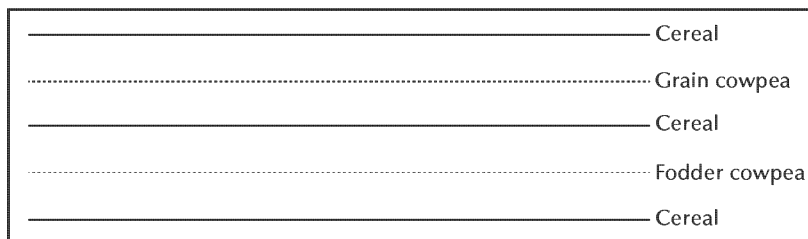
The dry savannas consist of the drier part of the northern Guinea savanna, plus the Sudan savanna representing more than 50% of the total land area of sub-Saharan Africa, with a significant proportion located in West Africa. Over 40% of the total ruminant livestock in West and Central Africa are in this region (Winrock 1992). Annual rainfall is less than 1000 mm with a growing period of 180 days or less meaning that much of the region experiences a long (7–9 months) harsh dry season. The growing period shortens on a

south–north axis. The sandy soils are generally poor, with low organic carbon, and cation exchange capacity, and are deficient in nutrients, especially nitrogen and phosphorus.

Cropping is cereal-based with sorghum and millet dominating, and the former decreasing in prominence towards the north. Intercropping cereals with grain legumes is common in over 90% of fields, with cowpea and groundnut being the most common legume components. As well as grain, the residues from cropping, especially from cowpea (and groundnut), are important components of the farming systems in particular as fodder resources for the ruminant livestock which are also an integral part of the farming systems. Cattle, sheep, goats, and to a lesser extent camels, provide milk, meat, traction, manure, and cash.

Major constraints to agricultural productivity in the region include the long dry season, which results in crop stress due to drought at the beginning and/or end of the wet season and a shortage of ruminant fodder during the harsh dry period. The poor soils and incidences of pests and diseases also have negative effects on crop production (both grain and fodder). In much of sub-Saharan Africa, inputs such as fertilizers and pesticides to counteract these negative forces are generally scarce or priced well above the means of the smallholder farmer.

Farm sizes in the region are generally small, ranging from about 3 to 6 ha; each field is usually 1 ha or less and one farmer rarely owns contiguous fields (Ogungbile et al. 1999). A typical cropping pattern is as follows (Singh and Tarawali 1997). At the onset of the rainy season, cereal (millet or sorghum) is sown in rows with wide interrow spaces; two–three weeks later, a grain type of cowpea (short duration) is sown in alternate interrow spaces, followed by a fodder (or dual-purpose, late maturing) type of cowpea in the remaining interrows about three weeks later (Fig. 1). The cropping layout may be complicated by replacing some of the cowpea rows with groundnut and the timing of planting (but not the order) may vary, with the interval between planting the crops often much shorter than three weeks. Cereals will mature and be harvested first, together with the grain type cowpea, which will give a reasonable grain yield, but virtually no crop residue. The remaining dual-purpose/fodder type cowpea is left to grow over the rest of the field, until the rains cease and the leaves begin to show signs of wilting. At this stage, any grain on the plants is harvested, and the residue is cut and rolled up for storage on house roofs or in tree forks. The stored residue is fed to ruminants during the dry season, or, in some cases, sold in local markets where the high price during this period of feed scarcity means it will make a substantial contribution to a farmer's income. The cereal stalks remaining after harvest are fed to ruminants, but often, the leaves may be stripped off and fed to animals and



**Figure 1. Schematic representation of common cropping pattern in the dry savannas. Spacing between the cereal rows can be as much as 3 m.**

the stalks used as building or fencing materials. Ruminants within farm compounds are supplemented with the cowpea residues and, within the compound, the manure is collected with household waste. At the start of the next cropping season, the “compost” of manure and household waste is spread on the crop fields, before land preparation.

Thus, in the dry savannas, crop and livestock enterprises are closely integrated, with reciprocal benefits from crop residues as livestock fodder, and the latter providing manure and in some cases, traction, that contribute directly to crop production. While the benefits of such integration are recognized, and mixed crop–livestock farming systems, which currently contribute over 50% of the world’s meat and over 90% of its milk (ILRI 2000) are recognized to have the greatest potential for intensification (de Haan et al. 1997), food demands of expanding populations place increased pressure on these systems to raise productivity. Such productivity increases, if they are to be sustainable, need to be achieved without damaging the natural resource base. In some cases, where production of mixed farming systems has intensified, the full implications have not been considered as, for example, soil is mined and severely degraded and livestock waste products become a problem, etc. (Delgado et al. 1999). In this context, the situation in the dry savannas of West Africa, where integrated crop and livestock production systems have existed for many decades, but now face the pressure to produce more, is ripe for interventions that address these opportunities. Cowpea, which can contribute both to crop–livestock production systems, and directly to soil fertility, has the potential to make major contributions in this respect.

## **Contributions of cowpea towards increased and sustainable productivity in mixed systems**

As a legume, cowpea can contribute to soil fertility, mainly through its nitrogen fixing abilities. Part of the nitrogen fixed will remain in the soil in the roots, and thereby contribute to the soil fertility for subsequent crops. Some fixed nitrogen will eventually return to the soil as manure after residues are fed to livestock. In terms of the direct effects of cowpea in rotation with cereals, Manu et al. (1994) report a comparison of on-station and on-farm studies in Niger where cowpea–millet intercrop and cowpea–millet rotations were used. Their results are summarized in Table 1. On farmers’ fields, rotation with cowpea gave 2.6 times more millet grain and 3.3 times more residue, than the intercropped, nonrotated treatment. Bagayoko et al. (1998) reported that cowpea can supply 35–40 kg N/ha in a cowpea–millet rotation, and Carsky and Berner (1995) presented similar figures for cowpea rotations with maize. See also Carsky et al. this volume.

**Table 1. Summary of results comparing cowpea intercropping with rotation in farmer- and researcher-managed fields.**

| Cropping system           |                | Yield (kg/ha)  |                    |
|---------------------------|----------------|----------------|--------------------|
|                           |                | Farmer-managed | Researcher-managed |
| Traditional intercropping | Millet grain   | 62             | 172                |
|                           | Millet residue | 162            | 827                |
| Rotation                  | Millet grain   | 163            | 308                |
|                           | Millet residue | 538            | 1531               |

Source: Extracted from Manu et al. (1994).

There is some evidence that cowpea may help to reduce the number of viable *Striga hermonthica* seeds in the soil through stimulating suicidal germination of the seed. *S. hermonthica* is parasitic on cereal plants, and causes huge crop losses (Berner et al. 1996). Carsky and Berner (1995) report that rotation with selected cowpea varieties has a substantial and rapid effect on reducing *S. hermonthica*, with the number of attached *S. hermonthica* plants per maize plant being reduced by at least 50% when maize was grown after cowpea.

Farmers' awareness of these roles of cowpea for soil fertility and *S. hermonthica* reduction is, to some extent, demonstrated by the fact that they usually rotate the legume and cereal rows within fields in alternate years. This means that the cereal and cowpea rows are interchanged each year, and the cereal will benefit at this "microlevel" from the cowpea grown in the previous year.

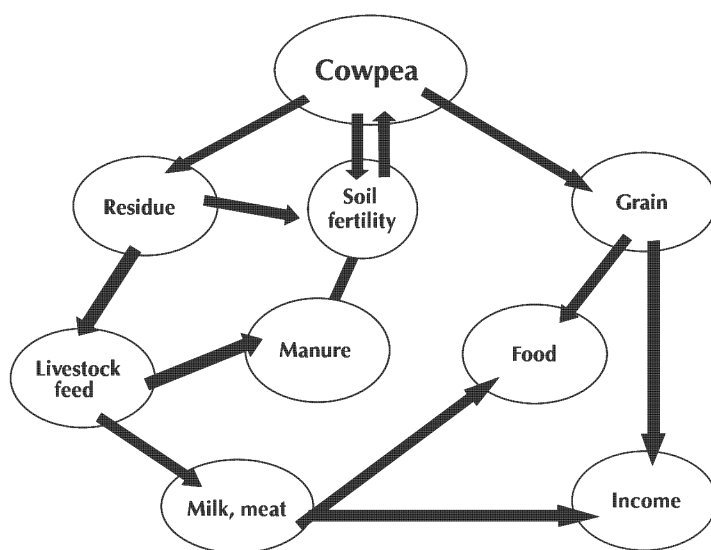
Cowpea residue is an important fodder resource for ruminant livestock (Tarawali et al. 1997). Farmers in the dry savannas deliberately grow varieties and use management practices that will ensure some cowpea fodder is available for harvest at the end of the growing season, even at the expense of grain production. Harvesting at the end of the wet season, before the dry season becomes severe, gives the best quality, and this is preserved throughout the storage period. If the fodder is harvested late, when the dry season is already underway, quality is poor (Tarawali et al. 1997). Recognition of the importance of fodder from cowpea led to the initiation of joint IITA–ILRI research in 1990 when fodder quantity and quality parameters were included in the breeding and selection program. These efforts resulted in the identification of promising dual-purpose cowpea varieties suitable for the dry savannas (Singh and Tarawali 1997).

Cowpea fodder as a feed supplement increases animal liveweight gain during the dry season. Schlecht et al. (1995) report an experiment where Zebu cattle (bulls of about 250 kg, equivalent to 1 TLU—Tropical Livestock Unit) were supplemented with 1 kg cowpea hay at night and 0.5 kg fresh rice feed meal in the morning per day/animal during the second half of the dry season. The animals were allowed to graze as usual for the rest of the day. From February 1988 to September 1989 the supplemented group gained 95 kg compared to 62 kg for the unsupplemented group. Taking animal numbers into account, this worked out to be equivalent to a difference of 67 g/animal/day. In many regions, cowpea fodder is particularly valued as a supplement in the period leading up to Muslim festivals when sheep are traditionally slaughtered. Some farmers sell cowpea fodder during the dry season when feed shortage is critical, and there have been suggestions that income from fodder sales makes a substantial contribution to the annual income in such cases (ICRISAT 1991). In addition to the direct benefits of improved livestock production and health that result from feeding cowpea fodder, the quantity and quality of manure from such better fed animals will be improved and therefore, when returned to the land at the beginning of the growing season, contribute more towards the maintenance of soil fertility. In the same experiment referred to above, although not significant in this particular trial, the manure nitrogen, in g N/TLU/day was on average 25% higher in animals receiving supplements.

Indications are that from 1 ha of improved cowpea, a farmer could benefit by an extra 50 kg meat per annum from better nourished animals, with over 300 kg more cereal grain as a result of improved soil fertility directly from the cowpea and more/better manure from the animals (Tarawali, unpublished). Of course, considerations of the time scale—increased

crop yields—would be realized only the next year and the distribution of manure should be taken into account. It is, however, noteworthy that these preliminary calculations have not considered all the potential benefits, for example, better fed traction animals would work harder, meaning more timely land preparation and better crop yields; better fed ruminants would give more milk and are likely to be more productive (increased weight gains mean young animals come into oestrus earlier). Providing more nutritious fodder also means that the comparatively indigestible parts of cereals (stalks, etc.) that are used as fodder are likely to be better consumed—intake of more fibrous material usually improves with the addition of better quality material to the diet. The potential impact of reduced *S. hermonthica* because of rotation with cowpea has also not been quantified.

Some of the potential contributions of cowpea described above are summarized in Figure 2. In view of these contributions of cowpea and the availability of improved varieties, when seeking to address the opportunities posed by the intensification of crop–livestock systems in the dry savannas, it was apparent that a key component should be improved dual-purpose cowpea varieties. What was equally clear, however, was that cowpea, livestock, or cereal crops never function in isolation in farm fields or households in the dry savannas; likewise, there is a complex of interactions between the biophysical, economic, social, and policy environments that influence farmers' decisions in these environments. As a result of such considerations, in the late 1990s, international and national institutions working on various aspects of component research in the dry savannas began to develop



**Figure 2.** Schematic representation of the potential contributions of cowpea in crop–livestock systems in the dry savannas. Not all potential interactions are shown for simplicity. For example, *dussa* is a regular household product which can contribute to livestock feed. Similarly, other crops and weeds in the system are not shown.

*Dussa* is the testa of the grain which is separated from the endosperm by soaking prior to pounding and winnowing.

a new approach designed to bring together some of these key elements. This strategy is presented by Tarawali et al. (2000) in the context of natural resource management. In this paper, the emphasis is on the role of cowpea in promoting food and feed production as well as sustainable agriculture.

## **Development of the research paradigm**

The three international research centers with interest in various aspects of the system began meeting to consider how best to initiate such an integrated approach. Scientists from IITA, with the world mandate for cowpea research, ILRI for livestock, and ICRISAT for cereals and groundnut as well as the majority of the dry savanna ecoregion began to plan joint research in 1997. International Fertilizer Development Centre, Niger, with an interest in the soils component of the system, and Center for Overseas Research and Development, University of Durham, UK, with scientists from national research and development institutions have also joined this group more recently. From the outset, there has been consensus among the institutes that the aim of this joint research should be to “improve the lives of farm families in the dry savanna and Sahel of West Africa through sustainable management of the natural resource base for food security and income generation.”

The first step in implementing the joint research was the establishment of an experiment at one location in 1998, using existing resources from the institutes involved. At the meeting to plan this research, two major principles were elucidated: first, the idea of “best-bet” options and secondly, a holistic, on-farm approach to evaluate these options. Combining the best of each aspect of the integrated crop–livestock system, varieties, crop geometry, crop residue/manure management, and livestock feeding constituted the best-bet options and it was recognized that these would differ from region to region within the dry savanna, depending on the dominant crop species and management practices. In some regions, sorghum and cowpea would be appropriate, in others, millet and cowpea, etc. Corralling livestock on crop fields may be suitable in some cases but not in others. It was further recognized that, depending on, among other things, market access, it would not be unrealistic to anticipate that some inputs would be available to farmers, and that the options offered, both in terms of the crops used and their arrangement in the field, should seek to maximize the use of available inputs. Implementing this research in a holistic manner meant that not only would crop grain and residue yields be measured, but that the animal performance when fed this fodder and the manure produced to return to the field would be assessed. Furthermore, aspects of nutrient cycling, and the social and economic circumstances and implications of these best-bet options would need to be assessed as a whole.

## **Implementation of research**

The challenges posed by the best bet approach were recognized and so, the initial strategy was to start small and in 1998 the trial was established at just one location in northern Nigeria in Bichi Local Government (8°19'E; 12°12'N). This is about 50 km from Kano, on a good road. It was selected because information on village characterization (Ogungbile et al. 1999) from a survey carried out by ICRISAT and IAR in late 1996 was available. Originally, the intention was to use this survey dataset to define various groups of farmers so that representatives of each group could be selected to participate in the trial. However, after describing the aims of the trial to farmers from the village, only 11 volunteered to

participate and provided land; it was therefore decided to work with these 11 for the first year. In 1999, an additional 13 farmers participated.

A total of three treatments were established by the participating farmers and in all cases one treatment consisted of the traditional field of sorghum and cowpea (L). Two best-bet options were used; both had improved varieties of cowpea (IT90K-277-2) and sorghum (ICSV 400) and the rows were planted 75 cm apart with four rows of cowpea to two rows of sorghum, in contrast to the farmers' 1 to 1.5 m row spacing and one : one cereal : cowpea geometry. One best-bet option (BB+) included minimum inputs in the form of fertilizer, with nitrogen (N) applied only to the sorghum rows, and insecticide spray (for post-flowering insect pests) applied only to the cowpea; the other best-bet option (BB) had no inputs. It was anticipated that, in addition to maximizing the benefits from cowpea to the soil and minimizing the detrimental effects of sorghum shading on the cowpea, this row arrangement would allow optimal use of scarce inputs. The farmers appreciated the inputs (even though they were required to pay for them) so that in 1999, the BB treatment was modified to include local sorghum but with the same inputs of fertilizer and pesticide. Part of the best-bet options also included the concept of double cropping the cowpea—planting another crop of the same cowpea variety after harvesting the grain and fodder of the first. Previous trials had shown that this could give a good fodder yield with some grain, depending on the rainfall pattern (Singh and Tarawali 1997). All treatment plots received 3 t/ha of manure (1.6% N and 0.7% P) at the start of the 1998 growing season. All operations, land preparation, planting, weeding, application of inputs, harvesting, etc. were carried out by the farmers themselves with some technical guidance from technicians and scientists.

Prior to planting, bulked soil samples were collected from the top 20 cm of soil and analyzed for C, N, and P. Plots were sampled for grain and stover at maturity, using randomly placed quadrants (of about 20 m<sup>2</sup>), at the same time they were harvested by the farmers. Samples of grain and biomass were taken for analysis of N and P. When all the sorghum and cowpea residues were dry in the field, they were weighed, collected, and stored in treetops or on house roofs prior to use in the feeding trial. Residues from different treatments were kept separately.

## **On-farm livestock feeding**

During the first part of the dry season, farmers usually release their small ruminants into the fields once the grain harvest is completed to enable them to graze the remaining crop residues and weeds. Once these resources are used up, usually by the middle of the dry season, the animals are tethered within the homestead and fed with the stored crop residues. The initial intention was to tether animals on the respective treatment plots early in the dry season, but farmers indicated that there would be no way to prevent other animals from grazing the plots also, as livestock roam freely once the crop harvest is complete. It was therefore decided to follow the farmers' usual practice and allow free grazing until the weeds and crop residue remaining in situ were used up. Harris (1998) reported that manure deposition on crop fields from free grazing animals is fairly insignificant at an estimated 17 kg/ha. Accordingly, the period for feeding the crop residues harvested from the present experiment began in early February in 1999 and early March in 2000, when the animals were confined to the compounds. By using estimates of 10 kg dry matter per TLU (TLU = Tropical Livestock Unit = 250 kg animal liveweight) per day for a period

of 180 days, the recommended liveweight of animals to be fed using the available residue was estimated. The 10 kg daily allowance was made up of a mixture of sorghum and cowpea residues in proportion to the available total weight of biomass of each component on a plot by plot basis. At their suggestion, the farmers provided areas within their compounds where the animals were tethered. In those cases where a farmer had more than one treatment, the area was divided to separate different treatment groups. Animals were tagged; and tags, bowls for feed, and ropes to tie the fodder were color-coded according to treatment. It was recognized that for the L treatment, the fodder was unlikely to be sufficient and farmers were not prevented from providing their own inputs to animals on these treatments, once the material from the experimental plots had been used up. In these instances, the material provided, amounts, and costs were monitored. Even for the animals on BB+ and BB treatments, some farmers opted to provide additional feed resources in the form of *dussa* from millet or sorghum grain. In these instances, the quantities fed were estimated, and samples taken for analysis of N and P. The animals were weighed at the start of the feeding period and thereafter every two weeks. Manure and urine produced during the course of the feeding trial were allowed to accumulate in situ, and kept in the treatment compartment, together with any feed refusals. At the end of the feeding period, in late May, samples of this manure/compost were collected for analysis of N and P. The manure/compost collected during the feeding period was applied to the same treatment plots shortly before planting in 1999.

The costs of inputs used were recorded on a plot by plot basis, and included the planting material, fertilizer, pesticides, purchased manure, and labor. Local market prices for grain and fodder were recorded year round. Information on the sociocultural circumstances relating to farmers' crop–livestock management was also collected during the experiment, largely through village-based technicians and extension officers who interacted closely with both participating and nonparticipating farmers.

In 1999, in addition to the farmers at Bichi, a similar experiment commenced at Unguwan Zangi (8°05'E, 11°15'N), a village 60 km northeast of Zaria, in northern Nigeria, with 23 farmers participating. Unguwan Zangi is further south than Bichi, has a longer growing season, and slightly poorer market access. Treatments were the same as for Bichi in 1999, but the varieties were cowpea IT86D-719 and sorghum KSV8. Unguwan Zangi had been characterized in the medium to high resource use intensity domain as part of a survey carried out in 1997 within the context of the Ecoregional Program for the Humid and Subhumid Tropics of Africa (EPHTA) (Manyong et al. 1998).

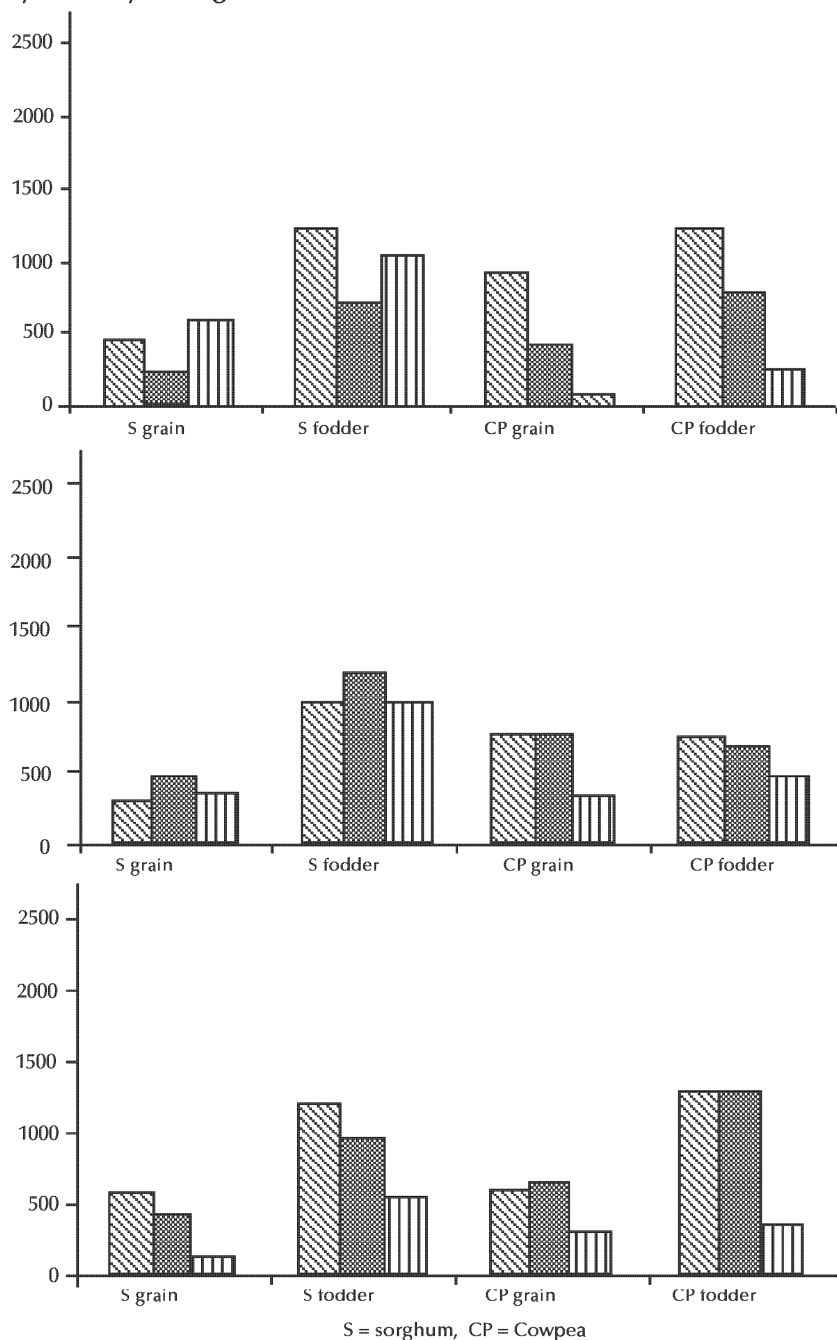
## **Preliminary results**

### ***Crop yields***

The estimated quantities of cowpea grain and fodder in the BB treatments were greater than those in the local treatment (Fig. 3). The most dramatic difference was for cowpea grain at Bichi in 1998 where the BB+ treatment yielded more than double the BB and about 16 times the L. Fodder yields for BB+ were one and a half times more than BB and five times more than L. In 1999, these differences were less marked, partly because the yields from L were higher. In many instances, although not quantified, this could be related to an increase in the number of farmers adopting some aspects of the best-bet options—varieties and/or cropping patterns. In terms of quantity, the grain and fodder from improved sorghum did not differ much from the local sorghum, but the farmers



# Dry-matter yield (kg/ha)



**Figure 3.** Estimates of dry-matter yields of grain and fodder. From top to bottom, Bichi, 1998; Bichi, 1999; Unguwan Zangi, 1999.  
 Diagonal hatching: BB+; Solid shading: BB; Vertical hatching: L.

indicated a preference for the improved sorghum, both in terms of cooking quality and time for the grain, and the fodder quality. The farmers' observation of the latter was backed up by analysis that showed about 30% of the local sorghum fodder, which had tall and thick stems, to be edible, compared to at least 60% of the improved, with shorter, thinner stems. Comparing actual fodder yields for both cowpea and sorghum in 1998 indicated that there were considerable losses of the dry fodder during transportation and storage. In some instances, the actual fodder yield when converted to kg/ha was as little as 20% of that predicted from the quadrant harvests. These losses were, to some extent, reduced in 1999 with careful handling, and minimized movement of the fodder for weighing.

Double cropping was not fully implemented to date. In 1998, farmers were reluctant to harvest the first cowpea crop, as the rains, atypically, continued later than usual. This had two effects; one was that the farmers wanted to continue picking the ripe pods and the other was that they did not want to harvest fodder when the environment was still wet meaning the fodder would not dry, but become rotten and be unpalatable to the animals. This limitation was further emphasized by labor requirements for harvesting tomato and pepper on other parts of the farm at the time the second cowpea crop was to be planted. A few farmers at Bichi in 1999 and 1998 implemented double cropping and were able to harvest both grain and fodder. At a recent field day, samples of fodder from the second cowpea crop were compared visually with those from the first. Farmers agreed that the second crop was clearly of better quality, based on a visual comparison of the leafiness and greenness—criteria they usually use to assess fodder quality.

## **Livestock productivity**

For livestock feeding, using the fodder harvested in 1998 to feed small ruminants during the 1998/99 dry season, only eight farmers at Bichi were able to participate so the results should be viewed with some caution, considering also the farm-to-farm variation. These preliminary data indicated that animals on the BB+ treatment gained significantly more weight during the last six weeks of the 16-week feeding period than those on BB or L (Fig. 4). Overall, the average liveweight gains (averaged over all farmers) were 3.54 kg per animal for BB+, 0.91 kg (BB) and 2.19 kg (L). While manure quantities produced by animals on the different treatments (manure here is used to refer to the manure plus feed refusals—all that was collected and returned to the field) did not differ significantly, the N content was 1.35% (BB+) 1.09% (BB) and 0.80% (L). P contents were estimated as 0.28% (BB+) 0.27% (BB) and 0.25% (L). These values are within the ranges reported by Tarawali et al. (2001).

Figure 4 shows the preliminary results from livestock feeding trials in the 1999/2000 dry season at Bichi (17 farmers participating) and Unguwan Zangi (11 farmers). At Bichi, again the BB+ was superior to BB or L, but at Unguwan Zangi it appeared that the two best-bet options were better than the local, but not different from each other. Average weight changes (kg) per animal over the entire feeding period at Bichi were 1.75 (BB+), 0.28 (BB), and 0.03 (L), representing gains of 8, 1.3, and 0.1%. At Unguwan Zangi, there were slight weight losses for BB+ (0.74 kg) and L (0.78 kg), whereas animals on BB gained an average of 0.9 kg per animal.

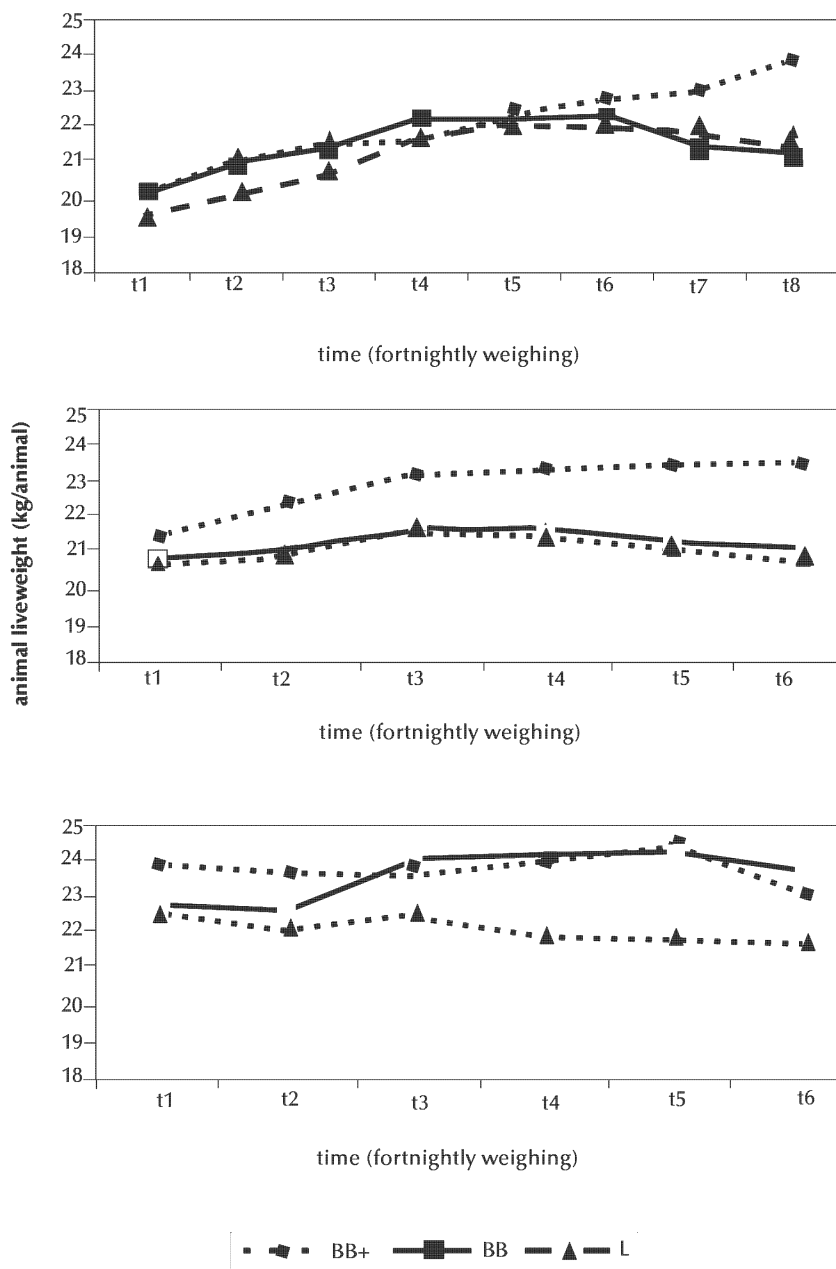


Figure 4. Average liveweight (kg/animal) for livestock feeding trials. Upper graph Bichi 1998/1999; center graph Bichi 1999/2000; lower graph Unguwan Zangi 1999/2000.

## **Nutrient dynamics**

Using data from the eight farmers who participated in the feeding trial at Bichi in 1998, it is possible to look at some aspects of nutrient dynamics in these integrated options (Table 2). In simple terms, for nitrogen (N) and phosphorus (P), the inputs have been considered as the soil status for these elements at the time of trial establishment, the manure and fertilizer added, and a small input of P from the harmattan dust (Harris 1998). Outputs are the nutrients removed in grain and fodder. At present, there has been no attempt to take account of nutrient loss through leaching, volatilization, etc. These figures are within the range reported by Harris (1998) for similar farmers' fields in the Kano region and indicate that both N and P balances were positive at the end of the growing season. It would appear that the cowpea removed more nutrients than the sorghum, or this could be interpreted that the cowpea used the added nutrients more effectively than the sorghum. The strong positive balances are surprising and could be attributed to a number of factors. As indicated above, the N and particularly the P concentrations in the applied manure were quite high, compared to results in other reports (Tarawali et al. 2001). Furthermore, since leaching and volatilization were not considered, it may be inappropriate to include the initial soil N and P and the contribution from P in the harmattan dust. If these factors are excluded, and the manure N contents reduced to 1.5 and P to 0.2%, then the balances are only just positive (Table 2). This information is at present inadequate to enable estimation of the role of cowpea in promoting nutrient cycling, and the nutrient balances need to be monitored for several more seasons, including the returns to the system from the manure and crop residue refusals, removal of subsequent crop harvests, etc. At this point, the emphasis is that nutrient dynamics is being monitored in these studies and should provide quantitative information on whether nutrients are being mined by this more intensive production system, if the applied nutrients are being optimally used, and how the improved options compare with farmers' traditional systems.

## **Economics**

The objective of the economic evaluation is to compare the costs, returns, and profits among the three treatments as a basis for further assessing the desirability of introducing the best-bet options. Although a whole system analysis is planned, as an example, only a partial result on the treatments is presented here, based on the results of the crop yields in 1999 at Bichi. This approach will subsequently be expanded to include an estimation of the value of the livestock products (increased liveweight and manure nutrients), rather than, as treated in this example, considering the monetary value of the crop residues as if they were all sold. In order not to bias the comparison between the improved and local varieties, average market prices for the study area were used for inputs and outputs. Labor data were collected separately for hired and family labor and include the cost of ridging, planting, spraying, fertilizer application, weeding, remolding, and harvesting. Material costs include fertilizers, insecticide, seeds, and manure.

Results of the partial economic analyses are summarized in Table 3. Because farmers use a lot of family labor (about 70% of the total for most operations), the cost of which is often not estimated, figures are presented for both total costs which includes an estimate of family labor, and the actual costs where this value is excluded. One of the most striking features is the difference in costs for labor and materials between BB+ and BB. In 1999, the only difference between these two options was that BB+ had improved sorghum and

Table 2. Estimated nitrogen and phosphorus inputs and outputs (kg/ha) during the first year of the trial at Bichi (1998).

|   | BB+      |            | BB       |            | L        |            |
|---|----------|------------|----------|------------|----------|------------|
|   | Nitrogen | Phosphorus | Nitrogen | Phosphorus | Nitrogen | Phosphorus |
| <b>Inputs</b>   |          |            |          |            |          |            |
| Soil  | 7.9      | 0.1        | 7.8      | 0.1        | 8.3      | 0.1        |
| Manure (1.6% N; 0.7% P)   | 48.0     | 21.0       | 48.0     | 21.0       | 48.0     | 21.0       |
| Inorganic fertilizer  | 35.0     | 15.0       | 0.0      | 0.0        | 0.0      | 0.0        |
| Harmattan dust  |          | 0.8        |          | 0.8        |          | 0.8        |
| Total inputs  | 90.9     | 37.0       | 55.8     | 22.0       | 56.3     | 21.9       |
| <b>Outputs</b>  |          |            |          |            |          |            |
| Sorghum grain   | 6.6      | 0.9        | 5.0      | 0.7        | 6.1      | 0.9        |
| Sorghum fodder  | 3.8      | 0.8        | 3.4      | 1.0        | 5.0      | 0.8        |
| Cowpea grain  | 27.8     | 2.2        | 19.7     | 1.6        | 1.7      | 0.1        |
| Cowpea fodder   | 16.5     | 2.0        | 12.1     | 1.2        | 10.4     | 1.1        |
| Total outputs   | 54.6     | 5.9        | 40.1     | 4.6        | 23.2     | 2.9        |
| Balance   | 36.4     | 31.1       | 15.7     | 17.4       | 33.2     | 19.1       |
| Balance with 1.5% N and 0.2% P,<br>excluding soil and harmattan | 25.4     | 15.1       | 4.9      | 1.4        | 21.8     | 3.1        |

BB+ = Best bet option with inputs.

BB = Best bet options without inputs.

L = Traditional sorghum and cowpea.

**Table 3. Summary of partial economic analyses for the three treatments. Total costs include the value of family labor, which is not accounted for in the values for actual costs.**

|                      | BB+    | BB     | L      |
|----------------------|--------|--------|--------|
| <b>Total cost</b>    |        |        |        |
| Total revenue        | 32 069 | 35 181 | 19 872 |
| Materials            | 8 746  | 10 796 | 4 767  |
| Labor                | 12 581 | 16 675 | 10 644 |
| Total costs          | 21 327 | 27 471 | 15 411 |
| Gross margin         | 10 742 | 7 710  | 4 461  |
| Benefit : cost ratio | 1.50   | 1.28   | 1.29   |
| <b>Actual cost</b>   |        |        |        |
| Total revenue        | 32 069 | 35 181 | 19 872 |
| Materials            | 8 746  | 10 796 | 4 767  |
| Labor                | 3 355  | 3 617  | 3 004  |
| <b>Total cost</b>    | 12 101 | 14 413 | 7 771  |
| Gross margin         | 19 968 | 20 768 | 12 101 |
| Benefit : cost ratio | 2.65   | 2.44   | 2.56   |

Values are all in Naira/hectare (at the time of writing, ₦100 = US\$1.00).

BB local sorghum. Closer analysis of the information reveals that BB has 23% more material costs, with the highest component of this being a 30% increase in the cost of seed. Labor costs were even more different, with BB having 32% more labor costs than BB+. Within these costs, BB had higher costs than BB+ for remolding (86%), harvesting (34%), and weeding (39%). It can be speculated that these differences are related to the higher yield of the local sorghum and its tall stature (this could have necessitated more remolding to make sure the tall stalks did not get blown over late in the season). Because the local sorghum plants are generally bigger than the improved variety, they may have been planted less densely and therefore more space between plants could have meant more weeding. Alternatively, moving through these taller plants to weed could have been more difficult and therefore more time consuming. While BB+ required 38% more inputs than L, the revenue was 77% more, indicating that increased yields amply compensated for the investment in fertilizers and insecticides.

Total revenue from the crop enterprise (grain and fodder) was highest for BB, followed by BB+, representing increases of 77 and 61% respectively, over L. Income differences related almost entirely to differences in yield. All treatments, in both scenarios including and excluding family labor gave positive gross margins and benefit cost ratios greater than one, indicating that the system as a whole is quite profitable. BB+ had the highest benefit–cost ratio.

For both the best-bet treatments, about 70% of the revenue was from cowpea grain and fodder, with the balance being contributed by the sorghum component. By contrast, 59% of the revenue in the L treatment was obtained from cowpea. About one-fifth of the cowpea revenue in BB+ and BB was contributed by cowpea fodder, but as much as 25% of the cowpea revenue in the L treatment was from fodder. Such considerations suggest that it may be more profitable for a farmer to grow only cowpea, if maximum profit is the aim. Indeed, hypothetical calculations comparing potential partial budgets from 100% cowpea or 100% sorghum fields, based on these figures, give higher benefit–cost ratios

for cowpea only 1.82 (BB+); 1.42 (BB), and 1.46 (L). If only sorghum were to be grown, benefit-cost ratios fall to 1.20 (BB+), 1.28 (BB), and 1.3 (L). Nevertheless, it is important to keep these hypothetical examples in the context of the family needs; no farmer could afford not to grow some sorghum because it is the staple family diet. This stresses the importance of considering not only the economic values, but the social context of the introduced technologies. It could also be argued that maintaining the intercropping system used by farmers ensures some degree of risk diversification.

### **A win-win situation?**

In Nigeria, with an estimated 4 million ha planted annually to cowpea (FAO 2000), if we were to estimate that the best-bet options would be appropriate for one-third of this, and take the lower figure of a doubling in grain yield and apply it to the 538 kg/ha average national yield (FAO 2000), the implication would be an increase of 0.7 million tonnes of cowpea grain. Applying similar speculations to livestock figures, Winrock (1992) estimates 56% of the goats and 64% of the sheep in sub-Saharan Africa are in the dry savannas. If these estimates are applied to current FAO figures for the numbers of sheep and goats in Nigeria (FAO 2000), then an estimate is obtained of 13.6 million goats and 13.1 million sheep in the dry savannas of Nigeria. From the livestock feeding trials carried out in Bichi in 1998/99, those animals on BB+ gained 1.6 times more weight than the local treatment animals. If the intervention were to reach one-third of the small ruminants in the Nigerian dry savanna, this would mean 8.9 million animals gaining an extra 1.35 kg each per annum, a total of 11.6 million kg liveweight—in the region of 5 million kg of extra meat, or 0.6 million animals. If these 0.6 million animals produced manure at the rate of 1 kg/day/TLU and a nitrogen content of 7%, this could represent about 12 000 tonnes of nitrogen (although this figure does not take account of volatilization or leaching). Clearly, these figures are really speculation, and it is not possible to put a time scale on the adoption of these interventions at this point. Furthermore, these are based on calculations of productivity alone, and it is important to recollect that the aim of the best-bet options is not solely to increase productivity, but to do so in a way that is sustainable and does not destroy the natural resource base, as well as being economically and socially attractive to farmers.

In this context, it is important to take into consideration the nutrient dynamics, and to ask whether we are really intensifying production without mining the soil. This question requires several years of data to answer, and there are opportunities to continue to optimize the nutrient use. In order to identify what some of these options might be, complementary trials have been carried out in Niger, where, in farmer-managed trials involving 10 farmers in the Sahelian zone at Sadoré, hill placement of small quantities of fertilizers and broadcasting of phosphate rock of Tahoua were compared with farmers' practices in continuous, intercropping, and rotation systems. The farmers' practices without any input yielded 497 kg/ha of millet grain whereas about an additional 300 kg/ha was obtained with broadcasting of locally available phosphate rock of Tahoua plus 4 kg P/ha of compound P fertilizers. With the addition of nitrogen fertilizers, whereas in continuous cropping, 881 kg/ha of millet grain was harvested, 1135 kg/ha was obtained when millet was rotated with cowpea. In the intercropping system, in addition to 858 kg/ha of millet grain, 234 kg/ha of cowpea grain was harvested. It is important to note

that the benefit of selling the cowpea grain will be enough to purchase the needed external inputs in this case.

The calculations of partial budget data, based on the crop yields only, suggest that the best-bet options are profitable for farmers. Including the livestock values in the calculations is likely to enhance this even further. In trials established in 2000, the introduction of improved cowpea grain storage methodology, using a simple triple bagging method (Murdock et al. 1997) is anticipated to increase income from cowpea grain even more. By storing the cowpea grain without fear of insect attack, farmers can keep the grain for at least three months when the price could increase by as much as threefold.

Semistructured interviews with participating farmers are planned during 2000 and 2001 in order to assess the social context into which these interventions fit, and to better elucidate farmers' perceptions and priorities.

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## **References**

- Badiane, O. and C.L. Delgado. 1995. A 2020 vision for food, agriculture, and the environment in sub-Saharan Africa. International Food Policy Research Institute (IFPRI), Washington DC, USA. 56 pp.
- Bagayoko, M., S.C. Mason, and S. Traoré. 1998. The role of cowpea on pearl millet yield, N uptake and soil nutrient status in millet–cowpea rotation in Mali. Pages 109–114 in *Soil fertility management in West African land-use systems*, edited by G. Renard, A. Neef, K. Becker, and M. von Oppen. Margraf Verlag Weikersheim, Germany.
- Berner, D.K., R.J. Carsky, K.E. Dashiell, J. Kling, and V.M. Manyong. 1996. A land management-based approach to integrated *Striga hermonthica* control in sub-Saharan Africa. *Outlook on Agriculture* 25: 157–164.
- Carsky, R.J. and D.K. Berner. 1995. Benefits of crop rotation with soybean and cowpea in savanna cereal-based systems. Pages 391–402 in *Technology options for sustainable agricultural production in sub-Saharan Africa*, edited by T. Bezuneh, A.M. Emechebe, J. Sedgo, and M. Ouédraogo. Semi-Arid Food Grain Research and Development (SAFGRAD), Ouagadougou, Burkina Faso.
- Delgado, C., M. Rosegrant, H. Steinfeld, S.K. Ehui, and C. Courbois. 1999. *Livestock to 2020. The next food revolution*. Food, Agriculture and the Environment Discussion Paper 28. International Food Policy Research Institute (IFPRI), Washington DC, USA; Food and Agriculture Organization of the United Nations (FAO), Rome, Italy; and International Livestock Research Institute (ILRI), Nairobi, Kenya.
- de Haan, C., H. Steinfeld, and H. Blackburn. 1997. *Livestock and the environment. Finding a balance*. Report of a study coordinated by FAO, USAID, and the World Bank. FAO, Rome, Italy.
- Ehui, S., H. Li Pun, V. Mares, and B. Shapiro. 1998. The role of livestock in food security and environmental protection. *Outlook on Agriculture* 27: 81–87.
- FAO. 2000. FAOSTAT Database. <http://apps.fao.org> Food and Agriculture Organization of the United Nations, Rome, Italy. Accessed June 2000.



- Harris, F. 1998. Farm-level assessment of the nutrient balance in northern Nigeria. *Agriculture, Ecosystems and Environment* 71: 201–214.
- ICRISAT. 1991. ICRISAT West African program annual report. 1990. International Research Institute for the Semi-Arid Tropics (ICRISAT) Sahelian Center, Niamey, Niger.
- ILRI. 2000. ILRI strategy to 2010. Making the livestock revolution work for the poor. International Livestock Research Institute (ILRI), Nairobi, Kenya.
- McIntire, J., D. Bourzat, and P. Pingali. 1992. Crop–livestock interaction in sub-Saharan Africa. World Bank, Washington DC, USA.
- Manu, A., T.L. Thurow, A.S.R. Juo, I. Zanguina, M. Gandah, and I. Mahamane. 1994. Sustainable land management in the Sahel: a case study of an agricultural watershed at Hamdallaye, Niger. TropSoils Program, Soils and Crop Sciences Department, Texas A & M University, USA.
- Manyong, V.M., K.O. Makinde, and J.O. Olukosi. 1998. Delineation of resource-use domains and selection of research sites in the northern Guinea savanna ecoregional benchmark area, Nigeria. Paper presented during the launching of the northern Guinea savanna ecoregional benchmark area, 2 December 1998. Institute of Agricultural Research (IAR), Zaria, Nigeria. IITA, Ibadan, Nigeria.
- Murdock, L.L., R.E. Shade, L.W. Kitch, G. Ntougkam, J. Lowenberg-DeBoer, J.E. Huesing, W. Moar, O.L. Chambliss, C. Endondo, and J.L. Wolfson. 1997. Postharvest storage of cowpea in sub-Saharan Africa. Pages 302–312 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Naazie, A. and J.W. Smith. 1997. Modelling feed resources budgets in the moist savannas of West Africa. Pages 197–198 *in* Proceedings of the XVIII International Grassland Congress, June 1997, Winnipeg and Saskatoon, Canada.
- Ogungbile, A.O., R. Tabo, and N. van Duivenbooden. 1999. Multiscale characterization of production systems to prioritize research and development in the Sudan savanna zone of Nigeria. (summary in English and French). Information Bulletin no. 56. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. 112 pp.
- Okike, I., M.A. Jabbar, V. M. Manyong, J.W. Smith, J.A. Akinwunmi, and S.K. Ehui. 2001. Agricultural intensification and efficiency in the West African savannas: Evidence from northern Nigeria. Socioeconomics and Policy Research Working Paper 33. International Livestock Research Institute (ILRI), Nairobi, Kenya. 54 pp.
- Ousman Badiane, O. and C.L. Delgado (editors). 1995. A 2020 vision for food, agriculture and the environment. International Food Policy Research Institute (IFPRI), Washington DC, USA.
- Schlecht, E., F. Mahler, M. Sangaré, A. Susenbeth, and K. Becker. 1995. Quantitative and qualitative estimation of nutrient intake and faecal excretion of Zebu cattle grazing natural pasture in semiarid Mali. Pages 85–97 *in* Livestock and sustainable nutrient cycling in mixed farming systems of sub-Saharan Africa, edited by J.M. Powell, S. Fernández-Rivera, T.O. Williams, and C. Renard. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Singh, B.B. and S.A. Tarawali. 1997. Cowpea and its improvement: key to sustainable mixed crop–livestock farming systems in West Africa. Pages 79–100 *in* Crop residues in sustainable mixed crop–livestock farming systems, edited by C. Renard. International Crops Research Institute for the Semi-arid Tropics (ICRISAT), International Livestock Research Institute (ILRI), and CAB International, Wallingford, UK.

- Tarawali, S.A., B.B. Singh, M. Peters, and S.F. Blade. 1997. Cowpea haulms as fodder. Pages 313–325 *in* *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Tarawali, S.A., J.W. Smith, P. Hiernaux, B.B. Singh, S.C. Gupta, R. Tabo, F. Harris, S. Nokoe, S. Fernández-Rivera, and A. Bationo. 2000. Integrated natural resource management—putting livestock in the picture. Paper presented at the Integrated Natural Resource Management meeting, 20–25 August 2000, Penang, Malaysia.
- Tarawali, S.A., A. Larbi, S. Fernández-Rivera, and A. Bationo. 2001. The role of livestock in the maintenance and improvement of soil fertility. Pages 281–304 *in* *Sustaining soil fertility in West Africa*, SSSA Special Publication No. 58. Soil Science Society of America and American Society of Agronomy, Madison, USA.
- Winrock. 1992. *Assessment of animal agriculture*. Winrock International, Morrilton, Arkansas, USA.

## 4.2

# Cowpea rotation as a resource management technology for cereal-based systems in the savannas of West Africa

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### Abstract

A synthesis of results from the savanna zone of West Africa suggests that cowpea rotation can be considered to be an effective resource management technology in cereal-based systems. Part of the N requirement of cereal crops can be satisfied by cowpea crop rotation. Furthermore, benefits of cowpea rotation are sometimes higher than expected based on the N content of the cowpea crop alone. Reasons for this include substantial root biomass and N, substantial N-sparing by the legume, and other benefits such as reduction in *Striga hermonthica*, or pests and often diseases, and possibly access to sparingly soluble P. The characteristics to be encouraged to maximize the N benefit are the amount of nitrogen derived from the atmosphere and the amount of N returned in the residues. In addition the data suggest that (1) the maturity class of the cowpea variety should be as late as possible, (2) the cereal should be planted as soon as possible after cowpea has been harvested, and (3) minimum soil requirements for optimum cowpea growth should be respected. These can be considered as recommendations to be followed if appropriate for local agroecological and socioeconomic circumstances.

### Introduction

Herbaceous legumes as cover crops occupy land meant for food production, therefore, grain legumes are usually more acceptable to farmers than cover crops (Schulz et al. 2001). However, the potential benefit to the soil and subsequent crops from grain legumes is less. We reviewed the literature to learn more about the benefits of cowpea to cereal-based cropping systems in the savannas of West Africa to help design better systems. Several examples of short-term rotation trial results (Table 1) show a clear benefit of cowpea rotation. The benefit may be due to N supply by the legume, non-N effects, or a combination of the two. First we explore the nitrogen contribution and then non-N benefits of cowpea rotation. Based on this we give recommendations to optimize the benefits of cowpea rotation. The recommendations relate to the choice of the cowpea variety to use and how to manage the cowpea crop, with special emphasis on P fertilizer management.

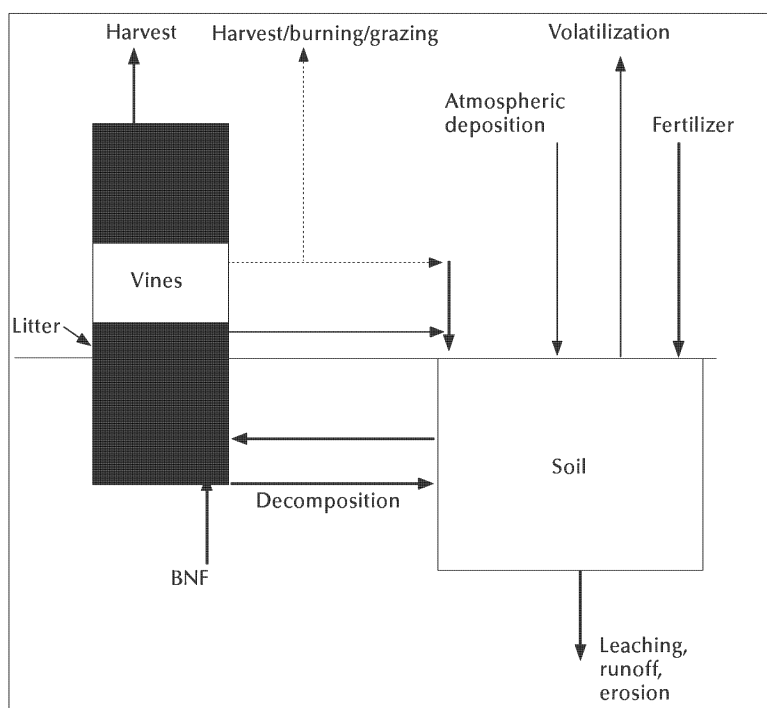
### Evidence and estimates of N benefit of cowpea rotation

The N benefit of cowpea includes the contribution to the soil–plant system through biological N fixation (Fig. 1). Because legumes fix N from the atmosphere, we expect an N contribution to subsequent cereal crops. The direct contribution to the soil–crop system is

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**Table 1. Benefit of cowpea rotation to cereal with low (< 30 kg/ha) or no N application.**

| Control crop | Test crop | Test crop yield (kg/ha) |              | Source                        |
|--------------|-----------|-------------------------|--------------|-------------------------------|
|              |           | Control                 | After cowpea |                               |
| Sorghum      | maize     | 2394                    | 2690         | Jones (1974)                  |
| Sorghum      | maize     | 1945                    | 2795         | Nnadi et al. (1981)           |
| Millet       | sorghum   | 482                     | 835          | Stoop and van Staveren (1982) |
| Grass        | maize     | 650                     | 943          | Carsky et al. (1999)          |
| Millet       | millet    | 410                     | 1590         | Rodriguez (1986)              |
| Millet       | millet    | 760                     | 2190         | Reddy et al. (1994)           |
| Millet       | millet    | 1148                    | 1685         | Bagayoko et al. (1997)        |
| Maize        | maize     | 952                     | 1857         | Dakora et al. (1987)          |
| Maize        | maize     | 1830                    | 2740         | Horst and Hardter (1994)      |
| Maize        | maize     | 1167                    | 1879         | Osei-Bonsu and Asibuo (1997)  |
| Maize        | maize     | 1220                    | 1940         | Jeranyama et al. (2000)       |

**Figure 1. Schematic of major and minor N fluxes to be found in a cowpea sole crop. Major N inputs to the soil-plant system are biological N fixation (BNF) and major N outputs are harvest of grain and export of vines.**

the amount of N in the legume crop derived from the atmosphere (Ndfa). The rest of the N content of the cowpea plant is absorbed from the soil. Part of the plant N is exported in the grain, which has a high N content (3–4%). The N in the cowpea residue is then available to the soil and subsequent crop. Thus, it is important to know the partitioning of N within the plant. The proportion of N in the grain to the total aboveground N is the

nitrogen harvest index (NHI). As a rule of thumb, a legume increases the soil N pool if the proportion of N fixed from the atmosphere exceeds the NHI (Giller et al. 1994).

The N balance from a cowpea rotation is an estimate of the N accrual to the soil–plant system. Some examples of N balance from the literature are presented in Table 2. This synthesis shows that N balance is generally positive because approximately 60 to 70% of N is derived from the atmosphere (not the soil) and the N exported in the grain is generally less than 50%. The low Ndfa observed by Carsky et al. (2001) was for a very early local variety on relatively poor soils, although soil P was apparently adequate. On low P soil, Sanginga et al. (2000) found that N balance of cowpea increased slightly with P application (Table 3).

The available estimates of N balance take only the aboveground cowpea crop into consideration. The roots will contain some N derived from the soil and some N derived

**Table 2. Examples of N balance calculations for cowpea in the West African savanna.**

| Uptake from soil (kg/ha) | Aboveground Ndfa (kg/ha) | Grain N removed (kg/ha) | Aboveground N in residue (kg/ha) | N balance (kg/ha) | Source |
|--------------------------|--------------------------|-------------------------|----------------------------------|-------------------|--------|
| 29                       | 17                       | 19                      | 27                               | –2                | 1      |
| 25                       | 201                      | 76                      | 150                              | 125               | 2      |
| 29                       | 70                       | 50                      | 49                               | 20                | 3      |
| 64                       | 93                       | 63                      | 94                               | 30                | 4 (a)  |
| 36                       | 61                       | 48                      | 49                               | 13                | 4 (b)  |
| 62                       | 92                       | 88                      | 66                               | 4                 | 5      |

Sources:

1. Carsky et al. (2000): mean of three early maturing cowpea varieties (60–70 days); estimate of soil uptake from nearby fallow plots.
2. Dakora et al. (1987): one medium-late duration (approximately 90–110 DAP) cowpea crop grown in the Guinea savanna of Ghana.
3. Eaglesham et al. (1982): mean of four cowpea varieties grown in pots.
4. Horst and Hardter (1994): two consecutive early duration (60 DAP) crops grown in the Guinea savanna of Ghana during 1984(a) and 1985(b).
5. Awonaike et al. (1990): three cowpea varieties at 57 DAP in the derived savanna of Nigeria.

**Table 3. Effect of P application on the N-balance (kg N/ha)<sup>†</sup> of cowpea lines grown in a low P soil (source: Sanginga et al. 2000).**

| Cowpea lines                      | P application (kg P/ha) |       |      |      |
|-----------------------------------|-------------------------|-------|------|------|
|                                   | 0                       | 20    | 40   | 60   |
| <b>Non-P-responders</b>           |                         |       |      |      |
| IT81D-715                         | –2.9                    | 0.1   | 5.5  | 11.1 |
| Danila                            | 3.2                     | –5.8  | –2.3 | 6.3  |
| IT90K-59                          | –2.6                    | –10.6 | –9.3 | –3.6 |
| IT89KD-349                        | –2.4                    | –4.0  | –8.4 | 8.1  |
| <b>P-responders</b>               |                         |       |      |      |
| IT89KD-374                        | –1.6                    | 7.6   | –0.1 | 0.9  |
| IT82D-716                         | –9.3                    | 1.9   | –9.0 | –2.1 |
| IT82KD-391                        | –4.8                    | 0.9   | 2.8  | –5.0 |
| IT82D-849                         | –10.4                   | –6.4  | –1.8 | 7.7  |
| LSD <sub>0.05</sub> (P level)     | 6.0                     |       |      |      |
| LSD <sub>0.05</sub> (cowpea line) | 8.5                     |       |      |      |

<sup>†</sup>N balance is calculated from the difference between total N fixed and total N exported in seeds.

from the atmosphere and thus, accounting for root N may make the N balance more positive. The belowground cowpea biomass may be a source of N for a subsequent cereal crop. Estimates of cowpea root dry matter are extremely variable ranging from 0.3 Mg/ha (Carsky 2000) to 2.9 Mg/ha (Groot et al. 1995). Poulain (1980) assumed 0.5 Mg/ha of cowpea roots as a probable range. Root N concentration was 1.5 and 2.5% for two varieties grown and sampled by Nnadi and Balsubramanian (1978). Root N, if measured, may help to explain the beneficial effect of cowpea rotation when above-ground N balance does not appear sufficient. Franzluebbers et al. (1994a) estimated the contribution of the cowpea roots to the following sorghum crop to be in the range of one-fifth of the whole cowpea plant used as green manure. In contrast to this, in a field study conducted by John et al. (1992), the aboveground cowpea material was removed and cowpea roots only accounted for an increase in soil mineral N content, but did not affect the yield of the subsequent rice crop. When the aboveground cowpea biomass was included, however, the rice yield increased significantly.

All or part of the cowpea residue may be exported as animal feed or it may be grazed off or burned off during the dry season. In these cases, the recycled cowpea residue consists only of leaves fallen before harvest (i.e., the litter) and the roots. Estimates of cowpea litter in the literature are rare and those shown in Table 4 indicate extremely variable results in different trials, ranging from less than 0.1 to more than 1 Mg/ha and from less than 5% to more than 60% of total aboveground residue. The nitrogen concentration of cowpea litter in the Nigeria study was 1.7% compared with N content in leaves of 2% (Carsky et al. 2001).

The nitrogen fertilizer replacement value (NFRV) is an estimate of the benefit of legume rotation for the farmer. It compares cereal yield after a legume to cereal yield after a cereal or fallow control treatment. N fertilizer applied to the control allows estimates of the N benefit of the legume (Fig. 2). The N benefit consists of N derived from the atmosphere (the aboveground and below ground cowpea crop), the N-sparing effect of the cowpea crop and other non-N benefits, and therefore, overestimates the N contribution of the rotation (Wani et al. 1995). The N-sparing effect may result in more N in the soil for a subsequent crop if the N is not lost from the soil profile before the subsequent cereal crop (e.g. by leaching). Although it is an apparent benefit to the subsequent cereal crop, it is not a contribution to the soil-plant system.

While N supply is the major benefit of cowpea rotation with cereals, non-N benefits are possible. In order to ascertain whether there are non-N benefits, there should be a full

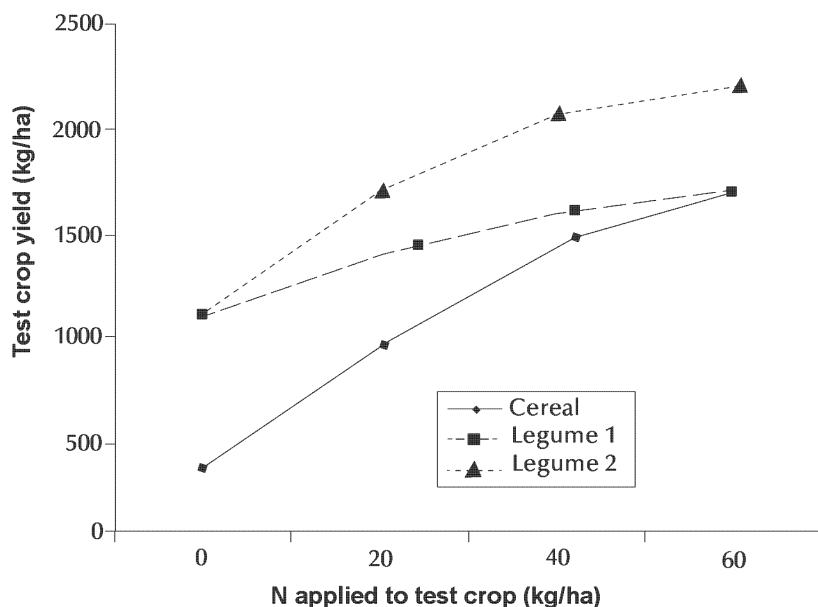
**Table 4. Haulm and surface litter of cowpea measured in West Africa.**

| Site; year                 | P applied<br>(kg/ha) | Haulm<br>(kg/ha) | Litter<br>(kg/ha) |
|----------------------------|----------------------|------------------|-------------------|
| Nigeria, 1996 <sup>†</sup> | n.a.                 | 1249             | 46                |
| Nigeria, 1997 <sup>†</sup> | n.a.                 | 1601             | 63                |
| Benin, 1998 <sup>‡</sup>   | 0                    | 534              | 1273              |
| Benin, 1998 <sup>‡</sup>   | 30 <sup>§</sup>      | 1038             | 1971              |

<sup>†</sup> On-farm trials at 10°24'N; 7°42'E, mean of three early varieties in two replicated trials measured at harvest approximately 10 weeks after planting. Source: Carsky et al. (unpublished data).

<sup>‡</sup> Research station field at 6°36'N, 2°14'E, variety NI-86-650-3, measured at 12 weeks after planting. Source: Vanlauwe et al. (unpublished data).

<sup>§</sup> Application of 30 kg P/ha as triple superphosphate.



**Figure 2.** Hypothetical response of cereal to previous cereal or legumes, legume 1 having only N effects and legume 2 having N and non-N effects.

range of N levels after both preceding crops (cowpea and control) as shown in Figure 2. If the curves converge as N fertilizer is applied (as for legume 1 in Fig. 2), then one can characterize the benefit as being due to soil N supply and NFRVs can be estimated from those studies. We will examine results from short ( $\leq 5$  months) and long ( $> 5$  months) rainy season zones.

In the short rainy season zone, it is only possible to grow one sole crop per year or one relay intercrop. In Zimbabwe, Jeranyama et al. (2000) grew cowpea and *Crotalaria juncea* as relay intercrops with maize for two years and in the third year calculated an NFRV of 36 kg/ha compared to continuous maize (Fig. 3). The cowpea yield after maize and after legumes converged at higher N levels, suggesting that only N benefits were realized. At Cinzana, Mali (latitude 13°N) Bagayoko et al. (1997) grew millet after cowpea for four years with a continuous millet control and found that 40 kg N/ha applied to the continuous millet gave a yield similar to cowpea rotation (Fig. 4). Thus, they estimated the NFRV to be approximately 40 kg/ha. It should be noted, however, that soil N was not higher in the cowpea system than the continuous millet system after four years.

The two previous estimates of NFRV are substantial, approaching 40 kg/ha. In contrast, the mean NFRV from two sites in northern Nigeria was only 9 kg/ha for one season of cowpea in the first year followed by one season of maize in the second year in the Guinea savanna of northern Nigeria at latitude 11°N (Carsky et al. 1999; Fig. 5). In this case, the cowpea effect was compared to native fallow rather than a continuous cereal control. A continuous cereal control is likely to give higher estimates of NFRV than fallow because of higher N export by the cereal.

When the rainy season is six months long or longer it is possible to grow a cowpea and a cereal crop in succession in the same year. Dakora et al. (1987) grew cowpea in the first growing season followed immediately by maize in the second season after

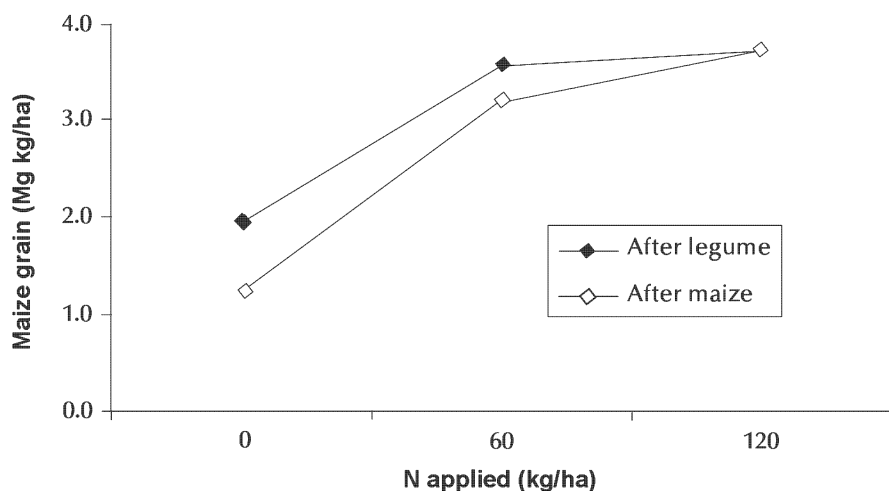


Figure 3. Effect of previous legume (cowpea and *crotalaria* data combined) relayed into maize for two years on response of subsequent maize to N fertilizer in Zimbabwe compared to continuous maize. Points derived from equations published by Jeranyama et al. (2000).

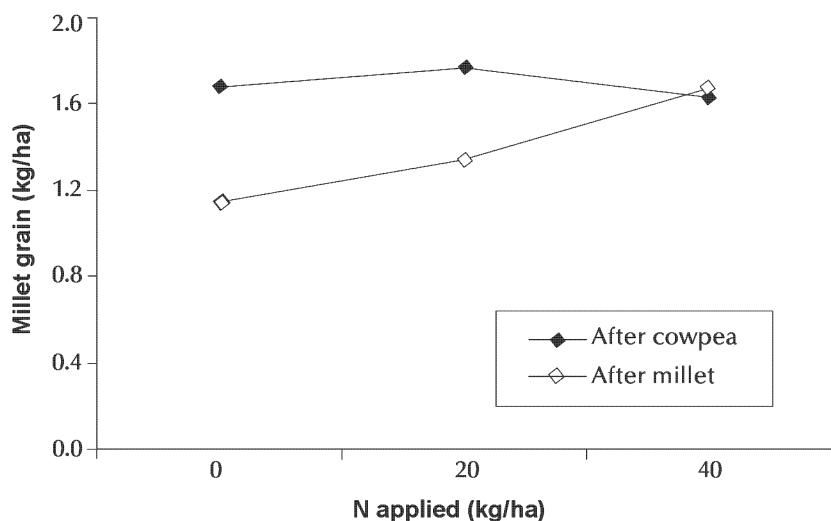


Figure 4. Effect of cowpea rotation on response of millet to N fertilizer in Mali from Bagayoko et al. (1997).

incorporating cowpea (and maize control) residues into the soil. Their estimate of NFRV was approximately 60 kg/ha (Fig. 6). In a similar set of conditions in Nigeria, Carsky et al. (2001) observed that the yield of maize after cowpea was not significantly different from the yield of maize with 0.30 kg N/ha applied after a previous fallow. The apparent NFRV of 30 kg/ha is slightly greater than the N content of the aboveground cowpea residues (Carsky et al. 2001). Root N was not measured in that study and may have accounted for the NFRV observed. Although an early cowpea variety was used ("Achishuru" described



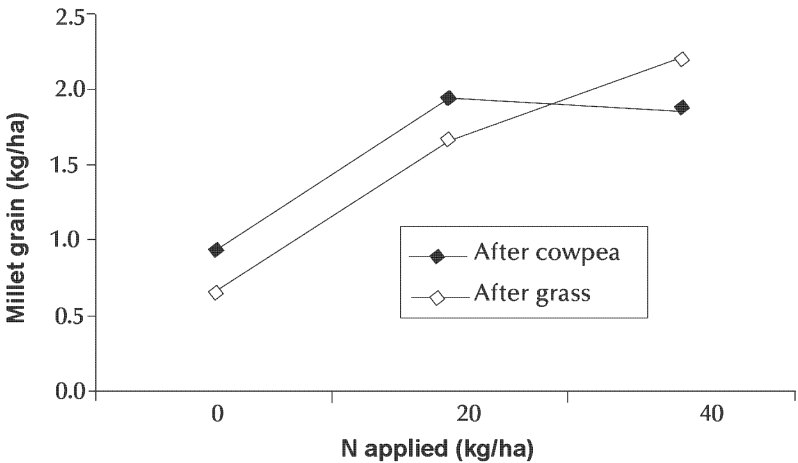


Figure 5. Response of maize to N after cowpea and after natural grass fallow at two northern Guinea savanna sites in northern Nigeria (Carsky et al. 1999).

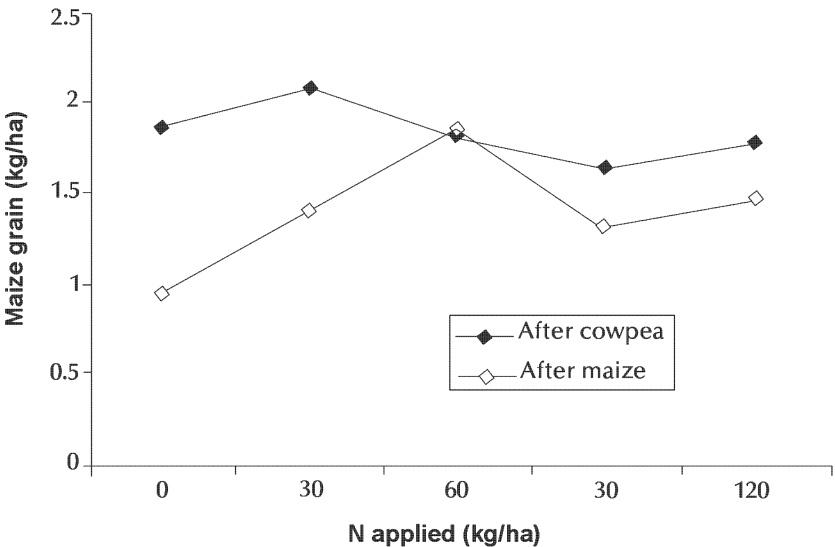


Figure 6. Effect of cowpea in the first growing season on maize response to N in the second growing season in northern Ghana (Dakora et al. 1987).

by Blahut and Singh 1999), it is easy to imagine that immediate incorporation into the soil followed soon by the cereal is responsible for maximizing the benefit. In a study conducted in Niger, Franzluebbers et al. (1994b) observed that at 26 days after incorporation, 13 to 26% of the N released from cowpea was already found in the shoots and roots of the subsequent sorghum crop.

### How important are non-N benefits of cowpea rotation?

Cereal yields are almost always higher after a cowpea crop than after a cereal crop, but this may not be due entirely to N supply. The benefit of cowpea rotation, often thought to

be due solely to biological N fixation, may be related to its influence on pest and disease problems of cereals (including reduction in *Striga hermonthica*) and other soil benefits such as access to sparingly soluble P. Experimental data supporting these aspects are discussed below.

An analysis of the results presented in Table 1 shows that the effect of cowpea rotation is greater when the control system was continuous mono-specific cereal (i.e., millet after millet or maize after maize). Yield increase after cowpea compared with continuous cereal of the same species was 80% while it was only 31% for continuous cereal of differing species (i.e., maize followed by sorghum or sorghum followed by millet). This suggests that a mono-specific continuous cereal control may have more pest and disease problems than a different-species continuous cereal control. If this is true, then the benefit of cowpea (providing a break in pest and disease cycles) could also be provided by many other non-leguminous crops. Cowpea would not be the only solution.

A study reported by Reddy et al. (1994) clearly shows a non-N benefit of cowpea rotation (Fig. 7). The curves for previous cereal and previous cowpea do not converge. In this case the effect of cowpea rotation appeared to be related to incidence of *Striga hermonthica* on the cereal test crop as there was more *Striga hermonthica* on millet after millet than on millet after cowpea. It is not clear whether cowpea actually reduced *Striga hermonthica* incidence or whether it simply did not result in build-up as the millet did. Ariga et al. (1994) showed how a preceding crop of cowpea variety TVx 3236 reduced *Striga hermonthica* density on a subsequent maize crop and increased maize yield. The effect increased with the duration of growth of the cowpea crop. However, in conditions of very low soil fertility, any source of N may increase emergence and growth of *Striga hermonthica* (Pieterse and Verkleij 1991). For example, cowpea rotation (or N application) was shown to increase *Striga hermonthica* density on subsequent maize in northern Nigeria (Carsky et al. 1999).

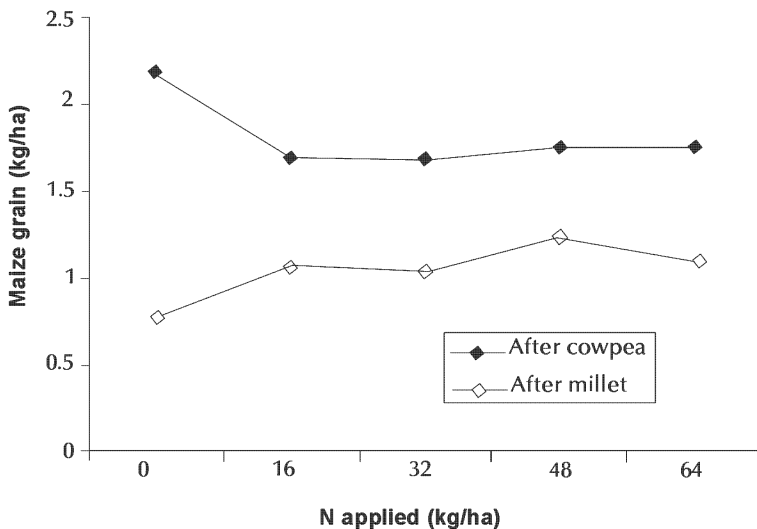


Figure 7. Response of millet to N fertilizer after cowpea compared with continuous millet (Reddy et al. 1994).

It has been reported that some legumes improve bioavailability of sparingly soluble soil P and the same effect might be expected for sparingly soluble P fertilizers (Vanlauwe et al. 2000). In a test of cowpea in southern Benin, application of P as rock phosphate did not increase cowpea biomass (Table 5), but subsequent maize yield after cowpea with rock phosphate is similar to the yield of maize after triple superphosphate. This may have been due to the release of P from rock phosphate over time independent of the cowpea crop. A maize control treatment, with and without rock phosphate, is needed to estimate the cowpea effect on rock phosphate. Aihou and Adomou (2000) conducted such a study for two years (two cycles of cowpea–maize rotation) in southern Benin. The response of maize to rock phosphate in a cowpea–maize rotation was not significantly different from the response in a maize–maize control system. Research should be conducted using many cowpea varieties to see if there is any potential for cowpea to make P more available (see Bationo et al. this volume).

The ability to make sparingly soluble P available may be a heritable trait. Lyasse et al. (2001) tested four cowpea varieties to assess the genotype specific potential to utilize rock phosphate as P source in a P-deficient soil in the derived savanna zone of Nigeria. Significant genotypic variation in terms of both P uptake and grain yield were observed in this study, and one variety was identified to react positively to the application of RP (Fig. 8). A similar trend was observed for the N-fixation as well as the biomass production at peak physiological growth stage (data not shown). Krasilnikoff et al. (2002) calculated that the same variety (IT90K-59) was also able to deplete the stable P fraction (non-Olsen P) in the rhizosphere.

Organic matter replenishment is often mentioned as a possible benefit of cowpea rotation. But the amount of organic matter generated by a cowpea crop is usually not as great as a cereal crop. Furthermore, cowpea residues, because of higher N concentration, may decompose more rapidly than low N cereal residues. This is good for supplying N and other nutrients to subsequent cereals but is not conducive to the build up of soil organic matter. In N'Dounga (Niger), Franzluebbers et al. (1994b) found that cumulative C loss from decomposing cowpea residues from the time of incorporation until the end of the rainy season was 78% of the initial cowpea C and no additional soil organic carbon build up was observed when compared to the control treatment without organic amendment.

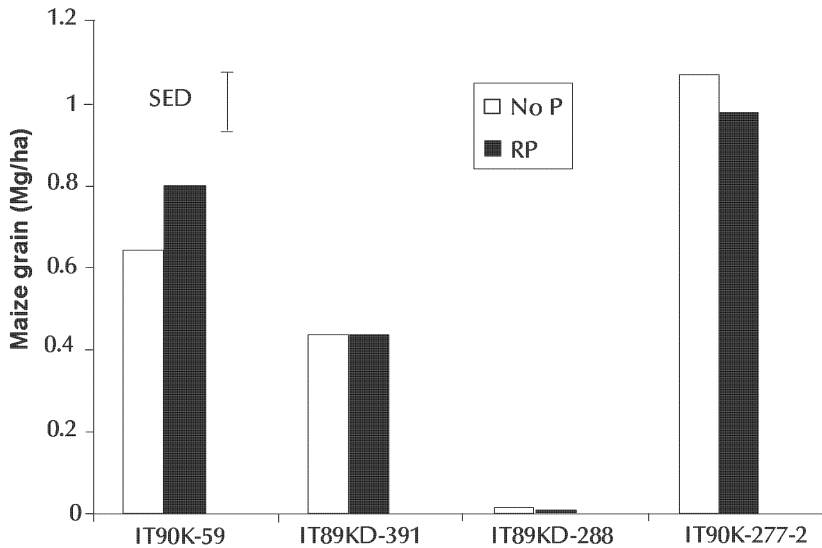
It is possible to calculate the amount of residues needed to maintain or increase SOC given an estimate of soil carbon mineralization rate and the rate of carbon loss from applied residues (De Ridder and van Keulen 1990). Assuming that 6% of soil carbon is mineralized each year (De Ridder and van Keulen 1990) and that 0.35 kg of humus carbon is generated from every kg of residue carbon (Himes 1997), it can be estimated that 3.2 Mg/ha of cowpea dry matter would be necessary to maintain soil organic carbon at 0.3% and 6.4 Mg/ha would be needed to maintain soil organic carbon at 0.6% (Table 6). Typical observations of one to four Mg/ha of aboveground cowpea dry matter (Table 7) and 0.5 to 2 Mg/ha belowground dry matter (as mentioned above) indicate that cowpea, if recycled, could maintain soil organic C at low levels but not at moderate levels. It can be seen from these calculations that it is not possible to increase soil organic C using cowpea.

When the cereal yield following cowpea is greater than that following a non-cowpea control even at high N, then non-N benefits should be suspected. When this occurs, follow-up research should be planned carefully after narrowing down the plausible

**Table 5. Maize grain yields as affected by previous application of 30 kg P/ha as triple superphosphate (TSP) or 90 kg P/ha as rock phosphate (RP) to cowpea at Sekou (6°36'N; 2°14'E), southern Benin, 1998.**

| P applied to cowpea | Cowpea haulms and litter (kg DM/ha) | Maize grain (kg DM/ha) |
|---------------------|-------------------------------------|------------------------|
| 0 P                 | 1808                                | 611                    |
| 30 TSP              | 3009                                | 1024                   |
| 90 RP               | 1853                                | 1192                   |
| SED                 | 329                                 | 181                    |

Source: B. Vanlauwe et al. (unpublished data).



**Figure 8. Grain yield of four cowpea cultivars as affected by RP application on a low-P soil in the derived savanna of Nigeria at Fashola, (Lyasse et al., 2001). [SED = standard error of the difference].**

**Table 6. Quantities of annual C loss by mineralization and organic residues (Mg/ha/yr) needed to maintain soil organic carbon at initial levels.**

| Initial OC (%) | C loss (Mg/ha) | Residue C (Mg/ha) | Residue DM (Mg/ha) |
|----------------|----------------|-------------------|--------------------|
| 0.3            | 0.50           | 1.44              | 3.20               |
| 0.6            | 1.01           | 2.88              | 6.40               |
| 1.2            | 2.02           | 5.76              | 12.80              |

Assumes

1. 6% loss of soil organic carbon per year (De Ridder and Van Keulen 1990).
2. One hectare at 0.20 m depth weighs 2 800 000 kg.
3. 0.35 kg humus C for every kg of residue C (Himes 1997).
4. 0.45 kg residue C for every kg of residue dry matter.

**Table 7. Aboveground dry matter (Mg/ha) of cowpea residue (after harvest) as a function of maturity class and insecticide treatment for several sites and years (number of observations in parentheses).**

| Maturity class | Spray    | No spray |
|----------------|----------|----------|
| Early          | 2.2 (29) | 2.3 (34) |
| Medium         | 2.5 (73) | 3.9 (41) |
| Late           | 3.3 (99) | 3.9 (69) |

Source: Schulz et al. (2001).

non-N benefits. Trials should be designed to isolate and understand individual effects. This can lead to manipulation of these phenomena to improve the ability of cowpea rotation to maintain soil productivity.

## Recommendations/strategies to optimize cowpea rotation benefits

### *Choice of variety*

The first condition for a cowpea rotation benefit is good growth of cowpea. For a positive N effect, the cowpea must nodulate well and fix N from the atmosphere. Varietal characteristics that determine N fixation in grain legumes are discussed in Chapter 8 of Giller (2001).

High biological N fixation will not lead to net N benefits if the N harvest is also high. To improve the benefit to the soil, a variety that puts more N in vegetation is preferred although the farm household may require more grain. Data derived from Schulz et al. (2001) show that aboveground residue dry matter increases with the maturity class of the cowpea (Table 7). Therefore, the benefit of cowpea rotation can be expected to increase in varieties that mature later, even if the harvest index remains constant. Indeed, Stoop and van Staveren (1982) demonstrated that the impact on subsequent millet increased as maturity cycle of preceding cowpea increased. We therefore recommend the variety with the longest agronomically appropriate maturity cycle.

Phosphorus deficiency is commonly observed in legumes in the savanna zone. As Sanginga et al. (2000) observed large varietal differences in P requirements for cowpea growth and N<sub>2</sub> fixation, this suggests the need to take the P requirements of these cowpea lines into account in plant introduction and plant selection for the moist savanna zone soils. The possibility to use less soluble and much cheaper P-sources (e.g., low reactive rock phosphate) in combination with selected P-efficient cowpea breeding lines could alleviate P depletion.

Another important consideration for the choice of cowpea variety is that it should be resistant to biotic and abiotic stresses that reduce aboveground biomass, including *Striga gesnerioides* and drought. In addition, an obvious advantage in a cereal-based system is the ability to promote *Striga hermonthica* seedbank reduction.

### *Management of soil and crop*

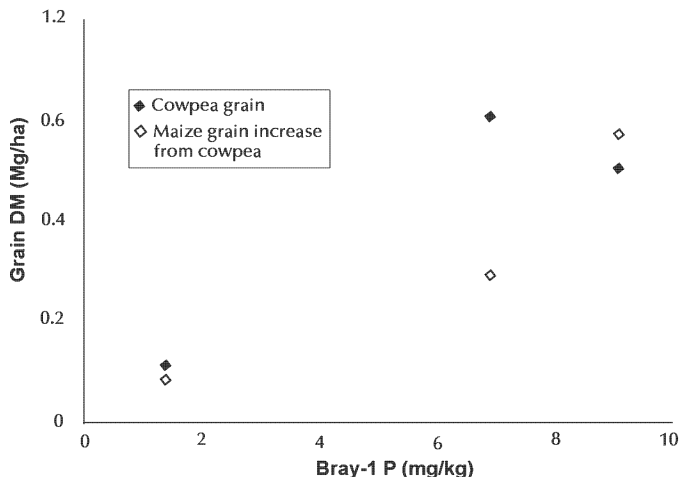
In order to optimize the benefit of cowpea rotation with cereals, it is of utmost importance to have sufficient soil P. This is clearly shown by the improvement in cowpea biomass with TSP application in southern Benin (Table 5). This benefit is believed to be related to improved nodulation as shown by many studies where P application increases nodule numbers and nodule fresh weight. The importance of P supply was also shown in a study of cowpea–maize rotation in Nigeria (Fig. 9) in which the effect of cowpea rotation was not important until plant available P was increased above 5 mg/kg. The critical level

of plant available P (the level above which P fertilizer application is not economically justified) has been estimated at 10.6 mg/kg by Aune and Lal (1995) from a small data set. This level should be confirmed as it would eventually be used to guide P fertilizer application by farmers.

It is sometimes observed that cowpea, if not adequately protected from insect damage, produces less grain and more leaf and vine dry matter as suggested by the data of Schulz et al. (2001), which is summarized in Table 7. The subsequent benefit to a cereal in rotation may be increased as was observed by Carsky et al. (1999). Although this should not be a goal of cropping systems development, it may provide an internal recovery mechanism for farmers who suffer from insect losses.

It may be questioned why cowpea intercropping is not recommended as a resource management technology. Whereas intercropping does benefit the need of the household for balanced food production and risk avoidance, rotation of sole cowpea generally has a much greater effect on a subsequent maize crop than rotation with a cereal–cowpea intercrop. This was shown by Bagayoko et al. (1997) in Mali and by Rodriguez (1986) in Burkina Faso (Table 8). The benefits of cowpea cereal rotation may be realized in a “within-field” rotation where cowpea and cereal rows are swapped over time especially in a spatial arrangement of two rows of cereal and four rows of cowpea.

The time between cowpea harvest and cereal planting is obviously important. One only needs to look at the large effect of cowpea rotation in the trials of Dakora et al. (1987) and Carsky et al. (2001) when cowpea was grown in the first growing season followed immediately by maize. Thus, first season cowpea should be considered if the length of growing season permits two crops. Possible problems with the system, which can not be ignored, include loss of cowpea grain quality when harvested mid-season and the need for the household to produce cereals first. Researchers should be aware of these as good reasons to grow cowpea in the second part of the season rather than the first.



**Figure 9.** Effect of soil P on cowpea grain yield (Mg/ha) and increase in maize grain yield (Mg/ha) from preceding cowpea compared with preceding native fallow in northern Nigeria from Carsky et al. (2001). Cowpea and natural (grass dominated) fallow from May to July were followed by maize from August to October. Plant available P was 6.8 mg/kg at a moderate-P site and 1.4 mg/kg at a low-P site where it was increased to 9.1 mg/kg by applying 200 kg of SSP/ha.

**Table 8. Comparison of sole cowpea (C) and millet–cowpea intercrop (M-C) on millet yield (Mg/ha) under low input conditions.**

| M–M  | C–M  | M–C–M | Source                                    |
|------|------|-------|---|
| 0.41 | 1.59 | 0.98  | Rodriguez (1986) <sup>†</sup>             |
| 1.17 | 1.88 | 1.91  | Osei-Bonsu and Asibuo (1997) <sup>†</sup> |
| 1.38 | 1.69 | 1.46  | Bagayoko et al. (1997) <sup>‡</sup>       |

Notes:

<sup>†</sup> Yield in fourth year only with N application rate of 28 kg/ha; averaged for two cowpea cultivars.<sup>‡</sup> Yield of maize in second year without N applied.<sup>‡</sup> Yield averaged for four years and across N application rates of 0, 20, and 40 kg/ha.

## Conclusion

Adoptability of cowpea is high. Dembele (2000), for example, recorded grain legume systems adoption in Mali to be many times higher than adoption of forage legumes. Oyewole et al. (2000) found that farmers preferred cowpea–maize to *Mucuna*–maize double cropping to keep grain producing cowpea in the system although the benefit of cowpea was less than that of *Mucuna*. It appears from our synthesis that cowpea rotation should be considered as an important resource management technology. However, for this to function, systems should be designed that optimize the benefit of cowpea to the soil–plant system. These will include: (1) cowpea varieties with the longest agronomically acceptable maturity cycles, (2) maintenance of adequate P supply, and (3) the shortest possible time between the cowpea and cereal crops in rotation. It will be possible to pursue these strategies in some, but not all socioeconomic and agroecological circumstances.

## References

- Aïhou, K. and M. Adomou. 2000. Contribution du phosphore à l'amélioration de l'assimilation de l'azote par le maïs en rotation avec le *mucuna* et le niébé. Pages 138–147 in *Cover crops for integrated natural resource management in West Africa*. Proceedings of a regional workshop October 1999, Cotonou, Republic of Benin, edited by R.J. Carsky, J.D.H. Keatinge, V.M. Man-yong, and A.C. Eteka. IITA, Ibadan, Nigeria.
- Ariga, E.S., D.K. Berner, and J. Chweya. 1994. Effects of previous season cotton and cowpea on *Striga hermonthica* parasitism on maize. *Phytopathology* 84: 1151.
- Aune, J.B. and R. Lal. 1995. The tropical soil productivity calculator—A model for assessing effects of soil management on productivity. Pages 499–520 in *Soil management: experimental basis for sustainability and environmental quality*, edited by R. Lal and B.A. Stewart. CRC Lewis Publishers, Boca Raton, Florida, USA.
- Awonaike, K.O., K.S. Kumarasinghe, and S.K.A. Danso. 1990. Nitrogen fixation and yield of cowpea (*Vigna unguiculata*) as influenced by cultivar and *Bradyrhizobium* strain. *Field Crops Research* 24: 163–171.
- Bagayoko, M., S.C. Mason, and S. Traoré. 1997. The role of cowpea on pearl millet yield, N uptake, and soil nutrient status in millet–cowpea rotation in Mali. Pages 109–114 in *Soil fertility management in West African land-use systems*, edited by G. Renard, A. Neef, K. Becker, and M. von Oppen. Margraf-Verlag, Weikerseim, Germany.
- Bationo, A., B.R. Ntare, S.A. Tarawali, and R. Tabo. 2002. Soil fertility management and cowpea production in the semiarid tropics. Pages 299–316 in *Challenges and opportunities for enhancing sustainable cowpea production*, edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. IITA, Ibadan, Nigeria.
- Blahut, G.R. and B.B. Singh. 1999. Achishuru cowpeas in central Nigeria. I. Origin, diversity and production practices. *Samaru Journal of Agriculture* 15: 21–28.
- Carsky, R.J., B. Oyewole, and G. Tian. 1999. Integrated soil management for the savanna zone of W. Africa: legume rotation and fertilizer N. *Nutrient Cycling in Agroecosystems* 55: 95–105.

- Carsky, R.J. 2000. Potential of herbaceous legume cover crop fallow systems in the savanna zone. Pages 594–602 in *La Jachère en Afrique tropicale*. Proceedings of the International Seminar, April, 1999, Dakar, edited by C. Floret and R. Pontanier. John Libbey Eurotext, Paris.
- Carsky, R.J., B.B. Singh, and B. Oyewole. 2001. Contribution of early-season cowpea to late-season maize in the savanna zone of West Africa. *Biological Agriculture and Horticulture*. 18: 303–315.
- Dakora, F.D., R.A. Aboyinga, Y. Mahama, and J. Apaseku. 1987. Assessment of N fixation in groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp.) and their relative N contribution to a succeeding maize crop in northern Ghana. *MIRCEN Journal*. 3: 389–399.
- Dembele, E. 2000. Activités liées à l'utilisation des légumineuses herbacées au Mali-sud. Pages 254–258 in *Cover crops for integrated natural resource management in West Africa*, edited by R.J. Carsky, J.D.H. Keatinge, V.M. Manyong, and A.C. Eteka. IITA, Ibadan, Nigeria.
- De Ridder, N. and H. van Keulen. 1990. Some aspects of the role of organic matter in sustainable intensified arable farming systems in the West African semiarid tropics (SAT). *Fertilizer Research* 26: 299–310.
- Eaglesham, A.R.J., A. Ayanaba, V. Ranga Rao, and D.L. Eskew. 1982. Mineral N effects on cowpea and soybean crops in a Nigerian soil. II. Amounts of N fixed and accrual to the soil. *Plant and Soil* 68: 183–192.
- Franzluebbers, K., R.W. Weaver, and A.S.R. Juo. 1994a. Mineralization of labeled N from cowpea (*Vigna unguiculata* [L.] Walp.) plant parts at two growth stages in sandy soil. *Plant and Soil* 160: 259–266.
- Franzluebbers, K., A.S.R. Juo, and A. Manu. 1994b. Decomposition of cowpea and millet amendments to a sandy alfisol in Niger. *Plant and Soil* 166: 255–265.
- Giller, K.E., J.F. McDonagh, and G. Cadisch. 1994. Can nitrogen fixation sustain agriculture in the tropics? Pages 173–191 in *Soil science and sustainable land management in the tropics*, edited by J.K. Syers and D.L. Rimmer. CAB International, Wallingford, UK.
- Giller, K.E. 2001. *Nitrogen fixation in tropical cropping systems*. 2nd edition. CAB International, Wallingford, UK. 423 pp.
- Groot, J.J.R., D. Koné, M. Traoré, and N. Kamissoko. 1995. Description du système racinaire de l'*Andropogon gayanus*, du *Vigna unguiculata* et du *Stylosanthes hamata* en zone soudano-sahélienne. Rapports Production Soudano-Sahélienne (PSS) No. 8. AB-DLO, Wageningen, The Netherlands.
- Himes, F.L. 1997. Nitrogen, sulfur, and phosphorus and the sequestering of carbon. Pages 315–319 in *Soil processes and the carbon cycle*, edited by R. Lal, J.M. Kimble, R.F. Follett, and B.A. Stewart. CRC Press, Boca Raton, Florida, USA.
- Horst, W.J. and R. Hardter. 1994. Rotation of maize with cowpea improves yield and nutrient use of maize compared to maize monocropping in an Alfisol in the northern Guinea savanna of Ghana. *Plant and Soil* 160:171–183.
- Jeranyama, P., O.B. Hestermann, S.R. Waddington, and R.R. Harwood. 2000. Relay intercropping of sunnhemp and cowpea into a smallholder maize system in Zimbabwe. *Agronomy Journal* 92: 239–244.
- John, P.S., R.K. Pandey, R.J. Buresh, and R. Prasad. 1992. Nitrogen contribution of cowpea green manure and residue to upland rice. *Plant and Soil* 142: 53–61.
- Jones, M.J. 1974. Effect of previous crop on yield and nitrogen response of maize at Samaru, Nigeria. *Experimental Agriculture* 10: 273–279.
- Krasilnikoff, G., T.S. Gahoonia, and N.E. Nielsen. 2002. Phosphorus uptake from sparingly available soil-P by cowpea (*Vigna unguiculata*) genotypes. Pages 239–250 in *Integrated*



- nutrient management in sub-Saharan Africa: from concept to practice, edited by B. Vanlauwe, J. Diels, N. Sanginga, and R. Merckx. CAB International, Wallingford, UK. In press.
- Lyasse, O., B.K. Tossah, B. Vanlauwe, J. Diels, N. Sanginga, and R. Merckx. 2002. Options for increasing P availability from low reactive rock phosphate. Pages 225–237 in *Integrated nutrient management in sub-Saharan Africa: from concept to practice*, edited by B. Vanlauwe, J. Diels, N. Sanginga, and R. Merckx. CAB International, Wallingford, UK. In press.
- Nnadi, L.A. and V. Balasubramanian. 1978. Root nitrogen content and transformation in selected grain legumes. *Tropical Agriculture (Trinidad)* 55: 23–32.
- Nnadi, L.A., L. Singh, and V. Balasubramanian. 1981. Effect of grain legumes and sorghum on soil nitrogen status and the yield of subsequent maize crop. *Samaru Journal of Agriculture* 1: 183–190.
- Osei-Bonsu, P., and J.Y. Asibuo. 1997. Studies on *Mucuna* (*Mucuna pruriens* var. *utilis*) in Ghana. Pages 435–441 in *Technology options for sustainable agriculture in sub-Saharan Africa*, edited by T. Bezuneh, A.M. Emechebe, J. Sedogo, and M. Ouédraogo. OAU/STRC-Semiarid Food Grain Research and Development, Ouagadougou, Burkina Faso.
- Oyewole, B., R.J. Carsky, and S. Schulz. 2000. On-farm testing of *Mucuna* and cowpea double cropping with maize in the Guinea savanna of Nigeria. Pages 137–147 in *Cover crops for integrated natural resource management in West Africa*, edited by R.J. Carsky, J.D.H. Keatinge, V.M. Manyong, and A.C. Eteka. IITA, Ibadan, Nigeria.
- Pieterse, A.H. and J.A.C. Verkleij. 1991. Effect of soil conditions on *Striga* development: a review. Pages 329–339 in *Proceedings of the 5th international symposium of parasitic weeds*, edited by J.K. Ransom, L.J. Musselman, A.D. Worsham, and C. Parker. CIMMYT, Nairobi, Kenya.
- Poulain, J.-F. 1980. Crop residues in traditional cropping systems of West Africa—effects on the mineral balance and level of organic matter in soils—proposals for their better management. Pages 38–71 in *Organic recycling in Africa. Papers presented at the FAO/SIDA workshop on the use of organic materials as fertilizers in Africa*. FAO Soils Bulletin 43, FAO, Rome, Italy.
- Reddy, K.C., P.L. Visser, M.C. Klaij, and C. Renard. 1994. The effects of sole and traditional intercropping of millet and cowpea on soil and crop productivity. *Experimental Agriculture* 30: 83–88.
- Rodriguez, M. 1986. Agronomie du maïs. Pages B1–B45 in *Rapport Annuel 1986 du projet “Recherche et développement des cultures vivrières dans les zones semiarides d’Afrique”*. SAFGRAD/IITA, Ouagadougou, Burkina Faso.
- Sanginga, N., O. Lyasse, and B.B. Singh. 2000. Phosphorus-use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant and Soil* 220: 119–128.
- Schulz, S., R.J. Carsky, and S. Tarawali. 2001. Herbaceous legumes: the panacea for West African soil fertility problems? Pages 179–195 in *Soil fertility maintenance in West Africa*, edited by G. Tian et al. ASA, Madison WI.
- Stoop, W.A. and J.P. van Staveren. 1982. Effect of cowpeas in cereal rotations on subsequent crop yields under semiarid conditions in Upper Volta. Pages 653–657 in *Biological nitrogen fixation technology for tropical agriculture*, edited by P.H. Graham and S.C. Harris. CIAT, Cali, Colombia.
- Vanlauwe, B., J. Diels, N. Sanginga, R.J. Carsky, J. Deckers, and R. Merckx. 2000. Utilization of rock phosphate by crops on a representative toposequence in the northern Guinea savanna zone of Nigeria: response by maize to previous herbaceous legume cropping and rock phosphate treatments. *Soil Biology and Biochemistry* 32: 2079–2090.
- Wani, S.P., O.P. Rupela, and K.K. Lee. 1995. Sustainable agriculture in the semiarid tropics through biological nitrogen fixation in grain legumes. *Plant and Soil* 174: 29–49.

## 4.3

# Advances in cowpea cropping systems research

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### Abstract

Cowpea (*Vigna unguiculata*) [L.] Walp.) is a major component of the traditional cropping systems in Africa, Asia, and Central and South America where it is widely grown in mixtures with other crops in various combinations. The productivity of cowpea in these mixtures is low, mainly due to low plant population, competition under intercropping, and lack of crop protection measures. Studies have shown that the productivity of cowpea in these systems could be enhanced through the use of improved varieties, appropriate date of planting with respect to the cereal, higher plant populations, improved soil fertility, and suitable spatial arrangements. This paper highlights recent research leading to improvements in cowpea cropping systems. These include improved productivity as a result of early cowpea planting, strip cropping, dense planting, and appropriate soil fertility management. For example, in West Africa, the use of high yielding improved varieties in a strip cropping system with two cereal rows : four cowpea rows offers an opportunity for selective input application and appears to be economically superior to the traditional one cereal row: one cowpea row.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is a major component of the cropping systems of the drier parts of the tropics, particularly sub-Saharan Africa. West and Central Africa account for over 64% of the estimated 12.5 million ha cultivated to cowpea worldwide (Singh et al. 1997). This is followed by Central and South America (19%), Asia (10%), and East and Southern Africa (6%). Cowpea is mainly grown in mixtures with other crops and a great diversity of crop mixtures has been reported (Mortimore et al. 1997). In a recent survey, Henriët et al. (1997) reported the existence of up to 43 crop mixtures in the Sudan savanna of Nigeria with a millet–cowpea mixture being predominant, representing 22% of the fields sampled (Table 1).

Other dominant crop mixtures included millet–sorghum–cowpea (18.6%), sorghum–cowpea (10.4%) and millet–cowpea–groundnut (7.6%). The importance of cowpea in the cropping systems of the dry savanna is well illustrated by the fact that this crop occurred in 71.4% of the fields sampled. However, the cowpea grain yields in these systems ranged from 0 to 132 kg/ha (Table 2) compared with a sole yield potential of 1500 to 3000 kg/ha under optimum management (Muleba and Ezumah 1985).

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**Table 1. Major cropping systems identified at several locations in the Sudan savanna ecological zone of Nigeria in 1992 and 1993.**

| Crop mixtures                   | % of different cropping systems |      |
|---------------------------------|---------------------------------|------|
|                                 | 1992                            | 1993 |
| Millet–cowpea                   | 22.0                            | 22.5 |
| Millet–cowpea–groundnut         | 15.4                            | 7.6  |
| Millet–sorghum–cowpea           | 12.4                            | 18.6 |
| Sorghum–cowpea–groundnut        | 9.7                             | 2.8  |
| Sorghum–cowpea                  | 8.0                             | 10.4 |
| Millet                          | 5.4                             | 6.0  |
| Millet–sorghum                  | 2.7                             | 4.7  |
| Millet–groundnut                | 5.4                             | –    |
| Sorghum                         | –                               | 4.5  |
| Sorghum–groundnut               | 2.7                             | –    |
| Millet–sorghum–cowpea–sesame    | –                               | 2.5  |
| Millet–sorghum–cowpea–groundnut | 7.0                             | 2.1  |
| Others <sup>†</sup>             | 8.1                             | 18.3 |

<sup>†</sup>Others: those involving sesame, cassava, okra, maize, and bambara nut.

Source: Henriët et al. 1997.

**Table 2. Mean grain yield of cowpea in different crop mixtures in farmers' fields in parts of the Nigerian Sudan savanna (1992 and 1993).**

| Crop mixtures            | Grain yield (kg/ha) <sup>†</sup> |             |
|--------------------------|----------------------------------|-------------|
|                          | 1992                             | 1993        |
| Millet–cowpea            | 22 (5–29)                        | 42 (6–129)  |
| Millet–cowpea–groundnut  | 18 (0–36)                        | 40 (8–103)  |
| Millet–sorghum–cowpea    | 18 (0–40)                        | 54 (16–132) |
| Sorghum–cowpea–groundnut | 13 (0–25)                        | 63 (16–104) |
| Sorghum–cowpea           | 29 (22–39)                       | 52 (30–84)  |

<sup>†</sup>Figures in parentheses represent the range in values.

Source: van Ek et al. 1997.

The major yield-limiting factors of cowpea cropping systems are low plant population, low yield potential of local cultivars, insect pests and diseases, shading by the cereals, drought stress, and low soil fertility. In this respect, there are opportunities for improved management practices that overcome these production constraints and enhance cowpea productivity. These include sowing date, row geometry, pest incidences, and variety improvement.

The objective of this paper is to highlight developments in these management areas for cropping systems research mainly in West Africa conducted with cowpea as one of the component crops thus complementing earlier reviews by Muleba and Ezumah (1985) and Blade et al. (1997).

### Relative sowing date in intercropping

Date of sowing is dictated by many factors including weather, soil moisture, time, and labor constraints faced by the farmer, variety, and crop production system. Cowpea is generally grown as the understorey crop in a cereal- or tuber-based system. In the West African savannas, cowpea is usually relay planted into the cereal crops. It has been noted

that in the Sahel, millet yield is reduced if millet and cowpea are planted simultaneously (Ntare 1990; Ntare and Williams 1992). However, Reddy and Visser (1997) concluded that intercropped cowpea should be sown simultaneously or soon after millet for maximum yield of cowpea. They found that, compared to simultaneous sowing, delaying cowpea sowing to seven weeks after millet led to significantly lower crop growth rates (19 to 10 kg/ha), lower grain (1110 to 100 kg/ha), and dry matter (2110 to 560 kg/ha) yields of cowpea. In contrast, grain yield of intercropped millet did not vary significantly with cowpea interplanting time (Table 3). Terao et al. (1997) also advocated simultaneous planting of cowpea and millet if there is no severe competition for water. Over two years, millet yield was not reduced when millet and cowpea were sown simultaneously at Minjibir (800 mm rainfall) while millet yield reduction at Mallam Madori (426 mm) was only 16%. In the Sudan savanna, Blade et al. (1997) found that delaying cowpea planting by two or three weeks resulted in a reduction of cowpea grain yield of over 50% in comparison to simultaneous millet and cowpea planting. In choosing the appropriate time to introduce cowpea into millet, an important consideration is the objective of the farmer—which is to have a full millet grain yield with some additional cowpea grain and fodder. Thus, farmers would be reluctant to adopt any practice that may reduce millet grain yield.

Most of the reported work on maize–cowpea mixtures indicated a reduction in cowpea yields while maize yields were unaffected (Haizel 1974; Isenmilla et al. 1981; Olufajo 1988; Cardoso et al. 1993). However, the competitive effects from the maize component could be reduced by sowing cowpea early. Myaka (1995) showed that when sown four weeks after maize, cowpea yields were 67% less than cowpea planted two weeks after maize. In both cases, maize yields were not affected by the cowpea component.

Being strongly competitive, cowpea reduces cotton yields when grown as an intercrop and the extent of yield reduction depends on the cowpea sowing date. Results from Endondo and Samatana (1999) suggest that cowpea should be sown five to six weeks after cotton in a cotton–cowpea intercrop. With simultaneous sowing, the intercropped cotton yield was 50% of the sole crop yield whereas the cotton yield was reduced by

**Table 3. Grain yield (kg/ha) of cowpea and millet as affected by the planting date of cowpea in a millet/cowpea intercropping system in Kolo, Niger Republic, in 1986 and 1988.**

| Time of planting cowpea <sup>a</sup> | Cowpea grain yield |      | Millet grain yield |      |
|--------------------------------------|--------------------|------|--------------------|------|
|                                      | 1986               | 1988 | 1986               | 1988 |
| Simultaneous                         | 1920               | 690  | 450                | 520  |
| 8–14 days after millet               | 1410               | 550  | 530                | 710  |
| 15–20 " " "                          | 1340               | 420  | 560                | 720  |
| 26–28 " " "                          | 680                | 240  | 610                | 680  |
| 31–36 " " "                          | 770                | 80   | 520                | 700  |
| 42–47 " " "                          | 250                | 0    | 570                | 730  |
| 56 " " "                             | 300                | 0    | 590                | 580  |
| Cowpea sole                          | 2850               | 1360 | —                  | —    |
| Millet sole                          | —                  | —    | 480                | 580  |
| Mean                                 | 1190               | 420  | 540                | 650  |
| CV(%)                                | 20                 | 24   | 21                 | 18   |
| SE                                   | 139                | 50   | 65                 | 59   |

<sup>a</sup>Cowpea interplanting dates varied between years depending on the occurrence of a rain of 12 mm or more to ensure cowpea germination.

Source: Reddy and Visser (1997).

16% and cowpea yield by 54% when cowpea was sown five to six weeks after cotton. However, year-to-year differences in the response of cotton–cowpea intercrop have been reported (Natarajan and Naik 1992). In wetter years, Myaka and Kabissa (1996) found that cowpea yield was reduced when cowpea sowing was delayed from two to four weeks after cotton, whereas in drier years, cowpea yield was not affected by sowing date. An important consideration with respect to cotton–cowpea intercropping is the time of insecticide application to the cotton component. Since farmers routinely apply insecticide to cotton whereas cowpea rarely receives insecticide protection, the main advantage of this mixture is the “incidental” benefit derived by the cowpea crop from the insecticide applied directly to cotton. It is noteworthy that the increase in cowpea grain yield as a result of the insecticide applied to cotton could be as high as 400% (Table 4). Further improvement in cowpea grain yield in this mixture could probably be achieved by using early maturing cowpea varieties whose reproductive phase would coincide with the period of insecticide application to cotton. In order to avoid contamination of cowpea, it is important to apply nonpersistent chemicals to cotton.

The only recent data on a cassava–cowpea intercrop are those of Okoli et al. (1996) who reported significant reductions in cassava yield whereas intercropped cassava had no effect on cowpea yield. However, cowpea yields were higher when established simultaneously with cassava than when introduced later into cassava.

### Row arrangement and density

The traditional production system involves varied arrangements of the component crops in time and space with implications for crop and livestock productivity, and sustainability (Shetty et al. 1995). Spatial arrangements and densities of the component crops have been manipulated in order to enhance complementarity and to reduce competition between the

**Table 4. Plant density, total aboveground biomass, pod weight, 1000 grain weight, and gross return of cowpea as a sole crop and when interplanted between cotton rows in Zimbabwe.**

|                          | Time of planting cowpea | Plant density established ('000/ha) | Total above-ground biomass (t/ha) | Pod weight (t/ha) | Grain weight (t/ha) | 100 seed weight (g) | Gross return (Z\$/ha) |
|--------------------------|-------------------------|-------------------------------------|-----------------------------------|-------------------|---------------------|---------------------|-----------------------|
| Sole cowpea <sup>†</sup> | Simultaneous            | 69.0                                | 1.16                              | 0.31              | 0.19                | 127                 | 376                   |
| Cotton–cowpea 1 : 1      | “                       | 37.6                                | 3.24                              | 1.32              | 0.96                | 135                 | 1866                  |
| Cotton–cowpea 1 : 1      | Staggered <sup>‡</sup>  | 37.9                                | 1.93                              | 0.71              | 0.49                | 129                 | 948                   |
| Cotton–cowpea 1 : 2      | Simultaneous            | 68.3                                | 2.97                              | 1.26              | 0.87                | 127                 | 1695                  |
| Cotton–cowpea 1 : 2      | Staggered               | 69.8                                | 1.93                              | 0.77              | 0.52                | 128                 | 1012                  |
| SE                       |                         | 1.8                                 | 0.32                              | 0.14              | 0.11                | 6                   | 213                   |

<sup>†</sup>Not sprayed.

<sup>‡</sup>Cowpea sown two weeks after cotton.

Source: Natarajan and Naik (1992).

component crops so that the physiological advantage from combining crop components is maximized (Willey and Osiru 1972; Willey 1979; Ofori and Stern 1987a, b).

In a millet–cowpea intercrop, Odo and Bibinu (1998) reported optimum spatial arrangements of one : three and three : three (millet : cowpea rows). Myaka (1995) showed that in a maize : cowpea intercrop, cowpea yields were 57% higher in two : two (maize : cowpea rows) compared with one : one (maize : cowpea rows). Asafu-Agyei et al. (1997) found that two : two (maize : cowpea rows) gave higher yields of maize and cowpea, land equivalent ratio (LER) and net benefit than one : one (maize : cowpea rows). Obuo et al. (1998) investigated the effect of intrarow spacing on cowpea–sorghum intercrop and found that yields of both components were highest at  $60 \times 20$  cm inter- and intrarow spacing.

Myaka and Kabissa (1996) found that alternating single rows of cotton with single rows of cowpea was superior to two : two or one : two (cotton : cowpea) in terms of crop yield and control of cowpea pests by insecticide applied directly to the cotton component in cotton–cowpea intercrop. Bezerra-Neto and Robichaux (1996) studied the effect of spatial arrangement and density on cotton–cowpea–maize intercrop and reported that the land equivalent ratio for yield was higher in the spatial arrangement of single rows of cowpea and maize between single rows of cotton. Land equivalent ratios for total biomass and grain yields were not affected as cotton density increased from 25 000 to 75 000 plants/ha. However, Bezerra-Neto and Robichaux (1997) noted that component yields and biomass production could be significantly affected by alteration of spatial arrangement and density. They therefore concluded that the most appropriate sowing arrangements in cotton–cowpea–maize intercrop should be determined by individual requirements for total biomass and grain yields.

Attempts have been made to plant the component crops in strips. This is advantageous in terms of ease of crop management, fertilizer and insecticide application, weeding, and reduction of the shading effect of cereal on cowpea. There is evidence that strip cropping with two rows cereal : four rows cowpea offers an opportunity for selective input application and better economic advantage than the traditional one row cereal : one row cowpea spatial arrangement (Singh and Emechebe 1998; Singh and Ajeigbe, this volume). Mensah (1997) noted that alternating three rows of cowpea with two or three rows of sorghum and one to two insecticide applications gave a yield advantage of 58 to 69% and proposed this as the most productive method to be adopted by subsistence farmers.

### **Soil fertility**

Soil fertility management has a major influence on the overall productivity of the intercropping system. In the traditional cereal–cowpea systems of the dry savanna of West Africa, millet is planted in less fertile fields with little or no fertilizer while sorghum is planted in relatively more fertile soils and with application of farmyard manure and fertilizers when available (van Ek et al. 1997). In the Sahel, phosphorus is the most limiting soil nutrient; nitrogen increases crop yield only in the presence of adequate phosphorus (Fussell et al. 1987; 1992). The application of a small quantity of phosphorus (13 kg/ha) has been suggested for increased productivity of the millet–cowpea system (Shetty et al. 1995; Subbarao et al. 1999). Biielders (1998) obtained 27% increase in cowpea and 52% increase in millet grain yield, as a result of the application of Tahoua rock phosphate. Millet benefited from the residual effects of rock phosphate applied to cowpea when millet and cowpea rows were rotated. Buerket et al. (1998) found that the application of Tahoua rock

phosphate led to between 25 and 78% increase in total dry matter of millet and sorghum, and between 12 and 46% increase for cowpea.

In cereal–cowpea intercrops, N application generally favors cereals, resulting in decreased cowpea yield due to shading by the cereal crop (Blade et al. 1997). However, in a farmer-managed trial, the yield of intercropped maize and cowpea increased by 35% and cowpea by 24%, with the application of 120-26-50 kg/NPK/ha compared with 60-13-25 kg/NPK/ha (Olufajo et al. 1997). In rotation and as intercrops with cereals, cowpea provides N and it contributes to overall fertility improvement. However, subsequent benefits from cowpea in sole cropping were greater than from intercropped cowpea (Carsky and Vanlauwe 2002). A further elaboration of soil fertility issues in relation to cowpea production is given by Bationo et al. (2002).

### Implications for pest incidence

Intercropping has long been known to be a major component of integrated pest control. Singh and Emechebe (1998) screened ten cowpea breeding lines under intercropping with millet as well as sole cropping with and without insecticide application. They found that intercropped cowpea grain yields were generally higher than yields from the sole crop when no insecticide was applied, indicating less insect damage under intercropping (Table 5). Mensah (1997) reported a low population density of post-flowering pests (*Maruca vitrata* and a complex of pod-sucking insects) but a high population density of flower pests (*Megalurothrips sjostedti*) in a crop mixture consisting of one row of sorghum alternated with two rows of cowpea. Although he observed a reduction in pests and damage to cowpea in mixtures compared with monoculture, he recommended one to two insecticide applications to maximize cowpea yields. Agboh-Noameshie et al. (1997) studied pest populations on cowpea intercropped with cassava and found that the micro-environment created by the intercrop reduced the populations of flower thrips (*M. sjostedti*) and pod-sucking bugs (Heteroptera) but increased those of the pod borer (*M. vitrata*). Intercropped maize, pepper, and cassava have also been reported to reduce the population of cowpea flower thrips while maize, cassava, and pigeonpea intercrops reduced the incidence of blister beetles (*Mylabris* sp.) on cowpea (Emeasor and Ezueh 1997). It is,

**Table 5. Grain and fodder yields of promising medium maturing cowpea varieties in different cropping systems at Minjibir, Nigeria, 1995.**

| Variety       | Sole crop<br>2 sprays |        | Sole crop<br>no spray |        | Intercrop<br>no spray |        |
|---------------|-----------------------|--------|-----------------------|--------|-----------------------|--------|
|               | Grain                 | Fodder | Grain                 | Fodder | Grain                 | Fodder |
| IT93K-23      | 2739                  | 3277   | 21                    | 4416   | 144                   | 406    |
| IT90KD-277-2  | 2571                  | 1492   | 163                   | 4250   | 293                   | 437    |
| IT92KD-371-1  | 2316                  | 3590   | 36                    | 4139   | 42                    | 171    |
| IT90K-391     | 2278                  | 3423   | 8                     | 4000   | 423                   | 703    |
| IT90K-365     | 2026                  | 2588   | 28                    | 3861   | 237                   | 437    |
| IT93K-621-7   | 1944                  | 2818   | 347                   | 1833   | 117                   | 406    |
| Dan Ila       | 1835                  | 1429   | 157                   | 1222   | 151                   | 265    |
| IT90K-372-1-2 | 1499                  | 960    | 261                   | 1000   | 133                   | 265    |
| IT89KD-349    | 1981                  | 2713   | 33                    | 2861   | 393                   | 656    |
| IT93K-734     | 1866                  | 1022   | 160                   | 1431   | 207                   | 203    |
| LSD 5%        | 458                   | 521    | 180                   | 2314   | 129                   | 535    |

Source: Singh and Emechebe 1998.

however, noteworthy that none of these companion crops significantly reduced cowpea damage by the mung moth, *M. vitrata*, African pea moth, *Cydia ptychora* (*Leguminivora ptychora*), and the pod-sucking bug complex, all of which constitute the major pests of the cowpea crop.

Jackai and Adalla (1997) reviewed the effect of intercropping on insect pests of cowpea and emphasized that intercropping does not necessarily reduce the pest load in any given situation; it depends on the crop(s) and pest(s) in question. Although intercropping can contribute to the control of a pest in an integrated control context, in most cases, pest damage to intercropped cowpea is generally no less than that to the monocrop at the time of harvest.

### Improved varieties adapted to intercropping systems

Variety development is discussed in detail in other parts of this conference by Singh et al. (2002). However, considering the fact that variety selection is a key to modifications that can be made in the cropping system, it is appropriate to consider the efforts currently being made to develop cowpea varieties that are suitable for intercropping. This is especially relevant, as different plant traits are required for cultivars intended for use under intercropping compared to those intended for use under sole cropping (Nelson and Robichaux 1997).

The local cowpea varieties are highly adapted to intercropping but they have a low harvest index. Terao et al. (1997) concluded that the type of cowpea adapted to intercropping is the spreading type of cowpea, improved to retain a substantial root system and high translocation efficiency. The number of branches and nodes and increased internode length are plant traits that are important under intercropping (Nelson and Robichaux 1997). Thus, the cultivar with a bush-type habit has been reported to be higher yielding under sole cropping, whereas the cultivar with a spreading habit was higher yielding under intercropping (Nelson and Robichaux 1997).

Subsistence farmers require crop varieties, which produce acceptable grain and fodder yields under a wide range of environmental conditions. New cowpea breeding lines are currently being evaluated under three systems at IITA, Kano: (i) pure crop with two sprays of insecticide, (ii) pure crop with no insecticide, and (iii) intercrop with no insecticide. Singh and Emechebe (1998) noted good performance of a number of improved varieties, particularly IT90K-277-2 under both sole- and intercropping. Thus, there is a good potential for increasing intercropped cowpea grain and fodder yields through the introduction of appropriate improved varieties.

### Implications and research needs

Future research should focus on the following:

- To enable better understanding of the dynamics of the complex farming system and ensure the introduction of innovations that are compatible with the farmers' socio-economic environment, work must continue on the characterization of the farming system. It is important to involve farmers in technology testing and validation to enhance the process of technology dissemination and adoption.
- Since cowpea is mainly grown in mixture with cereals, there is need for proper investigation of cereal-cowpea genotypic interactions in order to identify plant types of both components that will contribute to increased efficiency and production of the cropping



systems. Improved genotypes of both cowpea and cereals adapted to intercropping and amenable to management that suit the farmers' objectives should be developed.

- There is need for better understanding of soil fertility management in intercropping systems. Areas such as the possible contribution of some cowpea cultivars to P uptake under low soil P conditions including the effect of mycorrhizal association on P uptake, as well as the identification of cowpea cultivars with enhanced  $N_2$ -fixing efficiency need special attention. (see also Bationo et al. 2002).

## Conclusions

Cowpea is predominantly a crop of drier areas. For the foreseeable future, its production will continue to be based mainly on the diverse and complex intercropping systems. Over the years, food requirements have increased while land availability has become less. Thus, the only way to increase agricultural production is to increase the yield of individual crops. Being the understorey crop in most intercropping systems, growth and yield of cowpea are usually suppressed by the dominant crop. Complementarity in an intercropping situation can occur when the growth patterns of the component crops differ in time or when they make better use of resources in space. It is evident that the depressive effect of cereals on cowpea could be reduced by planting cowpea simultaneously or soon after cereals, particularly if there is no severe competition for water. Denser planting also improves productivity. Consistent increases in cowpea grain yields under strip cropping with cereals have also been reported, although there is scope for a better understanding of the physiological mechanisms that could limit the production and efficiency of such cropping systems. Concurrent with the development of cowpea genotypes that are adapted to intercropping, there is need to screen and develop cereal and tuber crops that are suitable for intercropping with cowpea. This will contribute to the overall improvement of the cropping system.

## References

- Agboh-Noameshie, A., L.E.N. Jackai, A.A. Agboola, and H.C. Ezumah. 1997. Manipulating canopy structure in cassava intercropped with cowpea and its effects on cowpea insect population densities. *Tropical Agriculture* 74: 210–215.
- Asafu-Agyei, J.N., K. Ahenkora, B. Banful, and S. Ennin-Kwabiah. 1997. Sustaining food production in Ghana: the role of cereal–legume-based cropping systems. Pages 409–416 *in* Technology options for sustainable agriculture in sub-Saharan Africa, edited by T. Bezuneh, A.M. Emechebe, J. Sedogo, and M. Ouedraogo. Semi-Arid Food Grain Research and Development Agency (SAF-GRAD) of the Scientific, Technical and Research Commission of OAU, Ouagadougou, Burkina Faso.
- Bationo, A., B.R. Ntare, S.A. Tarawali, and R. Tabo. 2002. Soil fertility management and cowpea production in the semiarid tropics. Pages 299–316 *in* Challenges and opportunities for enhancing sustainable cowpea production, edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. IITA, Ibadan, Nigeria.
- Bezerra-Neto, F. and R.H. Robichaux. 1996. Spatial arrangement and density effects on an annual cotton–cowpea–maize intercrop. I. Agronomic efficiency. *Pesquisa Agropecuária Brasileira* 31: 729–741.
- Bezerra-Neto, F. and R.H. Robichaux. 1997. Spatial arrangement and density effects on an annual cotton–cowpea–maize intercrop. II. Yield and biomass. *Pesquisa Agropecuária Brasileira* 32: 1029–1037.

- Biielders, C.L. 1998. Improving the productivity of millet–cowpea intercrops through the application of inputs to cowpeas and rotation between rows. Pages 101–107 *in* Soil fertility management in West African land-use systems, edited by G. Renard, A. Neef, and K. Becker. University of Hohenheim, ICRISAT Sahelian Centre and INRAN, Niamey, Niger.
- Blade, S.F., S.V.R. Shetty, T. Terao, and B.B. Singh. 1997. Recent developments in cowpea cropping systems research. Pages 114–128 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Buerkert, A., M. Bagayoko, A. Bationo, and H. Marschner. 1998. Site-specific differences in the response of cereals and legumes to rock phosphate, crop residue mulch and nitrogen in the Sudano-Sahelian zone of West Africa. Pages 53–59 *in* Soil fertility management in West African land-use systems, edited by G. Renard, A. Neef, and K. Becker. University of Hohenheim, ICRISAT Sahelian Centre and INRAN, Niamey, Niger.
- Cardoso, M.J., F.R. Freire Filho, V.Q. Ribeiro, A.B. Frota, and F. de B. Melo. 1993. Plant density of maize–cowpea intercrops under irrigation. *Pesquisa Agropecuária Brasileira* 28: 93–99.
- Carsky, R.J., B. Vanlauwe, and O. Lyasse. 2002. Cowpea rotation as a resource management technology for cereal-based systems in the savannas of West Africa. Pages 250–264 *in* Challenges and opportunities for enhancing sustainable cowpea production, edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. IITA, Ibadan, Nigeria.
- Emeasor, K.C., and M.I. Ezueh. 1997. The influence of companion crops in the control of insect pests of cowpea in intercropping systems. *Tropical Agriculture* 74: 285–289.
- Endondo, C. and M. Samatana. 1999. Effect of cowpea sowing date on cotton yield in a cotton–cowpea intercrop. *Cahiers Agricultures* 8: 215–217.
- Fussell, L.K., P.G. Serafini, A. Bationo and M.C. Klaij. 1987. Management practices to increase yields and yield stability of millet in Africa. Pages 255–268 *in* Proceedings of the International Pearl Millet Workshop, 7–11 April 1986. ICRISAT, Patancheru, India.
- Fussell, L.K., M.C. Klaij, C. Renard, and B.R. Ntare. 1992. Millet-based cropping systems for improving food production in the southern Sahelian zone. Pages 109–127 *in* Proceedings of the workshop on appropriate technologies for developing sustainable food production systems in the semiarid regions of sub-Saharan Africa. Purdue University Press, West Lafayette, Indiana, USA.
- Haizel, K.A. 1974. The agronomic significance of mixed cropping. I. Maize interplanted with cowpea. *Ghana Journal of Agricultural Science* 7: 169–178.
- Henriet, J., G.A. van Ek, S.F. Blade, and B.B. Singh. 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria. I. Rapid survey of prevalent cropping systems. *Samaru Journal of Agricultural Research* 14: 37–45.
- Isenmilla, A.E., O. Babalola, and G.O. Obigbesan. 1981. Varietal influence of intercropped cowpea on the growth, yield, and water relations of maize. *Plant and Soil* 62: 153–156.
- Jackai, L.E.N., and C.B. Adalla. 1997. Pest management practices in cowpea: a review. Pages 240–258 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Mensah, G.W.K. 1997. Integrated pest management in cowpea through intercropping and minimal insecticide application. *Annals of Plant Protection Sciences* 5: 1–14.
- Mortimore, M.J., B.B. Singh, F. Harris, and S.F. Blade. 1997. Cowpea in traditional cropping systems. Pages 99–113 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

- Muleba, N., and H.C. Ezumah. 1985. Optimizing cultural practices for cowpea in Africa. Pages 289–295 *in* Cowpea research production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons Ltd., Chichester, UK.
- Myaka, F.A. 1995. Effect of time of planting and planting pattern of different cowpea cultivars on yield of intercropped cowpea and maize in tropical sub-humid environment. *Tropical Science* 35: 274–279.
- Myaka, F.A. and J.C.B. Kabissa. 1996. Fitting short duration cowpea into a cotton-based cropping system in Tanzania: effect of planting pattern, time of planting cowpea, and insecticide application to the cotton. *Experimental Agriculture* 32: 225–230.
- Natarajan, M. and D.M. Naik. 1992. Competitive effects of a short duration bush type cowpea when intercropped with cotton in Zimbabwe. *Experimental Agriculture* 28: 409–416.
- Nelson S.C. and R.H. Robichaux. 1997. Identifying plant architectural traits associated with yield under intercropping: implications of genotype–cropping system interactions. *Plant Breeding* 16: 163–170.
- Ntare, B.R. 1990. Intercropping morphologically different cowpea with pearl millet in short season environment in the Sahel. *Experimental Agriculture* 26: 41–47.
- Ntare, B.R. and J.H. Williams. 1992. Response of cowpea cultivars to planting pattern and date of sowing in intercrops with pearl millet in Niger. *Experimental Agriculture* 28: 41–48.
- Obuo, J.E., E. Adipala, and D.S.O. Osiru. 1998. Effect of plant spacing on yield of cowpea–sorghum intercrop. *Tropical Science* 38: 67–73.
- Odo, P.E. and A.T.S. Bibinu. 1998. Effects of sowing date and planting pattern in millet/legume mixtures. Pages 114–119 *in* Pearl millet in Nigerian agriculture: production, utilization, and research priorities, edited by A.M. Emechebe, M.C. Ikwele, O. Ajayi, M. Aminu-Kano, and A.B. Anaso. Lake Chad Research Institute, Maiduguri, Nigeria.
- Ofori, F. and W.R. Stern. 1987a. Cereal–legume intercropping systems. *Advances in Agronomy* 41: 41–90.
- Ofori, F. and W.R. Stern. 1987b. Relative sowing time and density of component crops in maize–cowpea intercrop system. *Experimental Agriculture* 23: 41–52.
- Okoli, O.O., M.A. Hossain, A.F.K. Kissiedu, and A. Asare-Bediako. 1996. Effect of planting dates and growth habits of cassava and cowpea on their yield and compatibility. *Tropical Agriculture* 73: 169–174.
- Olufajo, O.O. 1988. Effects of component populations on the productivity of maize–cowpea intercrop. *Nigerian Agricultural Journal* 23: 25–34.
- Olufajo, O.O., A.O. Ogungbile, and B. Ahmed. 1997. On-farm testing of variety and NPK fertilization for maize–cowpea mixture in the Nigerian savanna. Pages 235–246 *in* Technology options for sustainable agriculture in sub-Saharan Africa, edited by T. Bezuneh, A.M. Emechebe, J. Sedgo, and M. Ouedraogo. Semi-Arid Food Grain Research and Development Agency (SAFGRAD) of the Scientific, Technical, and Research Commission of OAU, Ouagadougou, Burkina Faso.
- Reddy, K.C. and P.L. Visser. 1997. Cowpea intercrop growth and yield as affected by time of planting relative to millet. *African Crop Science Journal* 5: 351–357.
- Shetty, S.V.R., B.R. Ntare, A. Bationo, and C. Renard. 1995. Millet and cowpea in mixed farming systems of the Sahel: A review of strategies for increased productivity and sustainability. Pages 293–303 *in* Livestock and sustainable nutrient cycling in mixed farming systems of sub-Saharan Africa, edited by J.M. Powell, S. Fernández-Rivera, and T.O. Williams. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA)

- and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Singh, B.B. and A.M. Emechebe. 1998. Increasing productivity of millet–cowpea intercropping systems. Pages 68–75 *in* Pearl millet in Nigerian agriculture: production, utilization and research priorities, edited by A.M. Emechebe, M.C. Ikwele, O. Ajayi, M. Aminu-Kano, and A.B. Anaso. Lake Chad Research Institute, Maiduguri, Nigeria.
- Singh, B.B. and H.A. Ajeigbe. 2002. Improving cowpea–cereals-based cropping systems in the dry savannas of West Africa. Pages 276–284 *in* Challenges and opportunities for enhancing sustainable cowpea production, edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. IITA, Ibadan, Nigeria.
- Singh, B.B., J.D. Ehlers, B. Sharma, and F.R. Freire Filho. Recent progress in cowpea breeding. 2002. Pages 22–40 *in* Challenges and opportunities for enhancing sustainable cowpea production, edited by C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa, and M. Tamò. IITA, Ibadan, Nigeria.
- Subbarao, G.V., C. Renard, A. Bationo, N. van-Duivenbooden, and C. Biielders. 1999. Alternative technologies for Sahelian crop production systems in West Africa. Pages 121–132 *in* Recent advances in management of arid ecosystem, edited by A.S. Faroda, N.L. Joshi, and S. Kattju. Arid Zone Research Association of India, Jodhpur, India.
- Terao, T., I. Watanabe, R. Matsunaga, S. Hakoyama, and B.B. Singh. 1997. Agrophysiological constraints in intercropped cowpea: an analysis. Pages 129–140 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria.
- van Ek, G.A., J. Henriët, S.F. Blade, and B.B. Singh. 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria. II. Management and productivity of major cropping systems. *Samaru Journal of Agricultural Research* 14: 47–60.
- Willey, R.W. 1979. Intercropping—its importance and research needs. Part 1. Competition and yield advantages. *Field Crop Abstracts* 32: 1–10.
- Willey, R.W. and D.S.O. Osiru. 1972. Studies on mixtures of maize and beans (*Phaseolus vulgaris*) with particular reference to plant population. *Journal of Agricultural Science (Cambridge)* 79: 519–529.

# Improving cowpea–cereals based cropping systems in the dry savannas of West Africa

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## Abstract

Most of the farmers in the dry savannas of West Africa plant local varieties of cowpea, millet, sorghum, and groundnut in various intercropping systems with little or no purchased inputs. In this system, the cowpea and groundnut yields are low due to shading by cereals and lack of plant protection measures. The cereal yields are low mainly due to lack of fertilizer. Efforts are being made, therefore, to develop a combination of improved varieties and improved cropping systems for higher productivity and profitability with a minimum use of insecticides and fertilizers. We evaluated four cereal–cowpea intercropping row arrangements involving one cereal : one cowpea, one cereal : four cowpea, two cereal : four cowpea intercrops, and sole crops of improved and local varieties of millet, cowpea, and sorghum with selective application of two sprays of insecticides on cowpea only and 100 kg/ha fertilizer (N.P.K.15:15:15) basal and 20 kgN/ha top-dressed to cereals only. The results indicated sole crop improved cowpea to be most profitable followed by the two cereal : four cowpea intercrop system. Farmer participatory evaluation of the improved intercrop system involving two rows of sorghum : four rows of improved cowpea with inputs as indicated above, gave 100 to 300% gross economic superiority over the traditional intercropping systems. Smalholder farmers prefer the improved intercropping system over sole crops because it provides them with sufficient sorghum and cowpea for home use and additional cowpea for cash income.

## Introduction

Crop production in West and Central Africa is still based on traditional intercropping systems which may be quite diverse and complex (Norman 1974; Mortimore et al. 1997). In the Sudan savanna and the Sahelian zones, these systems involve intercropping of sorghum, millet, cowpea, and groundnut in various spatial and temporal arrangements and have evolved over centuries of experience to ensure maximum use of rainfall and available resources for sustainable production of food and fodder. The cereals are the staple diet, complemented with cowpea as a source of protein. Most of the groundnut and some cowpea are sold for cash. Cereal stovers are used as building material, for fencing, fodder, and fuel but the haulms of cowpea and groundnut are always used as fodder, being the most valuable source of livestock feed during the dry season.

The cropping systems depend on a number of factors, including local traditions, level of technology, resource availability, and physical environment. The general objective of farmers is a sustained production (Baker and Norman 1975) of reasonable levels, at minimal risk, to satisfy subsistence and commercial needs (Beets 1990). These needs have increased due to the rise in population and consequent reduction in arable land on

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a per capita basis. Therefore, an important approach to increase agricultural production would be through improving the yield of individual crops per unit area. Farmers with few resources at their disposal have a limited capacity to tolerate production failure. They attach a risk factor to their assessment of agricultural innovations, preferring incremental changes to radical departures from existing practices (Edwards 1993). Therefore, research must build on these farming practices and aim at risk-free increases in productivity even if these have to be gradual.

Cowpea is an integral component of the traditional cropping systems due to its beneficial effect on sustainability and as a source of nutritious food and fodder (Henriet et al. 1997). The International Institute of Tropical Agriculture (IITA) with a global mandate for cowpea has been working on the improvement of cowpea varieties as well as the improvement of cropping systems to increase total productivity with limited use of purchased inputs. The strategy has been to study the role of cowpea in major cropping systems, identify the production constraints in traditional systems, and then develop improved cowpea varieties and improved systems (Singh 1993; 1994).

A quantitative assessment of the traditional cropping system revealed 22 types of crop mixtures in northern Nigeria of which millet–cowpea, sorghum–cowpea, millet–sorghum–cowpea, and millet–sorghum–groundnut–cowpea were predominant (Henriet et al. 1997). The mean grain yields in these systems ranged from 0 to 132 kg/ha for cowpea, 0 to 197 kg/ha for groundnut, 131 to 2600 kg/ha for millet, and 0 to 4903 kg/ha for sorghum, depending on the fertility level of the fields (van Ek et al. 1997). The major production constraints in the intercropping system were low fertility, low population, lack of fertilizer and pesticides, shading of cowpea and groundnut by the millet and sorghum, as well as late maturity and poor yield potential of local varieties. Efforts are being made, therefore, to develop a combination of improved varieties and improved cropping systems for higher productivity and profitability with limited use of insecticides and fertilizers. This paper describes improved varieties and optimum dates of planting for intercropping and the superiority of an improved strip cropping system that maximizes the benefits of limited fertilizers and pesticides, and minimizes competition between cereals and legumes.

### **Improving productivity of traditional intercropping system**

Traditional intercropping involves planting cowpea with millet and/or sorghum in a one row cereal : one cowpea row arrangement. Also, the cereals are planted at the onset of rains and cowpea is planted three to four weeks later between cereal rows when the rains have stabilized. Thus, cowpea is shaded by the cereals throughout the growing period. This causes severe reduction of shoot as well as root growth of cowpea resulting in very low grain and fodder yields. Recent studies have shown that even though cowpea occupies 50% of land area under intercropping, its grain and fodder yields are between 10 to 20% of those of sole crop cowpea (Singh et al. 1997; Terao et al. 1997). Even though sole crop cowpea is most profitable, most subsistence farmers plant cowpea as an intercrop with millet and sorghum. This is primarily because the land is limited and they want to produce a sufficient quantity of cereals for home consumption; and partly because sole crop cowpea requires one or two sprays of insecticide to control recalcitrant insect pests such as *Maruca* and pod bugs; furthermore, chemicals are often not available even if farmers have financial resources. Efforts are, therefore, being made to develop shade-tolerant cowpea varieties with resistance to diseases, insect pests, and parasitic weeds, giving grain and

fodder yields under intercropping with millet and sorghum. The general approach is to screen all the new breeding lines under intercropping with millet and sorghum, improve selected local varieties by defect elimination using the backcross method, and develop new improved cowpea varieties specifically adapted to intercropping without insecticide application.

### Improved cowpea varieties for intercropping

Selected improved cowpea breeding lines were screened under traditional one : one intercrop with millet without insecticide. The millet rows were 2 m apart and planted about three weeks before cowpea to reflect the actual farmers' practice. The improved varieties performed significantly better than the local variety, Dan Ila, under sole crop as well as intercrop (Table 1). However, grain yields under intercrop were less than 10% of the sole crop and fodder yields ranged from 10 to 20% even though expected yields of cowpea are 50% of the sole crop. This is primarily because millet grows faster, shades cowpea, and competes for nutrients and water, thereby reducing cowpea grain and fodder yields. Thus, the traditional intercropping of one row cereal : one row cowpea is less productive for cowpea even though the yields of improved varieties are three to four times higher than of the local variety.

The promising lines selected for good performance under intercropping in 1999 were separately evaluated with maize and sorghum at Samaru in 2000. IT95K-193-12 and IT95K-222-3 gave the highest grain yield followed by others with an average ranging from 300 kg to 500 kg/ha, compared to zero yield of the local varieties (Table 2).

**Table 1. Performance of promising cowpea breeding lines under sole crop and intercrop with millet.**

| Variety                                      | — Grain yield (kg/ha) — |           | — Fodder yield (kg/ha) — |           |
|--|-------------------------|-----------|--------------------------|-----------|
|  | Sole crop               | Intercrop | Sole crop                | Intercrop |
| <b>Extra-early maturing cowpea varieties</b> |                         |           |                          |           |
| IT95K-627-34                                 | 2335                    | 237       | 1662                     | 458       |
| IT98K-463-7                                  | 1968                    | 134       | 612                      | 208       |
| IT98K-205-8                                  | 2041                    | 120       | 1422                     | 146       |
| Dan Ila                                      | 1694                    | 82        | 2188                     | 677       |
| SED  | 349                     | 31        | 267                      | 73        |
| <b>Early-maturing cowpea varieties</b>       |                         |           |                          |           |
| IT97K-499-39                                 | 2715                    | 177       | 1406                     | 125       |
| IT97K-508-2                                  | 2265                    | 182       | 2074                     | 396       |
| IT97K-608-14                                 | 2357                    | 134       | 1102                     | 448       |
| Dan Ila                                      | 1823                    | 41        | 2104                     | 363       |
| SED  | 258                     | 29        | 321                      | 89        |
| <b>Medium-maturing cowpea varieties</b>      |                         |           |                          |           |
| IT95K-193-12                                 | 2381                    | 277       | 1308                     | 417       |
| IT98K-131-1                                  | 2409                    | 233       | 2689                     | 573       |
| IT98K-494-3                                  | 2079                    | 175       | 1982                     | 365       |
| Dan Ila                                      | 846                     | 58        | 1091                     | 406       |
| SED  | 237                     | 49        | 343                      | 106       |

In another trial at Minjibir, selected promising varieties were evaluated under intercropping with local and improved varieties of millet and sorghum to ascertain whether improved varieties of cereal would cause less competition with cowpea. The improved varieties yielded higher than the local landrace with almost 200 to 300% superiority in grain yield (Table 3). The mean grain yield of cowpea varieties was similar under improved and local sorghum but it was consistently less under local millet compared to the improved millet although the differences were not significant.

These results indicated that the improved cowpea varieties are more productive than the local variety under millet, sorghum, and maize intercropping.

## Effect of date of planting cowpea as intercrop in millet

As indicated earlier, under traditional intercropping, farmers normally plant millet first at the onset of rains in the beginning of June; about three weeks later, they plant cowpea as an intercrop. This causes shading of cowpea by the fast growing millet. An experiment was, therefore conducted to assess the effect of different dates of planting cowpea as an intercrop in millet. The treatments included planting of cowpea simultaneously with millet, and at three, six, and nine weeks after millet. There was a significant genotype  $\times$  date of planting interaction (Table 4). The early- and medium-maturing cowpea varieties such as IT93K-452-1, IT90K-277-2, and Dan Ila had highest grain and fodder yields when

**Table 2. Performance of improved cowpea varieties under intercrop at Samaru, 2000.**

| Variety       | Grain yield (kg/ha) |       |        |         |
|---------------|---------------------|-------|--------|---------|
|               | Cowpea              | Maize | Cowpea | Sorghum |
| IT95K-193-12  | 571                 | 5574  | 365    | 1950    |
| IT95K-222-3   | 414                 | 3193  | 420    | 3042    |
| IT97K-1129-51 | 365                 | 3842  | 425    | 2175    |
| IT98K-279-2   | 403                 | 2651  | 362    | 3240    |
| IT97K-207-21  | 325                 | 2137  | 375    | 3519    |
| IT97K-461-4   | 277                 | 2315  | 396    | 3300    |
| IT95K-627-34  | 303                 | 3900  | 305    | 1693    |
| IT97K-499-39  | 232                 | 3411  | 385    | 2165    |
| IT97K-499-38  | 282                 | 2937  | 298    | 2386    |
| IT97K-819-118 | 362                 | 2758  | 179    | 1981    |
| Dan Ila       | 0                   | 1647  | 0      | 2064    |
| Aloka         | 0                   | 1656  | 0      | 2150    |
| SED           | 99                  | 1073  | 26     | 373     |

**Table 3. Performance of promising cowpea varieties for intercropping with millet and sorghum (ICSV-111) at Minjibir, 2000.**

| Variety      | — Cowpea grain yield (kg/ha) — |      |        |       | — Cowpea fodder yield (kg/ha) — |      |        |       |
|--------------|--------------------------------|------|--------|-------|---------------------------------|------|--------|-------|
|              | Sorghum                        |      | Millet |       | Sorghum                         |      | Millet |       |
|              | Local                          | ICSV | Local  | Sosat | Local                           | ICSV | Local  | Sosat |
| IT95K-231-1  | 335                            | 353  | 214    | 261   | 417                             | 567  | 317    | 283   |
| IT97K-356-1  | 320                            | 434  | 194    | 200   | 350                             | 367  | 317    | 217   |
| IT97K-608-14 | 392                            | 245  | 171    | 178   | 517                             | 583  | 250    | 267   |
| IT97K-499-39 | 349                            | 306  | 148    | 167   | 200                             | 367  | 183    | 200   |
| IT97K-207-21 | 253                            | 375  | 139    | 143   | 350                             | 357  | 317    | 217   |
| Dan Ila      | 105                            | 126  | 120    | 155   | 417                             | 500  | 350    | 283   |



simultaneously planted with millet, whereas the late-maturing varieties IT89KD-288 and IT89KD-349 had highest yields when planted three weeks after millet. However, all the varieties showed drastic yield reduction when planted six and nine weeks after millet. This may be due to severe shading and competition for nutrients in later plantings, as the millet already had well developed stem and root systems. The results confirm earlier observations and suggest that for maximum yield under intercrop, cowpea should be planted as soon as millet has been sown in wider rows to reduce shading.

### Improved strip cropping system for higher productivity

Since the overall productivity under traditional intercropping is very low, due to shading and severe competition for nutrients, efforts were made to develop alternative systems which will minimize shading and maximize gains from limited application of fertilizer and agrochemicals. Among several systems evaluated, a strip cropping system involving two rows of densely planted cereal : four rows of densely planted cowpea appeared to be significantly more productive, particularly when limited amounts of fertilizer was applied to the cereal and one or two sprays were given to cowpea. Figure 1 illustrates this system.

Productivity of different cropping systems was tested on-station involving improved cowpea varieties in sole crop and intercrop systems using one row of millet : one row of cowpea; and two rows of millet : four rows of cowpea with a minimum basal application of 15 kgN; 15 kg P<sub>2</sub>O<sub>5</sub>, and 15 kg K<sub>2</sub>O, top dressing of the cereals only at the rate of 30 kg/N/ha. The cowpea was sprayed with insecticide twice—at flowering and at full podding. The performance of the improved cowpea variety was superior to the local cowpea in sole crop as well as in two : four intercrop system and similar to local one : one intercrop system (Table 5). However, the gross economic value of the two : four system was significantly higher than the one : one system and very close to that of the sole crop cowpea. The improved cowpea varieties IT89KD-391 and IT90K-277-2 appeared to be most promising for the two : four system. During farmer field days, farmers showed great interest in the two : four system because it provides them with sufficient millet for home consumption and a large amount of additional cowpea, part of which can be used as nutritious food at home and part can be sold for cash.

**Table 4. Grain and fodder yields of different cowpea varieties planted as intercrop at different dates in millet field.**

| Cowpea variety | Yield (kg/ha) under intercrop with millet |        |         |        |         |        |         |        |
|----------------|---|--------|---------|--------|---------|--------|---------|--------|
|                | Simultaneous                              |        | 3 weeks |        | 6 weeks |        | 9 weeks |        |
|                | Grain                                     | Fodder | Grain   | Fodder | Grain   | Fodder | Grain   | Fodder |
| IT90K-277-2    | 214                                       | 491    | 94      | 175    | 35      | 75     | 4       | 33     |
| IT93K-452-1    | 200                                       | 342    | 43      | 125    | 31      | 58     | 7       | 20     |
| Dan Ila        | 199                                       | 625    | 132     | 175    | 20      | 133    | 28      | 45     |
| IT89KD-349     | 109                                       | 650    | 153     | 242    | 43      | 117    | 7       | 38     |
| IT89KD-288     | 75  | 600    | 107     | 267    | 17      | 142    | 45      | 35     |
| SED            | 35  | 51     | 35      | 51     | 35      | 51     | 35      | 35     |

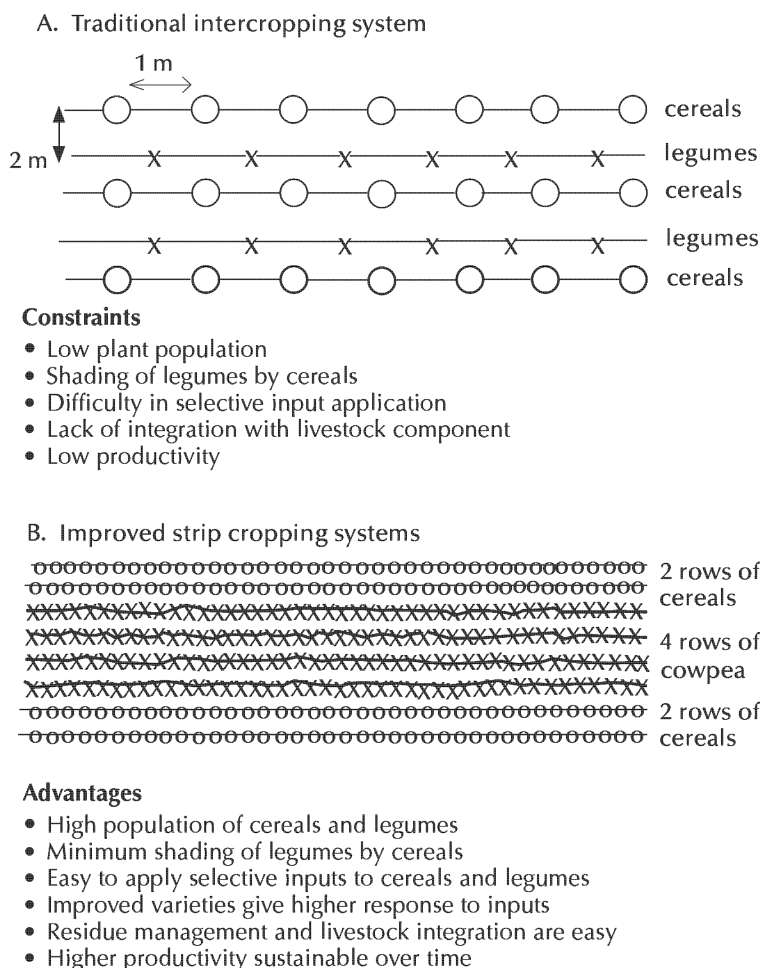


Figure 1. Field planting patterns of traditional and improved strip cropping systems.

Table 5. Relative productivity of different cropping systems with a range of improved cowpea varieties.

| Cowpea variety  | Grain yield in different cropping systems |                     |                     |       |       | Naira value of grain <sup>†</sup> |        |        |
|-----------------|---|---------------------|---------------------|-------|-------|-----------------------------------|--------|--------|
|                 | Sole cowpea                               | 1 millet : 1 cowpea | 2 millet : 4 cowpea |       |       | Sole cowpea                       | 1 : 1  | 2 : 4  |
| IT89KD-391      | 1504a                                     | 2282a               | 237a                | 1763a | 1198a | 52,640                            | 35,679 | 63,086 |
| IT90K-277-2     | 1817b                                     | 2473a               | 240a                | 1731a | 1080a | 63,595                            | 38,076 | 58,592 |
| IT89KD-349      | 2095b                                     | 2192a               | 267a                | 1539a | 1032a | 73,325                            | 35,649 | 54,588 |
| Dan Ila (Local) | 1319a                                     | 2305a               | 205a                | 1547a | 681b  | 46,165                            | 34,835 | 42,399 |

<sup>†</sup>Cowpea grain @ ₦35/kg and millet grain @ ₦12/kg.

a, b = Values with same letters are not significant.

## Screening millet and sorghum varieties for higher productivity under two : four strip cropping system

In order to select the best sorghum and millet varieties for strip cropping with cowpea, one local and one improved sorghum variety, and one local and one improved millet variety were strip cropped with the best improved cowpea variety IT90K-277-2 using two cereal rows : four cowpea rows arrangement. Sole crop plots of all the varieties were also planted for comparing the relative superiority of the strip cropping. The data indicated superiority of strip cropping over sole crops of sorghum and millet as evidenced by land equivalent ratios (LER) greater than one in most cases (Table 6). The best combination was two rows of Kaura sorghum : four rows of IT90K-277-2 cowpea with a gross return/ha of ₦60 406 compared to ₦49 289 for sole cowpea and ₦48 145 for sole Kaura sorghum.

In another experiment, two varieties of sorghum and two varieties of cowpea were evaluated under strip cropping involving two rows of sorghum : four rows of cowpea in order to identify the best combination for higher productivity. The improved cowpea variety IT90K-277-2 was superior to the local variety Dan Ila, but the local sorghum variety Kaura was superior to the improved sorghum variety ICSV-111 (Table 7). The best strip combination was two rows of Kaura sorghum : four rows of IT90K-277-2. Both combinations involving the improved cowpea were superior to the one involving the

**Table 6. Evaluation of different cereals in strip cropping with improved cowpea planted.**

| Variety                  | Cowpea |        | Cereal |       | LER   |        | Naira <sup>†</sup><br>equivalent |
|--------------------------|--------|--------|--------|-------|-------|--------|----------------------------------|
|                          | Grain  | Fodder | Grain  | Stalk | Grain | Fodder |                                  |
| IT90-277-2 (sole cowpea) | 1211   | 863    | 0      | 0     | —     | —      | 49,289                           |
| Farfara (sole sorghum)   | 0      | 0      | 1877   | 11513 | —     | —      | 34,037                           |
| Kaura (sole sorghum)     | 0      | 0      | 2846   | 13993 | 0     | 0      | 48,145                           |
| ICSV-111 (sole sorghum)  | 0      | 0      | 808    | 2770  | —     | —      | 12,466                           |
| L. millet (sole)         | 0      | 0      | 1245   | 5272  | 0     | 0      | 20,212                           |
| Sosat (sole millet)      | 0      | 0      | 2163   | 7919  | 0     | —      | 33,875                           |
| Farfara : 277-2 (2:4)    | 810    | 477    | 843    | 3502  | 1.1   | 0.86   | 45,784                           |
| Kaura : 277-2 (2:4)      | 846    | 472    | 1846   | 4868  | 1.35  | 0.89   | 60,406                           |
| ICSV-111 : 277-2 (2:4)   | 709    | 427    | 342    | 1879  | 1.0   | 1.17   | 34,214                           |
| L. millet : 277-2 (2:4)  | 707    | 455    | 931    | 3083  | 1.33  | 1.11   | 42,640                           |
| Sosat : 277-2 (2:4)      | 804    | 466    | 998    | 3028  | 1.13  | 0.92   | 46,876                           |
| SED                      | 135    | 155    | 323    | 1465  |       |        |                                  |

<sup>†</sup>Cowpea grain @ ₦35/kg, cowpea fodder @ ₦8/kg, millet and sorghum grain @ ₦12/kg; millet and sorghum fodder @ ₦1/kg; LER = land equivalent ratio, L = local.

**Table 7. Evaluation of cowpea and sorghum varieties for grain and fodder production under strip cropping.**

| Crop mixture (2 : 4)<br>Sorghum variety | Cowpea<br>Variety | — Cowpea — |        | — Cereal — |       | Naira<br>value |
|---|-------------------|------------|--------|------------|-------|----------------|
|   |                   | Grain      | Fodder | Grain      | Stalk |                |
| ICSV-111                                | IT90K-277-2       | 713        | 1916   | 1207       | 2623  | 57,390         |
| Kaura                                   | IT90K-277-2       | 507        | 2178   | 1581       | 3508  | 57,649         |
| ICSV-111                                | Dan Ila           | 462        | 509    | 849        | 2507  | 32,937         |
| Kaura                                   | Dan Ila           | 439        | 922    | 1648       | 3022  | 45,539         |
| SED                                     |                   | 42         | 263    | 113        | 261   |                |

local cowpea Dan Ila. These results support the earlier observation that strip cropping involving improved cowpea has higher production. The strip cropping involving two cereal rows : four cowpea rows combination also permits selective application of fertilizer only on cereals and pesticide only on cowpea, thereby increasing total productivity with limited application of inputs.

This system was tested on farmers' fields at three locations. The results indicated the clear superiority of the two rows cereal : four rows improved cowpea over the traditional practice of one row cereal : one row cowpea (Fig. 2). The gross return from the two : four system was 50 to 300% higher than from the traditional intercropping. This has attracted the attention of a large number of farmers; over 500 farmers adopted this system in the 2000 crop season.



**Figure 2.** Traditional sorghum–cowpea intercrop (above) and improved strip cropping (below).

## Conclusion

Results of cropping systems trials have shown that the sole crop cowpea is most profitable and the strip cropping involving two rows cereals : four rows cowpea is the next best in terms of economic productivity. The two rows of cereal : four rows of cowpea combination is preferred by farmers because they need sorghum and millet for home consumption and cowpea for home consumption as well as for cash. This system may also be more suitable and help maintain soil fertility because two-thirds of the area is legume and only one-third is cereal. Cowpea also causes suicidal germination of *Striga hermonthica* and reduces the seedbank, thereby reducing parasitization of sorghum and millet by *Striga*. The increased production of cowpea haulms helps feed more livestock during the dry season, leading to greater crop–livestock integration.

## References

- Baker, E.F.I. and D.W. Norman. 1975. Cropping systems in northern Nigeria. IAR, Samaru, Nigeria.
- Beets, W.C. 1990. Raising and sustaining productivity of smallholder farming systems in the tropics. AgBe Publishing, Singapore.
- Edwards, R. 1993. Traditional farming systems and farming systems research. Pages 95–108 in *Dryland farming in Africa*, edited by J.R.J. Rowland. The Macmillan Press Ltd, Hong Kong.
- Henriet, J., G.A. van Ek, S.F. Blade, and B.B. Singh. 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria I. Rapid survey of prevalent cropping system. *Samaru Journal of Agricultural Research* 14: 27–45.
- Mortimore, M.J., B.B. Singh, F. Harris, and S.F. Blade. 1997. Cowpea in traditional cropping systems. Pages 99–113 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Norman, D.W. 1974. Rationalizing mixed cropping under indigenous conditions: the example of northern Nigeria. *Journal of Development Studies* 11: 3–21.
- Singh, B.B. 1993. Cowpea breeding. Archival Report 1998–1992. Grain Legume Improvement Program, Crop Improvement Division, IITA, Ibadan, Nigeria.
- Singh, B.B. 1994. Breeding suitable cowpea varieties for West and Central African savanna. Pages 77–87 in *Progress in food grain research and production in semiarid Africa*, edited by J.M. Menyonga, Taye Bezuneh, J.Y. Yayock, and I. Soumana. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K. Dashiell and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Terao, T.I., R. Watanabe, Matsunagas Hakoyama, and B.B. Singh. 1997. Agrophysiological constraints in intercrop cowpea: an analysis. Pages 129–140 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- van Ek, G.A., J. Henriet, S.F. Blade, and B.B. Singh. 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria II. Management of productivity of major cropping system. *Samaru Journal of Agricultural Research* 14: 47–60.

## Cowpea varieties for drought tolerance

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### Abstract

Success in breeding for cowpea with drought tolerance has not been as pronounced as for many other traits. This is partly due to lack of simple, cheap, and reliable screening methods to select drought-tolerant plants and progenies from the segregating populations and partly due to the complexity of factors involved in drought tolerance. Measuring drought tolerance using physiological parameters is expensive, time consuming, and difficult to use for screening large numbers of lines and segregating populations. Since several factors and mechanisms (in shoots and roots) operate to enable plants cope with drought stress, drought tolerance appears as a complex trait. However, if these factors and mechanisms can be separated and investigated individually, they may be easier to manipulate by breeders. We have developed a simple “box screening method” for shoot drought tolerance in cowpea, which eliminates the effects of roots and permits nondestructive visual identification of shoot dehydration tolerance. Alongside this, we have also developed a “root-box pin-board” method to study the two-dimensional root architecture of individual plants. Using these methods, we have identified two mechanisms of shoot drought tolerance in cowpea, which are controlled by single dominant genes as well as major differences for root architecture among cowpea varieties. Combining deep and dense root systems with shoot dehydration tolerance offers the opportunity to breed and select for highly drought-tolerant plants.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is widely grown in the semiarid tropics where drought is a major production constraint (Ashley 1993; Singh 1994; Singh et al. 1997). Due to the erratic rainfall in the beginning and towards the end of the rainy season, crops are often subjected to drought stress in both seedling and terminal growth stages, which causes substantial reduction in grain yield as well as biomass production. Early-maturing varieties escape terminal drought (Singh 1987, 1994) but if exposed to intermittent moisture stress during the vegetative or reproductive stages, they perform very poorly. Cowpea is inherently more drought tolerant than other crops but it still suffers considerable damage due to frequent drought in the Sahelian region where rainfall is scanty and irregular (Singh et al. 1999a). Therefore, efforts are being made to breed cowpea varieties with enhanced drought tolerance.

Recent reviews (Ashley 1993; Subbarao et al. 1995; Boyer 1996) have brought together the available knowledge on different aspects of drought tolerance in crop plants and options to minimize yield losses due to drought. Major differences among and within crop species have been reported and different strategies to breed drought-tolerant varieties have been

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suggested (Blum 1985; Walker and Miller 1986; Arrau deau 1989; Acevedo and Ceccarelli 1989). However, the success in breeding for drought tolerance has not been as pronounced as for other traits. This is partly due to lack of simple, cheap, and reliable screening methods to select drought-tolerant plants and progenies from the segregating populations, and partly due to the complexity of factors involved in drought tolerance.

Several methods have been used to estimate drought tolerance and water-use efficiency. Typically, these involve measurement of water potential, relative turgidity, diffusion pressure deficit, chlorophyll stability index, and carbon isotope discrimination (Bates et al. 1973; Turk and Hall 1980; Morgan 1984; Yadava and Patil 1984; Hall et al. 1990, 1997). However, most of these methods are expensive and time consuming and are therefore not very efficient or practical for screening large numbers of plants in segregating populations. Furthermore, screening under field conditions is not always possible because of the unpredictability and the variable intensity of drought stress. Also, screening for drought tolerance in the off-season using controlled watering is often not relevant to the environment of the real growing season, particularly when temperature and photosensitivity play important roles in crop growth and productivity. Most studies in the past have dealt with screening for drought tolerance as a whole and not individual components involved in drought tolerance (Lawan 1983, Watanabe et al. 1997). This could also contribute to variable results, depending on which factors were operational during screening.

Traditionally, drought tolerance is defined as the ability of plants to live, grow, and yield satisfactorily with limited soil water supply or under periodic water deficiencies (Ashley 1993). Since several factors and mechanisms (in shoots and roots) operate independently or jointly to enable plants to cope with drought stress, drought tolerance appears as a complex trait (Krishnamurthy et al. 1996). However, if the factors and mechanisms contributing to drought tolerance can be separated and studied individually, the components leading to drought tolerance will appear less complex and may be easier to manipulate by breeders. Breeding for early maturity, photosensitivity, indeterminacy, epicuticular wax, pubescence, and awns, which indirectly affect the ability of plants to cope with drought is easy because these traits are inherited and can easily be screened and incorporated in improved varieties as indicated above. For other traits such as osmotic adjustment and stomatal regulations, which directly control the drought tolerance of plants, the ideal approach would be to study the shoot drought tolerance and root characteristics separately and identify gene(s) responsible for stomatal behavior, osmotic adjustment, and root architecture, and combine them in improved varieties. This paper reviews the recent progress made in breeding cowpea for drought tolerance involving simple screening methods for shoot drought tolerance (using an example with 12 varieties), and describes a new method for studying root characteristics as part of a simplified approach to breeding for drought tolerance.

### **Box screening for shoot dehydration tolerance**

Singh et al. (1999a) described a simple wooden box screening method—showing good correlation with drought tolerance at vegetative and reproductive stages—to select drought-tolerant plants or progenies in cowpea at the seedling stage. This method is briefly described here.

Wooden boxes of 130 cm length, 65 cm width, and 15 cm depth made of 2.5 cm thick planks were kept on benches in a rain-protected screenhouse. The boxes were lined with polyethylene sheets and filled with a one : one mixture of topsoil and sand which averaged

about 7.5% clay, 84% sand, 8.5% silt, and 0.8% organic matter. The boxes were filled to 12 cm depth, leaving about 3 cm space on the top for watering. The polyethylene lining along the sides and bottoms of the boxes ensured even distribution of water. A spirit level was used to ensure a flat soil surface on the boxes after watering. Equidistant holes were made in straight rows 10 cm apart with a hill-to-hill distance of 5 cm within the rows. A specially made wooden guide was pushed in the soil up to a stopper (2 cm from its bottom end) in order to make holes uniformly at 2 cm depth. Two handpicked healthy seeds were sown in each hole and after germination, thinned to one plant per hill. Each box contained one row of each of 12 cowpea varieties with 12 plants and constituted one replication. Treatments were arranged in three randomized complete blocks. The boxes were watered daily using a small watering can until the partial emergence of the first trifoliate leaf, after which watering was stopped. Thereafter, wilted plants in each variety were counted daily until all the plants of the susceptible lines appeared dead. Watering was then resumed to ascertain regeneration percentages for each variety. Based on the days taken to wilting and percentage, recovery, the varieties were rated as drought tolerant or susceptible.

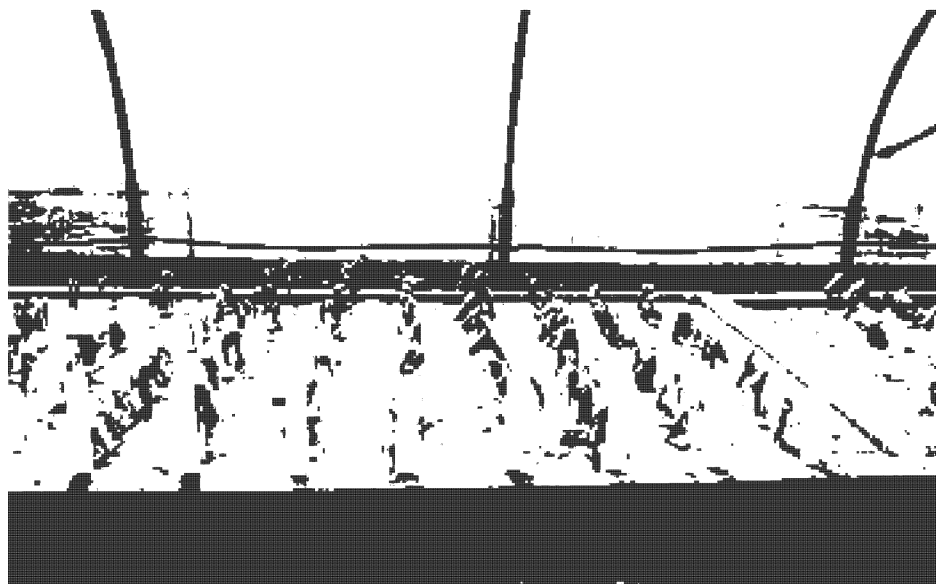
Seed germination and initial growth of plants of all 12 varieties were normal. About seven days after the termination of watering, stress effects started appearing in the seedlings of susceptible varieties, and differences among varieties became visible and progressively more pronounced with advancing days of moisture stress. The stress effects were first seen on the unifoliate leaves which became wilted, followed by the emerging trifoliate, and finally the growing tip dried. The most susceptible lines were TVu 8256 and TVu 7778, which showed wilting before other lines (Table 1). Interestingly, the unifoliate leaves of TVu 7778 turned deep yellow in response to moisture stress and then dried, whereas TVu 8256 and other varieties showed different shades of yellow, brown, and green. The differences among varieties with respect to drought tolerance were very clear (Fig. 1). The data on wilting percentage at different days after termination of watering indicated TVu 8256 and TVu 7778 to be the most susceptible to drought (Table 1) and

**Table 1. Relative drought tolerance of different cowpea varieties<sup>†</sup>.**

| Cowpea variety | Percentage wilting at days (D) after<br>termination of watering |     |     |     |     | Percentage recovery<br>after rewatering |
|----------------|---|-----|-----|-----|-----|---|
|                | D8  | D10 | D12 | D14 | D15 |   |
| IT90K-59-2     | 0   | 11  | 28  | 67  | 78  | 61                                      |
| Dan Ila        | 0   | 23  | 36  | 49  | 73  | 72                                      |
| TVu 11986      | 6   | 42  | 68  | 74  | 86  | 8                                       |
| TVu 13464      | 10  | 31  | 51  | 61  | 86  | 55                                      |
| Kanannado      | 11  | 29  | 29  | 29  | 47  | 89                                      |
| IT88D-867-11   | 11  | 51  | 69  | 69  | 76  | 72                                      |
| IT89KD-288     | 11  | 72  | 72  | 83  | 100 | 67                                      |
| TVu 11979      | 20  | 61  | 75  | 80  | 80  | 22                                      |
| TVu 12349      | 27  | 51  | 71  | 79  | 92  | 0                                       |
| TVu 12348      | 31  | 56  | 78  | 88  | 100 | 4                                       |
| TVu 8256       | 50  | 100 | 100 | 100 | 100 | 0                                       |
| TVu 7778       | 72  | 83  | 89  | 94  | 100 | 0                                       |
| LSD (5%)       | 31  | 39  | 33  | 29  | 30  | 30                                      |

<sup>†</sup> Source: Singh et al. 1999a.





**Figure 1. Box screening for drought tolerance.**

others to have different levels of drought tolerance. For example, on the eighth day after withholding water, 50% of TVu 8256 plants and 72% of TVu 7778 plants had wilted while IT90K-59-2 and Dan Ila had zero wilting and others ranged from 6 to 31%. On day 14, Kanannado had 29% wilting and Dan Ila had 49%, compared to 100% for TVu 8256 and 94% for TVu 7778. The wilting in other varieties ranged from 61 to 88%. The recovery percentage after rewatering ranged from 0% for TVu 8256, TVu 7778, and TVu 12349, to 89% for Kanannado, 72% for Dan Ila and IT88D-867-11. The recovery in other varieties ranged from 4 to 67%. The regenerated plants developed mainly from the growing tips as the unifoliate, and the first trifoliolate dried out during the moisture stress. Based on the actual counts of wilted plants, regenerated plants, and visual assessment on the row basis, Kanannado, Dan Ila, IT88D-867-11, and IT90K-59-2 were rated as highly drought tolerant; TVu 11986, TVu 13464, IT89KD-288, and TVu 11979 as moderately drought tolerant; TVu 12349 and TVu 12348 as slightly drought tolerant, and TVu 8256 and TVu 7778 as susceptible to drought.

### **Relationship between box screening and field performance**

To compare seedling screening for drought tolerance and field performance under drought stress, the same 12 varieties were planted in the field towards the end of the rainy season after which little or no rain was expected but there was adequate moisture for germination. The trial was planted in a randomized block design with three replications. Each plot consisted of four rows, which were 4 m long and 75 cm wide with a hill-to-hill distance of 20 cm within the rows and two plants/hill. Days to 50% flowering, days to 50% leaf senescence, pod yield, seed yield, and total biomass were recorded from the middle two rows of each plot. Observations were made on the degree of premature senescence due to drought stress.

The overall seed germination and initial growth of all the varieties were quite normal. Three light rains (17 mm, 20 mm, and 11.7 mm) were received within three weeks after planting and no rain thereafter. Stress symptoms started appearing about 50 days after planting when most of the varieties had flowered. Thus, the drought stress affected plant growth and development mostly at the reproductive stage. Premature senescence of leaves was noticed first in TVu 7778 and TVu 8256 with characteristic yellow coloration in TVu 7778. Both of these varieties showed 50% senescence at 60 days after planting whereas other varieties were still green and setting pods. The data on total biomass produced and pod and seed yields showed TVu 7778 and TVu 8256 to be most drought susceptible and TVu 12349, IT90K-59-2, and Kanannado to be most drought tolerant (Table 2, Fig. 2). Others were moderately tolerant to drought. Dan Ila showed less growth, probably because of its photosensitivity and short day lengths in October–November. Thus, there was a close correspondence between drought tolerance at the seedling and reproductive stages.

To further verify the results of box and field screening, five selected varieties representing different levels of drought tolerance and susceptibility were grown in plastic pots and subjected to drought stress at the onset of the reproductive stage. The results of pot screening reconfirmed the results of box screening and field screening. TVu 7778 and TVu 8256 were completely wilted 17 days after withholding water at the reproductive stage, whereas the plants of Dan Ila, TVu 11986, and TVu 12349 were still alive and showed only minor stress (Fig. 3).

The results of box screening indicated that varietal differences for plant responses to drought stress could be assessed at the seedling stage in cowpea. Also, the close correspondence between the results of seedling screening (box method), field screening, and pot screening further indicate that the phenomenon responsible for drought tolerance in the seedling stage is also manifested at the reproductive stage in cowpea. Therefore, screening cowpea varieties at the seedling stage appears to be a reliable method to identify drought-tolerant varieties. Since the results of the box screening, field screening, and pot screening are similar, box screening is more practical because of the ease of handling,

**Table 2. Performance of selected cowpea varieties in the field under drought stress at Minjibir, Nigeria<sup>†</sup>.**

| Cowpea variety | Days to |            | Total biomass (kg/ha) | Pod yield (kg/ha) | Seed yield (kg/ha) | Harvest index (%) |
|----------------|---------|------------|-----------------------|-------------------|--------------------|-------------------|
|                | flower  | senescence |                       |                   |                    |                   |
| TVu 12349      | 52      | 102        | 3248                  | 1371              | 847                | 26                |
| IT90K-59-2     | 44      | 75         | 2036                  | 1121              | 751                | 37                |
| Kanannado      | 47      | 77         | 2053                  | 1012              | 703                | 34                |
| TVu 11979      | 52      | 84         | 2267                  | 1042              | 653                | 29                |
| TVu 12348      | 48      | 80         | 3032                  | 761               | 455                | 30                |
| Dan Ila        | 46      | 72         | 1874                  | 841               | 586                | 31                |
| IT89KD-288     | 46      | 73         | 2472                  | 853               | 547                | 23                |
| IT88D-867-11   | 46      | 70         | 1737                  | 828               | 561                | 32                |
| TVu 13464      | 54      | 80         | 3032                  | 761               | 455                | 15                |
| TVu 11986      | 54      | 80         | 2331                  | 737               | 403                | 17                |
| TVu 7778       | 47      | 64         | 958                   | 195               | 111                | 12                |
| TVu 8256       | 44      | 60         | 1414                  | 168               | 84                 | 6                 |
| LSD (5%)       | 3       | 7          | 973                   | 381               | 271                | 12                |

<sup>†</sup>Source: Singh et al. 1999a.



**Figure 2.** Field screening for drought tolerance. The plants on the left are TVu 7778, (drought susceptible) those on the right, TVu 12349 (drought tolerant).



**Figure 3.** Pot screening for drought tolerance. Left to right: drought susceptible, drought tolerant, drought susceptible, and drought tolerant.

the possibility of using a controlled environment, and the ability to screen large numbers of lines or plants. Also, field screening for drought tolerance may be complicated due to differences in root length and architecture of the test materials. The shallow box method described here eliminates the effects of roots and thereby permits the identification of plants with shoot drought tolerance.

The box method is simple, nondestructive, and offers flexibility in terms of size of operation as boxes can be larger or smaller depending upon the need. The test materials can be homozygous lines or segregating populations and the drought-tolerant plants can be saved and transplanted for further progeny testing and selection.

### **Mechanisms of drought tolerance and its inheritance**

A close observation of cowpea plants and its inheritance in the boxes showed two types of drought-tolerance mechanisms (Mai-Kodomi et al. 1999a). Under drought stress, Type 1 drought-tolerant lines such as TVu 11986 and TVu 11979 stopped growth and conserved moisture in all the plant tissues, stayed alive for over two weeks, and gradually the entire plant parts dried. The Type 2 drought-tolerant lines such as Dan Ila and Kanannado continued slow growth of the trifoliates. However, with continued moisture stress, the unifoliates of these varieties showed early senescence and dropped off but the growing tips remained turgid and alive even longer (Fig. 4), suggesting that the moisture was being mobilized from the unifoliates to the growing tips.

Using the box screening method, the inheritance of drought tolerance in cowpea was studied (Mai-Kodomi et al. 1999b). Three cowpea lines: TVu 11986 with Type 1 drought tolerance, Dan Ila with Type 2 drought tolerance, and TVu 7778 as susceptible to drought were crossed in all possible combinations. The genetic segregation revealed that drought



**Figure 4.** Two types of drought tolerance. Left to right, Type 1 (1 plant), Type 2 (2 plants), Type 1 (2 plants), and a susceptible line.

tolerance is a dominant trait and both Type 1 and Type 2 reactions are controlled by a single dominant gene but the genes are independent in the two types. These are being designated as  $Rds_1$  (resistance to drought stress) and  $Rds_2$ . Test of allelism indicated that Type 1 is dominant over Type 2 and the  $F_2$  population between the two types segregated to 3 Type 1 : 1 Type 2 indicating that the genes  $Rds_1$  and  $Rds_2$  are either closely linked or are allelic at the same locus.

The simplicity of the wooden-box method and the inheritance of drought tolerance in this study may be due to its focus on only the shoot drought tolerance without involving the contribution of roots and other factors. Most of the earlier studies on drought tolerance have been conducted in the field where different mechanisms contribute to the overall drought tolerance of the plants and make it appear to be a complex trait. Screening for dehydration tolerance of the shoots only in the seedling stage using the wooden-box method should be related primarily to the stomatal behavior or osmotic adjustments as other mechanisms would not be operative. Once the plants sense water stress, it is likely that the genes controlling stomatal behavior or osmotic adjustments would be activated. The opening and closing of stomata, permitting solutes to accumulate in the cells may be simple phenomena, and therefore, they may be under major gene control as suggested by the results of this study.

### Relative shoot drought tolerance of major crops

The relative drought tolerance of some of the major crops grown in the semiarid tropics was reported by Singh et al. (1999b). They studied ten crop species for their relative shoot drought tolerance at the seedling stage using the box screening method. Based on percentage of dead plants at various time intervals and days taken to 100% dead plants for each of the crops, soybean appeared to be the most drought susceptible and cowpea the most drought tolerant (Table 3). The overall ranking of the crops in the increasing order of drought tolerance was soybean followed by blackgram, greengram, groundnut, maize, sorghum, millet, bambara nut, lablab, and cowpea. The water stress in the wooden-box

**Table 3. Proportion of dead plants of different crops at various intervals after terminating watering<sup>†</sup>.**

| Crop               | Days after terminating watering |     |     |     |     |     |
|--------------------|---------------------------------|-----|-----|-----|-----|-----|
|                    | 7                               | 9   | 11  | 15  | 19  | 23  |
| Cowpea: IT90K-59-2 | 0                               | 0   | 0   | 0   | 29  | 100 |
| Cowpea: TVu 11979  | 0                               | 0   | 0   | 13  | 53  | 100 |
| Cowpea: TVu 7778   | 0                               | 0   | 0   | 27  | 94  | 100 |
| Lablab bean        | 0                               | 0   | 0   | 17  | 66  | 100 |
| Bambara nut        | 0                               | 0   | 6   | 33  | 44  | 100 |
| Groundnut          | 14                              | 59  | 100 | 100 | 100 | 100 |
| Pearl millet       | 14                              | 28  | 68  | 100 | 100 | 100 |
| Sorghum            | 0                               | 0   | 93  | 100 | 100 | 100 |
| Greengram          | 8                               | 17  | 86  | 100 | 100 | 100 |
| Blackgram          | 14                              | 75  | 100 | 100 | 100 | 100 |
| Maize              | 17                              | 50  | 100 | 100 | 100 | 100 |
| Soybean            | 63                              | 100 | 100 | 100 | 100 | 100 |
| LSD 5%             | 46                              | 56  | 23  | 31  | 50  | NS  |

<sup>†</sup> Source: Singh et al. 1999b.

method using higher sand content was too drastic for crops other than cowpea and lablab. With increased clay content and gradual water stress, it may be possible to use this method to detect variable differences in crops such as maize, soybean, millet, and sorghum, which are less drought tolerant than cowpea.

### **Screening for root characteristics**

Screening for root characteristics is difficult because of the underground distribution of roots and associated soil variations. Several methods have been used to estimate root length, density, volume, and distribution in the field (Krishnamurthy et al. 1996). The “auger method” provides for a three-dimensional volumetric measure of soil–root relationship; however, this has large sampling variations. For the “monolith method”, soil samples of an area of 20 × 30 cm to a depth of 10 or 15 cm are successively recovered and the roots are washed in a 1 mm sieve. This method is less variable because it involves a larger sample size. However, these methods are suitable only for limited comparisons. The rhizotron or minirhizotron methods are more efficient and permit nondestructive continuous studies of root systems but these involve expensive setups and equipment and are not practical for screening large numbers of segregating populations. Also, the root density estimates using the minirhizotron method do not compare well with the auger or monolith methods (Krishnamurthy et al. 1996). Recently, the use of the “root-box pin board” method has permitted a two-dimensional study of root systems of large numbers of plants or progenies with limited resources and great simplicity. Results of these screening methods are highly correlated with actual field observations using in situ root pits and monolith methods.

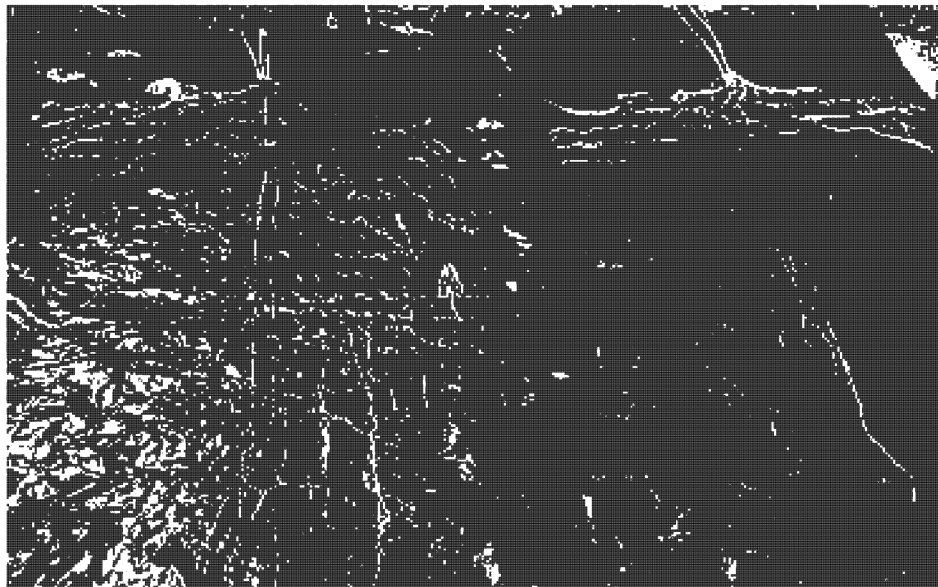
### **Root-box pin board method for root study**

Recently, a simple box method for studying root architecture was developed at IITA, Kano Station (unpublished), which permits fast screening of cowpea lines for root length, root density, and root spread.

The two-dimensional distribution of roots can be studied using a thin wooden box made by nailing two plywood sheets of 80 cm length, 60 width, and 5 mm thickness on a frame made of 5 cm thick square wooden stakes. One sheet is fixed with soft nails for easy removal and the other sheet is fixed with hard nails. Before fixing nails, the inner sides of the plywood sheets are lined with polyethylene and one side of the frame is open leaving a 5 cm gap. The 5 cm gap is then filled with a mixture of sand and topsoil (50 : 50) and watered. Three to five handpicked good seeds of the test crop are planted in a single hole in the middle, and after germination thinned to one to three plants, depending on the objective of the study. The box is watered daily for three to five weeks after which the roots can be studied. This is done by removing one side of the box (the soft nail side) and fixing the nail board (Fig. 5) in its place. The box is then turned over so that all the soil and the plant with its roots lie on the nail board when the sheet on the hard nail side is lifted out. The soil is washed off gently using sprinkling water leaving the roots on the polythene sheet on the nail board. The polythene sheet (with the roots) is lifted out and studied. Varietal or species differences are studied using one box for each with three to four replications. Major varietal differences have been observed in cowpea root architecture (Fig. 6). Some varieties have a well-spread deep root system while others have concentrated roots only on the upper soil strata. These differences affect



**Figure 5. Box screening for root architecture.**  
Left shows box with growing plant; right shows the two-dimensional structure of the roots on the nail board.



**Figure 6. Varietal differences in root architecture.**  
Left shows variety with well spread, deep root system; right shows variety with roots only in upper strata.

plant ability to absorb water from the receding water after the rains cease. Well distributed deep roots permit plants to survive longer than those with shallow roots.

### Breeding for drought tolerance

Using the box screening method for shoot drought tolerance and the root-box pin board screening method for root architecture, it has been possible to identify cowpea varieties with enhanced levels of shoot drought tolerance (Figs. 1 and 3), and varieties with well-distributed deep root systems (Fig. 6). These have been crossed to combine the two characteristics in order to develop new improved varieties with a high level of drought tolerance and the segregating progenies are being screened. In the meantime, the available improved breeding lines have been screened using box screening and a number of drought-tolerant lines have been identified (Table 4) and are being tested in drought-prone areas.

**Table 4. Reaction of improved cowpea breeding lines to drought.**

#### Drought-tolerant Type 1

IT93K-452-1, IT95K-627-34, IT96D-711, IT96D-724, IT97K-1068-27, IT97K-1068-7, IT97K-1075-7, IT97K-1106-6, IT97K-1129-51-1, IT97K-1133-7, IT97K-207-21, IT97K-491-4, 114, IT97K-499-38, IT97K-499-39, IT97K-634, IT98K-1025-6, IT98K-1106-3, IT98K-128-3, IT98K-131-1, IT98K-251-5, IT98K-258-19, IT98K-308-12-1, IT98K-310-7-1, IT98K-368-24-1, IT98K-368-43-1, IT98K-394-20, IT98K-402-2-2, IT98K-406-12, IT98K-406-3, IT98K-463-7, IT98K-471, IT98K-568-11, IT98K-580, IT98K-96-4, IT99K-1238, IT99K-1362, IT99K-254-2, IT99K-316-2, IT99K-332-3, IT99K-368-1, IT99K-429-2, IT99K-429-4, IT99K-467-7, IT99K-494-6, IT99K-499-5, IT99K-529-2, IT99K-536-5, IT99K-536-6, IT99K-539-1, IT99K-544-1, IT99K-564-2, IT99K-790-2.

#### Drought-tolerant Type 2

Aloka local, Dan Ila, Gorom local, IAR1696, IT89KD-288-40, IT89KD-288-42, IT89KD-349, IT89KD-374-57, IT95K-105-2, IT95K-1072-57, IT95K-181-9, IT95K-222-3, IT95K-223-19, IT95K-357-2, IT95K-426-2, IT95K-825-3, IT96D-602, IT96D-604, IT97K-1021-9, IT97K-1069-2, IT97K-1069-6, IT97K-209-4-1, IT97K-338-7, IT97K-377-4, IT97K-569-9, IT97K-573-1, IT97K-608-14, IT97K-8119-154, IT97K-819-118, IT97K-819-170, IT97K-819-172, IT97K-819-178, IT97K-819-220, IT97K-819-84, IT97K-820-13, IT97K-820-8, IT97K1025-18, IT98D-1219, IT98D-1232, IT98D-1300, IT98D-1355, IT98K-1091-2-1, IT98K-1091-3, IT98K-1093-5-2, IT98K-1108-4, IT98K-1399, IT98K-143-14, IT98K-210-1, IT98K-234-5, IT98K-317-8, IT98K-415-6, IT98K-418-2-2, IT98K-557-1, IT98K-690, IT99K-1008, IT99K-1016, IT99K-1152-14, IT99K-1235, IT99K-1260, IT99K-1288, IT99K-1296, IT99K-210-2, IT99K-210-3, IT99K-270, IT99K-298-2, IT99K-363-3, IT99K-364-2, IT99K-381-6, IT99K-411-2, IT99K-411-4, IT99K-412-1, IT99K-412-6, IT99K-415-2, IT99K-421-4, IT99K-421-5, IT99K-445-3, IT99K-451-4, IT99K-466-3, IT99K-476-2, IT99K-541-1, IT99K-556-2, IT99K-562-1, IT99K-636-7, IT99K-687, IT99K-695, IT99K-720-3, IT99K-723-13, IT99K-818-27, IT99K-826-3, IT99K-835, IT99K-957, IT99K826-2, Suvita-2, IT98K-412-13, IT98K-415-1.

#### Drought susceptible

IT82E-16, IT95K-1133-6, IT95K-231-1, IT95K-238-3, IT95K-356-1, IT95K-398-14, IT97K-1042-3, IT97K-356-2, IT97K-399-32, IT97K-419-3, IT97K-467-7, IT97K-556-4, IT98K-1079-10, IT98K-1088-5, IT98K-1107-2, IT98K-1107-8, IT98K-1110-2, IT98K-1111-1, IT98K-1128-18, IT98K-279-6, IT98K-311-8-1, IT98K-311-8-2, IT98K-399-1, IT98K-399-32, IT98K-439-3, IT98K-491-4, IT98K-555-1, IT98K-589-2, IT98K-598-1, IT98K-625-1, IT98K-642-2, IT99K-1122, IT99K-1152-23, IT99K-1152-8, IT99K-1256, IT99K-1366, IT99K-195-8, IT99K-390, IT99K-407-3, IT99K-573-2, IT99K-820-7, TVu-7778.



### **Evaluation of selected drought-tolerant varieties**

A number of selected drought-tolerant and susceptible varieties based on box screening were evaluated in the field at Minjibir towards the end of the rainy season and at Zinder, (Niger Republic) where rainfall is normally low. From the rainfall pattern, there was good level of moisture stress at both locations and cowpea varieties differed in their response. Generally, the drought-tolerant varieties had significantly higher grain yields than susceptible varieties at both locations but both had similar fodder yields (Table 5). The most promising drought-tolerant varieties were IT98K-452-1, IT97K-819-154, and IT98K-205-8. These results indicate that the box method can be used to screen for drought tolerance of new breeding lines to reduce their numbers before field testing.

**Table 5. Performance of drought-tolerant cowpea varieties, 2000.**

| Variety       | Grain yield (kg) |          | Fodder yield (kg/ha) |          | Reaction to drought |
|---------------|------------------|----------|----------------------|----------|---------------------|
|               | Zinder           | Minjibir | Zinder               | Minjibir |                     |
| IT98K-452-1   | 1209             | 650      | 1253                 | 919      | tolerant            |
| IT97K-819-154 | 1075             | 599      | 1614                 | 1091     | tolerant            |
| IT98K-20-8    | 1017             | 313      | 1252                 | 946      | tolerant            |
| IT89KD-349    | 730              | 669      | 1364                 | 768      | tolerant            |
| Aloka         | 903              | 597      | 1141                 | 668      | tolerant            |
| TVu 7778      | 583              | 214      | 751                  | 356      | susceptible         |
| IT95K-238-3   | 500              | 152      | 1280                 | 752      | susceptible         |
| IT92KD-357-2  | 330              | 271      | 1587                 | 752      | susceptible         |
| SED           | 167              | 96       | 187                  | 258      |                     |

### **Conclusion**

The traditional approach of studying drought tolerance on a whole plant basis makes it appear as a complex trait and therefore, difficult to manipulate by plant breeders. The studies described here indicate that it is possible to simplify this by separating shoot drought tolerance from the influence of roots and vice versa. Using the box screening method for cowpea, major varietal differences have been observed for shoot drought tolerance and the trait seems to be simply inherited. Similarly, using the root-box pin board method, major varietal differences have been observed for root architecture in cowpea. These methods have provided a simplified approach to the study of drought tolerance in cowpea and may lead to faster progress in breeding for drought tolerance in other crops.

### **References**

- Acevedo, E. and S. Ceccarelli. 1989. Role of physiologist–breeder in a breeding program for drought resistance conditions. Pages 117–139 *in* Drought resistance in cereals, edited by W.G. Baker. CAB International, Wallingford, UK.

- Arrauadeau, M.A. 1989. Breeding strategies for drought resistance. Pages 107–116 *in* Drought resistance in cereals, edited by W.G. Baker. CAB International, Wallingford, London, UK.
- Ashley, J. 1993. Drought and crop adaptation. Pages 46–67 *in* Dryland farming in Africa, edited by J.R.J. Rowland. Macmillan Press Ltd, UK.
- Bates, L.S., R.P. Waldren, and I.O. Teare. 1973. Rapid determination of free proline in water stress studies. *Plant and Soil* 38: 205.
- Blum, A. 1985. Breeding crop varieties for stress environments. *Critical Reviews in Plant Sciences* 2: 199–238.
- Boyer, J. S. 1996. Advances in drought tolerance in plants. *Advances in Agronomy* 56: 189–218.
- Hall, A.E., R.G. Mutters, K.T. Hubick, and G.D. Farquhar. 1990. Genotype differences in carbon isotope discrimination by cowpea under wet and dry field conditions. *Crop Science* 30: 300–305.
- Hall, A.E., S. Thiaw, A. Ismail, and J.D. Ehlers. 1997. Water-use efficiency and drought adaptation of cowpea. Pages 141–146 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Centre for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Krishnamurthy, L.C., C. Johansen, and O. Ito. 1996. Genotypic variation in root system development and its implication for drought resistance in Chickpea. Pages 235–250 *in* Roots and nitrogen in cropping systems of the semiarid tropics, edited by O. Ito, C. Johansen, J.J. Adu-Gyamfi, K. Katayama, J.V.K. Kumar Rao, and T. J. Rego. JIRCAS and ICRISAT, Hyderabad, India.
- Lawan, R.J. 1983. Responses of four grain legumes to water stress in higher plants. *Annual Review of Plant Physiology* 35: 299–319.
- Mai-Kodomi, Y., B.B. Singh, O. Myers Jr., J.H. Yopp, P.J. Gibson, and T. Terao. 1999a. Two types of drought tolerance in cowpea. *Indian Journal of Genetics* 59 (3): 309–313.
- Mai-Kodomi, Y., B.B. Singh, T. Terao, O. Myers Jr., J.H. Yopp, and P.J. Gibson. 1999b. Inheritance of drought tolerance in cowpea. *Indian Journal of Genetics* 59 (3): 317–232.
- Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* 35: 299–319.
- Singh, B.B. 1987. Breeding cowpea varieties for drought escape. Pages 299–306 *in* Food grain production in semiarid Africa, edited by J.M. Menyonga, T. Bezuneh, and A. Youdeowei. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso.
- Singh, B.B. 1993. Cowpea breeding archival report (1988–1992) of Grain Legume Improvement Program, IITA, Ibadan, Nigeria.
- Singh, B.B. 1994. Breeding suitable cowpea varieties for West and Central African savanna. Pages 77–85 *in* Progress in food grains research and production in semiarid Africa, edited by J.M. Menyonga, J. B. Bezuneh, J.Y. Yayock, and I. Soumana. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso.
- Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. Pages 30–49 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Science (JIRCAS). IITA, Ibadan, Nigeria.
- Singh, B.B., Y. Mai-Kodomi, and T. Terao. 1999a. A simple screening method for drought tolerance in cowpea. *Indian Journal of Genetics* 59 (2): 211–220.
- Singh, B.B., Y. Mai-Kodomi, and T. Terao. 1999b. Relative drought tolerance of major rainfed crops of the semi-arid tropics. *Indian Journal of Genetics* 59: 1–8.
- Subbarao, G.V., C. Johansen, A.E. Slinkard, R.C. Nageswara Rao, N.P. Saxena, and Y.S. Chauhan. 1995. Strategies for improving drought resistance in grain legumes. *Critical Reviews in Plant Sciences* 14: 269–523.
- Turk, K.J. and A.E. Hall. 1980. Drought adaptation of cowpea. Influence of drought on plant water status and relations with seed yield. *Agronomy Journal* 72: 421–427.

- Walker, D.W. and J.C. Miller Jr. 1986. Intraspecific variability for drought resistance in cowpea. *Scientia Horticulturae* 29: 87–100.
- Watanabe, I., S. Hakoyama, T. Terao, and B.B. Singh. 1997. Evaluation methods for drought tolerance in cowpea. Pages 141–146 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Yadava, R.B.R. and B.D. Patil. 1984. Screening of cowpea (*Vigna unguiculata* L.) varieties for drought tolerance. *Zeitschrift Fur Acker Und Pflanzenbau* 93: 259–262.

## 4.6

# Soil fertility management and cowpea production in the semiarid tropics

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### Abstract

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important grain legume in the semi-arid zone of West Africa as it is a major source of dietary protein for the people. It is usually grown as an intercrop with the major cereals, namely millet and sorghum. Despite its importance, its yields are very low due to several constraints including poor soil, insect pests, and drought. The soils in semiarid West Africa are inherently low in nitrogen and phosphorus. Soil, water, and nutrient management practices are inadequate to sustain food production and to meet the food requirements of the fast growing population. Research results show that proper management of organic amendments such as crop residues and manure, which are essential complements to mineral phosphorus fertilizers, can increase yields of cowpea and associated cereals more than three fold. Direct application of indigenous phosphate rocks can be an economical alternative to the use of imported, more expensive soluble phosphorus fertilizers for cowpea production in the region. The agronomic effectiveness of indigenous phosphate rock is about 50% compared to the imported single superphosphate. Furthermore, when the unreactive phosphate rocks are partially acidulated at 50%, their agronomic effectiveness can increase to more than 70%. Studies on cereal–cowpea rotation revealed that yields of cereals succeeding cowpea could, in some cases, double compared to continuous cereal cultivation. With efficient soil fertility management, cowpea can fix up to 88 kg N/ha and this results in an increase of nitrogen use efficiency on the succeeding cereal crop from 20% in the continuous cereal monoculture to 28% when cereals are in rotation with cowpea. Furthermore, the use of soil nitrogen increased from 39 kg N/ha in the continuous cereal monoculture to 62 kg N/ha in the rotation systems. Future research needs to focus on understanding the factors affecting phosphorus uptake from different sources of natural rock phosphate. There is also a need to quantify the below-ground nitrogen fixed by different cowpea cultivars. The increase of cowpea productivity in the cropping systems in this region will improve the nutrition of people, increase the feed quantity and quality for livestock, and contribute to soil fertility maintenance. This should contribute to reduction in poverty and environmental degradation.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important grain legume in the West African Semiarid Tropics (WASAT), where it occupies 6 million hectares. Cowpea is an important component of the predominantly cereal/legume production systems in the region. The

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most important cereals are sorghum and pearl millet and cowpea is often intercropped with these cereals (Steiner 1984).

Cowpea grain contains about 22% protein and constitutes a major source of protein for resource-poor rural and urban people. It is estimated that cowpea supplies about 40% of the daily protein requirements to most of the people in Nigeria (Muleba et al. 1997). The crop residues from cowpea constitute an important source of livestock feed especially in the dry savannas of WASAT.

The principal reasons for farmers to intercrop are flexibility, profit maximization, risk minimization, soil conservation and maintenance, weed control, and nutritional advantages (Norman 1984; Swinton et al. 1984; Shetty et al. 1995; Fussell and Serafini 1985). In mixed cropping systems, cowpea yields are very low due to low soil fertility, low planting densities, and pests and diseases (Ntare 1989, Reddy et al. 1992). Cowpea grain yield varies between 50 kg/ha and 300 kg/ha in farmers' fields in marked contrast to over 2000 kg/ha obtainable on research stations and by large-scale commercial enterprises in pure cropping. In the mixed farming systems of the WASAT, increasing legume component in the farming systems is important in order to increase the availability of fodder as livestock feed while increasing soil fertility.

Rotation of cereals with legumes has been extensively studied in recent years. Use of rotational systems involving legumes is gaining importance throughout the region because of economic and sustainability considerations. The beneficial effect of legumes on succeeding crops is normally exclusively attributed to the increased soil N fertility as a result of  $N_2$  fixation. The amount of  $N_2$  fixed by leguminous crops can be quite high, although it has been demonstrated that legumes can also deplete soil nitrogen (Rupela and Saxena 1987, Blumenthal et al. 1982).

Most of the data reported on the quantity of N fixed by legume crops in the WASAT concerned the aboveground part of the legume and very little is known about the nitrogen fixed by the roots. Where much of the legume biomass is returned to the soil as green manure, a positive N balance is to be expected. However, this may not be true for cowpea, where the bulk of above biomass is removed from the system. Nevertheless, there are many other positive effects of grain legumes such as the improvement of soil biological and physical properties and the ability of some legumes to solubilize occluded phosphorus and highly insoluble Calcium-bounded phosphorus by roots exudates (Arihara and Ohwaki 1989). Other advantages of crop rotation include soil conservation (Stoop and Staveren 1981), organic matter restoration (Spurgeon and Grimson 1965), and pest and disease control (Curl 1963). While considerable information is available on fertilizer requirements for sole cropping of various crops, it is limited for intercropping and rotations.

This paper will review the cowpea production environment, the effect of soil fertility improvement, and will conclude with new research opportunities.

## **Cowpea production environment**

Cowpea is predominantly grown in the WASAT. This zone is characterized by a growing period of 60–150 days. The rainfall is low, variable, and undependable. One striking feature of the soils is their inherent low fertility expressed in low levels of organic carbon (generally less than 0.3%), total and available phosphorus and nitrogen, and effective cation exchange capacity (ECEC) (Table 1). About 98% of the soil nitrogen is stabilized in organic matter. Thus, the total nitrogen in the soil and the amount of nitrogen released

**Table 1. Means and ranges of selected physical and chemical properties of West African semiarid soils from 30 representative sites.**

| Parameter                                    | Range      | Mean |
|--|------------|------|
| pH-H <sub>2</sub> O (2 : 1 water : soil)     | 3.95–7.6   | 6.17 |
| pH-KCl (2 : 1 water : soil)                  | 3.41–7.0   | 5.05 |
| Clay (%)                                     | 0.7–13     | 3.9  |
| Sand (%)                                     | 71–99      | 88   |
| Organic matter (%)                           | 0.14–5.07  | 1.4  |
| Total nitrogen (mg/kg)                       | 31–226     | 446  |
| Exchangeable bases (cmol/kg)                 |            |      |
| Ca   | 0.15–16.45 | 2.16 |
| Mg   | 0.02–2.16  | 0.59 |
| K  | 0.03–1.13  | 0.20 |
| Na   | 0.01–0.09  | 0.04 |
| Exchangeable Al (cmol/kg)                    | 0.02–5.6   | 0.24 |
| Effective cation exchange capacity (cmol/kg) | 0.54–19.2  | 3.43 |
| Base saturation (%)                          | 36–99      | 88   |
| Al saturation (%)                            | 0–46       | 3    |
| Total phosphorus                             | 25–941     | 136  |
| Available phosphorus                         | 1–83       | 8    |
| Maximum P sorbed                             | 27–406     | 109  |

Source: Bationo et al. (2000).

for plant nutrients uptake will depend on the organic matter level of the soil. Total and available P levels are very low and P deficiency is the most limiting soil fertility factor for cowpea production. Apart from low P stocks, the low-activity nature of these soils results in a relatively low capacity to fix added phosphorus (Bationo et al. 1995). Phosphorus sorption maxima of the WASAT soil ranged from 27 to 405 mg P/kg with a mean of 109 mg P/kg. Low quantities of P need to be added to the soil to maintain 0.2 ppm P in the soil solution. At present most cultivated land in the region lose more N, P, and K than gained and continuous cultivation has led to nutrient mining and loss of topsoil by wind and/or water erosion (Table 2). Under these conditions, productivity levels of both cereals and legumes are too low to sustain food production and to meet food requirements of the fast growing human populations.

Although organic amendments such as crop residue, manure, or compost are essential in the sustainability of the cropping systems, they cannot prevent nutrient mining. The addition of organic amendments corresponds in most cases to a recycling process, which cannot compensate for nutrient exported through crop products. As a result, the use of external inputs such as inorganic plant nutrients or local sources of P such as phosphate rock are essential requirements for soil productivity.

### Effect of soil fertility improvement on cowpea production

Research results in the region have shown the importance of the improvement of soil fertility for crop production (Mokwunye and Vlek 1986; Pieri 1989; Van Reuler and Jansen 1989; Van der Heide 1989; Bationo and Mokwunye 1991; Sedogo 1993). In the Sahelian zone, soil fertility appears to be more limiting to crop and fodder production than rainfall and the use of fertilizer will increase water-use efficiency (Penning de

**Table 2. Annual nutrient losses for some West African countries.**

| Country      | Area ('000 ha) | Losses for the region (10 <sup>3</sup> tonnes) |                               |                  |
|--------------|----------------|--|-------------------------------|------------------|
|              |                | N  | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O |
| Benin        | 2972           | 41388  | 10366                         | 32499            |
| Burkina Faso | 6691           | 95391  | 27754                         | 78764            |
| Ghana        | 4505           | 137140   | 32313                         | 90474            |
| Mali         | 8015           | 61707  | 17888                         | 66725            |
| Niger        | 985            | 176120   | 55331                         | 146617           |
| Nigeria      | 2813           | 1107605  | 316687                        | 946157           |

Source: Adapted from Stoorvogel and Smaling (1990).

Vries and Djiteye 1991, Breman and de Wit 1983). The use of mineral fertilizers can significantly increase water-use efficiency.

Significant cowpea responses to nitrogen applied as urea have been obtained in different agroecological zones of the WASAT (Table 3). These significant responses indicate that the predominantly sandy soils of the WASAT may be deficient in molybdenum required for efficient symbiotic fixation (Hafner et al. 1992). For example, on the sandy acid soil at Bengou in the Sudanian zone, significant molybdenum response was obtained at different levels of soil fertility management for cowpea (Fig. 1).

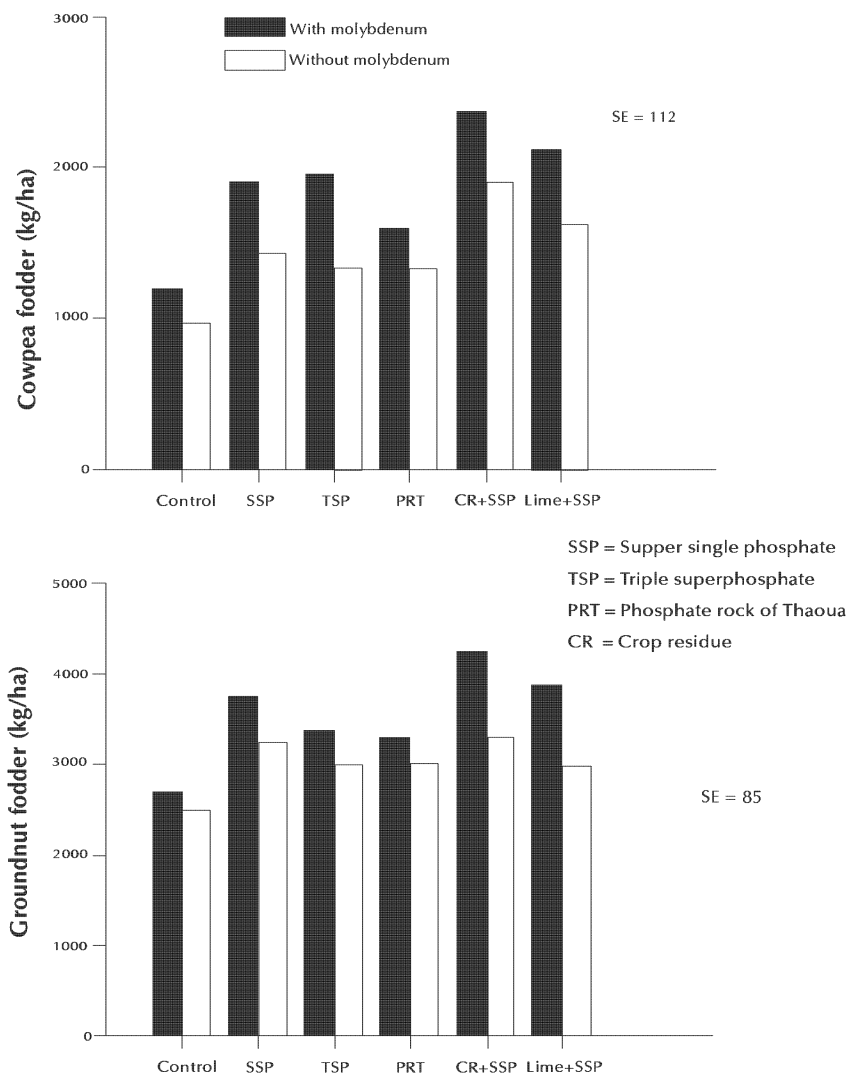
Legumes such as cowpea have a high P requirement. P is reported to stimulate root and plant growth, initiate nodule formation, as well as influence the efficiency of the rhizobium-legume symbiosis. It is also involved in reactions with energy transfer, more specifically ATP in nitrogenase activity (Israel 1987). Research conducted at Ikenne in the humid zone and Kamboinse in the Sudanian zone of West Africa indicated a strong differential response to P by cowpea cultivars (Fig. 2). The local Kamboinse variety is a fodder type and the application of P resulted in higher fodder yield but lower grain production. As reported by several scientists such as Dwivedi et al. (1975); Khan and Zende (1977); Stukenholtz et al. (1966); Takkar et al. (1976); and Youngdhal et al. (1977), the application of P resulted in significant decrease of zinc concentration in the cowpea grain which can affect the nutritional quality (Buerkert et al. 1998).

Despite the importance of P in these soils, the use of commercial P fertilizers in the WASAT is limited due to the high cost of imported fertilizers. Several countries in the region, however, are known to have natural phosphate deposits. Direct application of indigenous phosphate rocks (PR) can be an alternative to the use of more expensive water-soluble phosphorus fertilizers. This practice would also promote savings in scarce foreign exchange. The effectiveness of PR depends on its chemical and mineralogical composition, soil factors, and the crops to be grown (Khasawneh and Doll 1978; Lehr and McClellan 1972; Chien and Hammond 1978). The relative agronomic effectiveness of Tahoua PR and Kodjari PR in different agroecological zones of the WASAT has been evaluated (Table 4). The data indicate that Tahoua PR outperformed Kodjari PR in agronomic effectiveness at two of the three sites. These results are in agreement with the chemical composition of the two rocks where the molar PO<sub>4</sub>/CO<sub>4</sub> ratio is 25 for Kodjari PR and 4.9 for Tahoua PR. The agronomic cowpea is not better than that of the cereal pearl millet crop. This is in contradiction to other reports where legumes have highest strategy to solubilize PR than cereals by rhizosphere acidulation (Aguilar and Van Diest 1981; Kirk and Nye 1986; Hedley et al. 1982) and exudation of organic acids (Ohwaki and Hirata 1992).

**Table 3. Effect of nitrogen on cowpea yield at three sites in 1988.**

| N rates (kg N/ha) | Cowpea fodder |        |       |
|-------------------|---------------|--------|-------|
|                   | Sadore        | Bengou | Tara  |
| 0                 | 4069          | 2213   | 2974  |
| 15                | 4474          | 2510   | 2963  |
| 30                | 4288          | 2548   | 3025  |
| 45                | 4264          | 3008   | 3500  |
| S.E. (D.F.27)     | 218.3         | 153.7  | 161.3 |
| CV (%)            | 15            | 17     | 15    |

Source: Bationo and Ntare (2000).



**Figure 1. Effects of different phosphorus sources, crop residue, lime, and molybdenum on cowpea and groundnut fodder yield, Tara, Niger, 1993.**

Source: Bationo, (unpublished data).



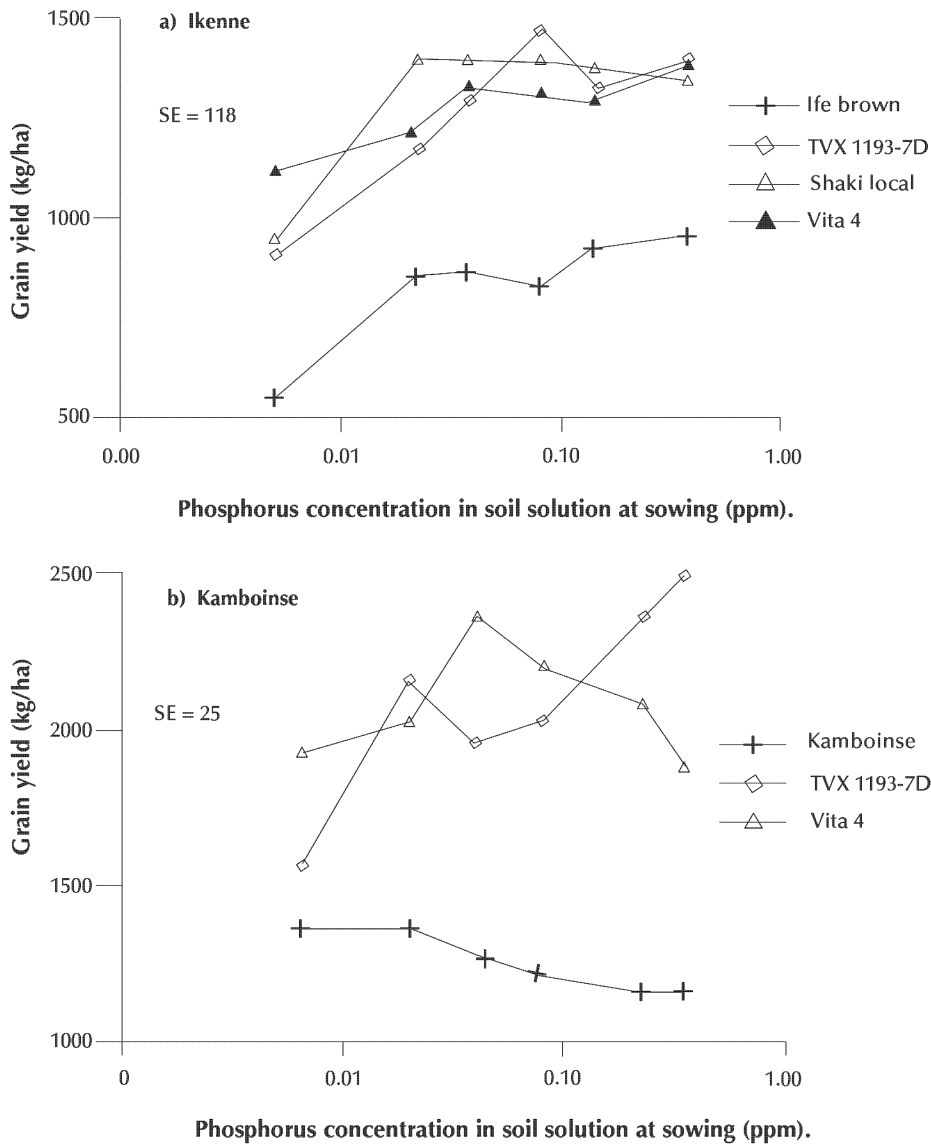


Figure 2. Relationship between grain yield and phosphorus concentration in soil solution at sowing in sandy loam Paleustatif Oxid Paleustalf at (a) Ikenne and (b) Kamboinse.

**Table 4. Relative agronomic effectiveness for pearl millet and cowpea as compared to single superphosphate (SSP) (%) of Tahoua phosphate rock (TPR) and Kodjari phosphate rock (KPR) in three agroecological zones of Niger.**

|                                 | Sadoré |     | Goberi |     | Gaya |     |
|---------------------------------|--------|-----|--------|-----|------|-----|
|                                 | TPR    | KPR | TPR    | KPR | TPR  | KPR |
| Cowpea fodder (kg/ha)           | 43     | 28  | 73     | 51  | 42   | 42  |
| Cowpea total dry matter (kg/ha) | 56     | 40  | 72     | 51  | 52   | 55  |

Source: Mahamane et al. (1997).

The response of cowpea grain and stover yield to different sources of P fertilizers is presented in Figure 3. The application of P fertilizers can triple cowpea stover production. The relative agronomic effectiveness of Phosphate rock annual application (PRA) indigenous to Niger varied from 42 to 54% as compared to the water soluble single superphosphate (SSP) (Table 5). The acidulation of PR at 50% (PAPR 50) with sulfuric acid can increase the relative agronomic effectiveness to 96% for cowpea stover production. For fodder production, triple superphosphate (TSP) relative agronomic effectiveness varied from 77 to 91% indicating that sulfur is needed for cowpea growth.

Research at ICRISAT-Niger has focussed on the placement of small quantities of P fertilizers at planting in order to develop optimum farmer-affordable P application recommendation for increased crop yield. For cowpea stover production, phosphorus-use efficiency increased from 44 with the addition of Kodjari PR to 93 kg/kg phosphorus plus 4 kg P/ha as 15-15-15, respectively, (Table 6).

Long-term experiments are a practical means of addressing the difficult issues associated with quantitative assessment of sustainability in agriculture. In summarizing the results of long-term soil fertility management in Africa, Pieri (1986) concluded that soil fertility in intensive arable farming in the WASAT can only be maintained through efficient cycling of organic materials in combination with mineral fertilizers and with rotation with leguminous N<sub>2</sub>-fixing species. Results from a long-term experiment at Sadore in Niger indicated that the application of small quantities of fertilizers and crop residues resulted in an increase of cowpea fodder yield from 1700 to 5300 kg/ha (Fig. 4). In on-farm trials, pocket applications of small quantities of manure (3 t/ha) plus 4 kg/ha of P at seedling time increased cowpea yield from 180 kg/ha in the control plot to 400 kg/ha (Fig. 5).

## Effect of cowpea production on soil fertility improvement

Despite the recognized need to apply chemical fertilizers for high yields, the use of mineral fertilizers in West Africa is limited by lack of capital, inefficient distribution systems, poor enabling policies, and other socioeconomic factors. Cheaper means of improving soil fertility and productivity is therefore necessary. Cereal-legume rotation effects on cereal yields have been reported for the WASAT (Bagayoko et al. 1996; 2000; Bationo et al. 1998; Klaij and Ntare 1995; Nicou 1977; Stoop and Staveren 1981; Bationo and Ntare 2000). In all these studies, the yield of cereal after cowpea was significantly higher than in continuous cereal cultivation. Cowpea yield also significantly responded to crop rotation, indicating that factors other than N alone contributed to the yield increases in the cereal-legume rotations.

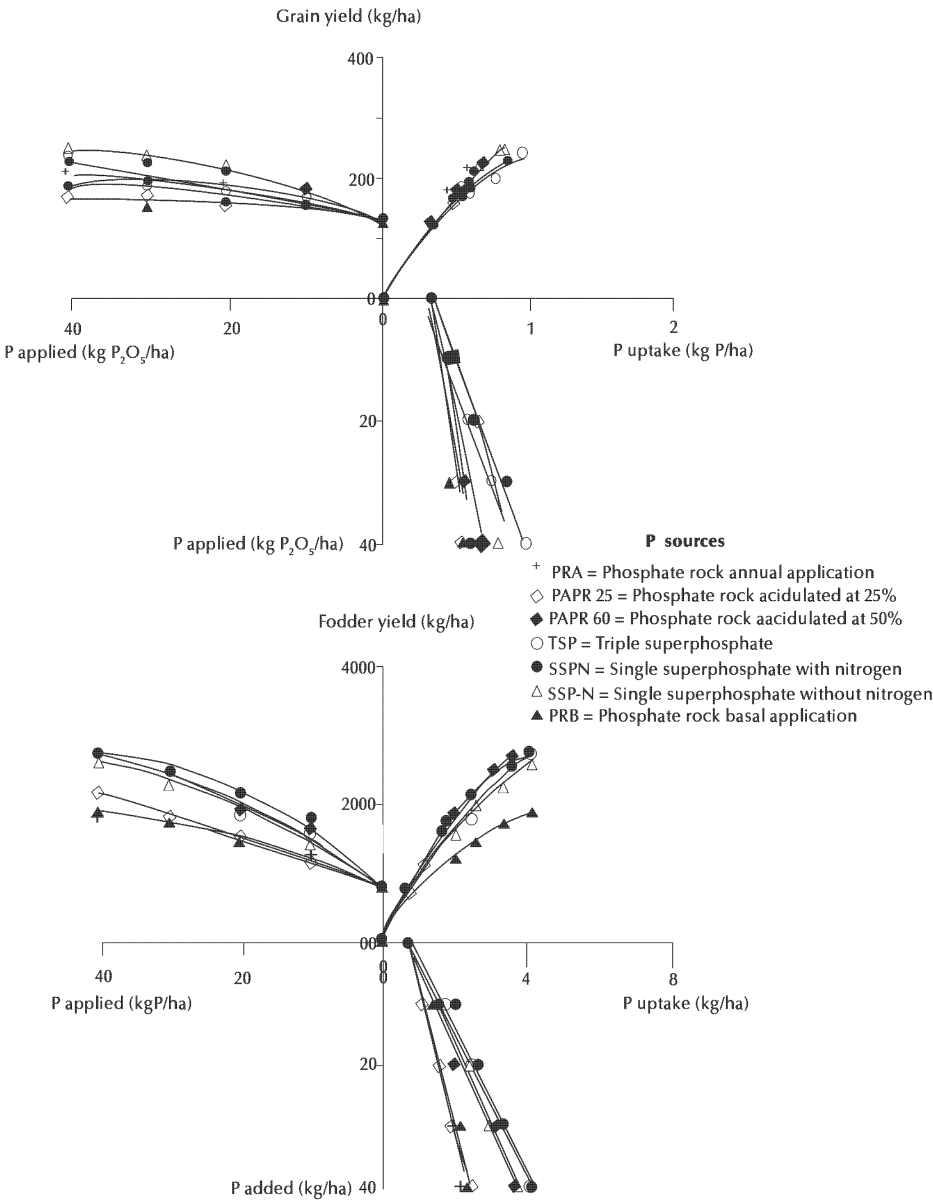


Figure 3. Relationship between cowpea grain and fodder yield with P applied, and between phosphorus applied and phosphorus uptake, Sadoré, Niger, 1983.

Source: Bationo (unpublished data).

**Table 5. Relative agronomic effectiveness of different sources of phosphorus on cowpea.**

| P sources  | 1993  |          | 1994  |          |
|--|-------|----------|-------|----------|
|  | Grain | % Fodder | Grain | % Fodder |
| Phosphate rock annual application (PRA)              | 70    | 54       | 49    | 42       |
| Partially acidulated phosphate rock at 25% (PAPR 25) | 45    | 58       | 61    | 75       |
| Partially acidulated phosphate rock at 50% (PAPR 50) | 72    | 92       | 88    | 96       |
| Triple superphosphate (TSP)                          | 68    | 91       | 65    | 77       |
| Single superphosphate (SSP)                          | 74    | 87       | 86    | 91       |
| Phosphate rock based application                     |       |          |       |          |

**Table 6. Effect of different sources of phosphorus and their placement\*\* on cowpea yield and Phosphorus-use efficiency (PUE), Karabedji, (1998 rainy season).**

| P sources and method of application | Grain         |             | Fodder                     |                          |
|-------------------------------------|---------------|-------------|----------------------------|--------------------------|
|                                     | Yield (kg/ha) | PUE (kg/ha) | Yield (kg/ha)<br>P applied | PUE (kg/ha)<br>P applied |
| Control                             | 505           |             | 1213                       |                          |
| SSP broadcast                       | 1073          | 44          | 2120                       | 70                       |
| SSP broadcast+SSP HP                | 1544          | 61          | 3139                       | 113                      |
| SSP HP                              | 1050          | 136         | 2021                       | 452                      |
| 15-15-15 broadcast                  | 1165          | 51          | 2381                       | 90                       |
| 15-15-15 broadcast+15-15-15 HP      | 2383          | 110         | 3637                       | 142                      |
| 15-15-15 HP                         | 1197          | 173         | 2562                       | 337                      |
| PRT broadcast                       | 986           | 37          | 2220                       | 77                       |
| PRT broadcast+SSP HP                | 1165          | 68          | 3127                       | 113                      |
| PRT broadcast+15-15-15 HP           | 1724          | 72          | 3163                       | 115                      |
| PRK broadcast                       | 920           | 32          | 1791                       | 44                       |
| PRK broadcast+SSP HP                | 1268          | 45          | 2588                       | 81                       |
| PRK broadcast+15-15-15 HP           | 1440          | 55          | 2792                       | 93                       |
| S.E.                                | 164           |             | 313                        |                          |

\*SSP Single superphosphate; 15-15-15 compound fertilizer containing 15% N, 15%  $P_2O_5$ , 15%  $K_2O$ ; Tahoua phosphate rock (TPR), Kodjari phosphate rock (KPR).

HP signifies hill placement of fertilizer.

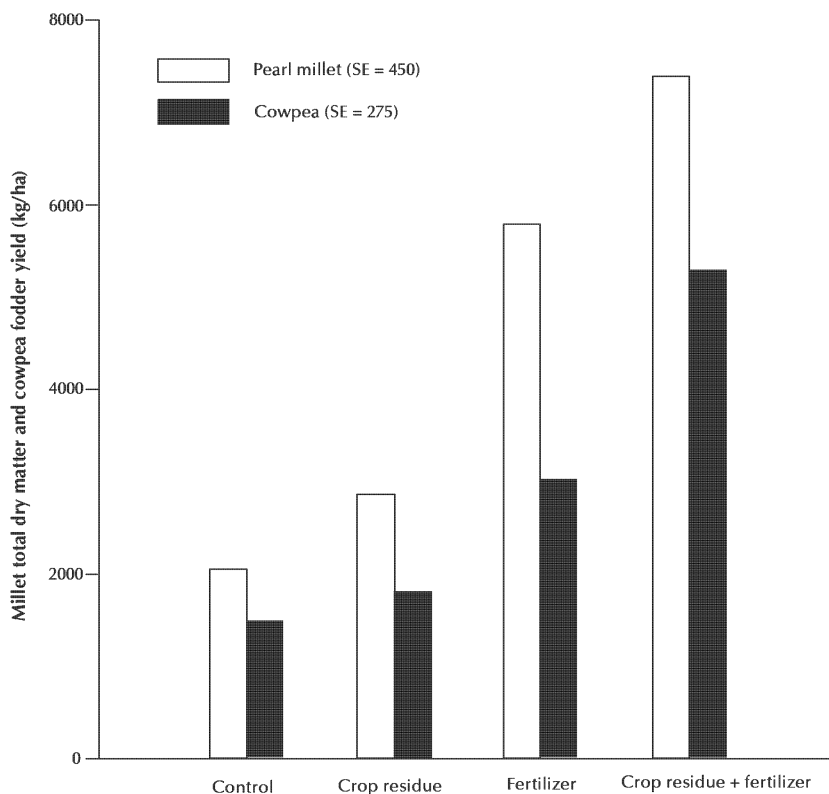
\*\*For broadcast, 13 kg P/ha was applied.

\*\* For HP, 4 kg P/ha as hill placement.

Source: Bationo (unpublished data).

Bationo and Ntare (2000) studied nitrogen dynamics in different cropping systems. In order to determine N availability, the soil was incubated and mineral nitrogen determined at 7, 21, and 35 days (Keeney 1982). Crop rotation significantly affected mineral nitrogen release (Fig. 6). The fallow millet rotation supplied more nitrogen than the cowpea–millet rotation, but the latter was more productive for millet production.

Isotopic dilution method with  $^{15}N$  was used to determine the nitrogen fixed by cowpea using pearl millet as a non-fixing crop. Nitrogen derived from the atmosphere by cowpea varied from 65 to 89% and the total nitrogen fixed by cowpea depended on the level of soil fertility improvement (Table 7). The quantity of nitrogen fixed by



**Figure 4. Long-term crop residue management at Sadoré, Niger, 1996.**

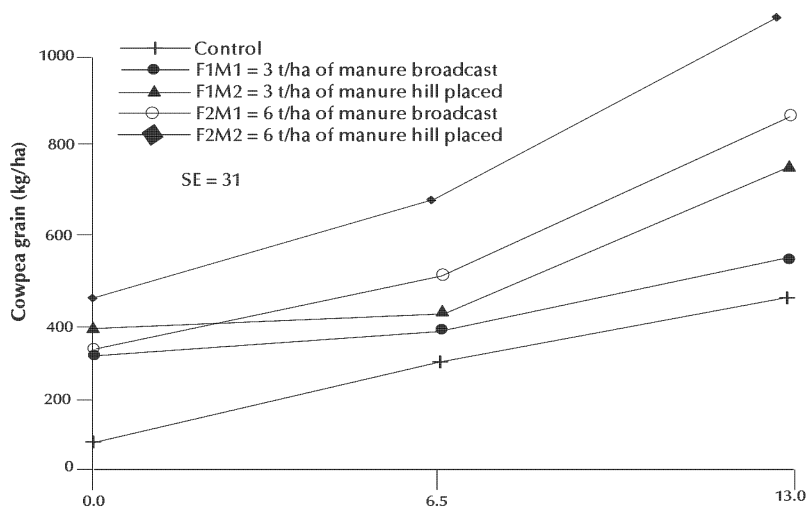
Source: Bationo et al. (2000).

(2 t/ha of crop residue was applied as mulch in crop residue treatment and 4 t/ha of crop residue was applied as mulch in the crop residue plus fertilizer treatment; fertilizer was applied at 30 kg N/ha and 13 kg P/ha).

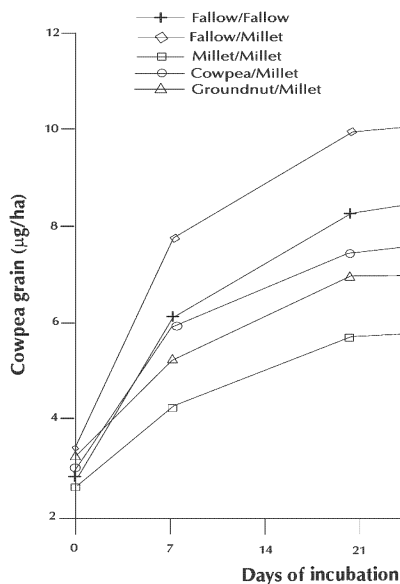
cowpea varied from 26 kg/ha in the control plot to 87 kg/ha in the treatment where the soils were amended with mineral and agronomic plant nutrients.

In order to determine  $^{15}\text{N}$  recovery from different cropping systems, labeled nitrogen fertilizers were applied to microplots where pearl millet was grown continuously (M–M) in rotation with cowpea (C–M), in rotation with groundnut (G–M), intercropped with cowpea (C/M–C/M), and intercropped with groundnut (G/M–G/M). Nitrogen-use efficiency increased from 20% in continuous pearl millet cultivation to 28% when pearl millet was rotated with cowpea (Bationo, unpublished data). Nitrogen derived from the soil was better used in rotation systems than with continuous millet cultivation.

In another trial on interaction between phosphorus fertilizers and different cropping systems, the application of P had a significant effect on yield of cowpea and pearl millet and rotation performed better than continuous cultivation of both crops (Fig. 7). A higher level of organic carbon was also found in the rotation systems compared to the continuous cropping systems, probably due in part to fallen cowpea leaves (Fig. 8).



**Figure 5.** Effects of fertilizer and manure placement on cowpea grain yield, Karabedji, 1999. Source: Bationo (unpublished data).

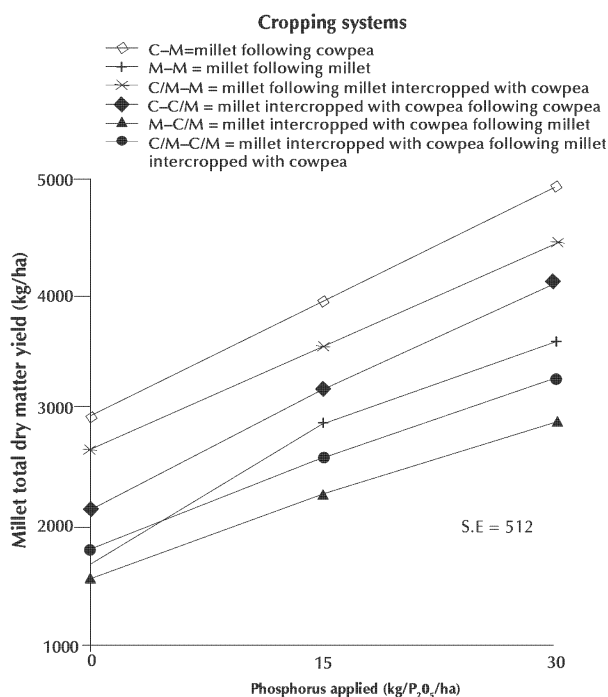


**Figure 6.** Relationship between cumulative mineral nitrogen and time of incubation of soils from different crop rotations pooled over three sites. Source: Bationo and Ntare (2000).

**Table 7. Nitrogen derived from the air (Nd<sub>fa</sub>) and total N fixed by cowpea stover using <sup>15</sup>N dilution technique, Sadoré, Niger, (1991 rainy season).**

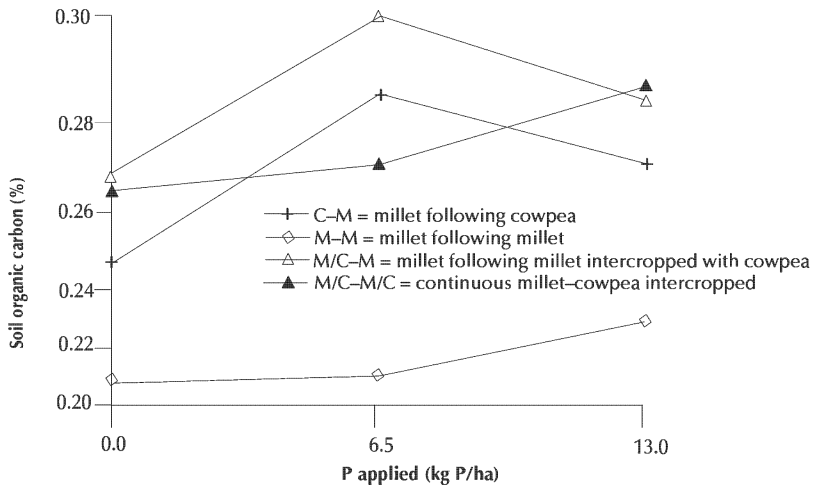
| Treatment  | Yield (t/ha) | N (%) | N yield (kg/ha) | NdFF (%) | Nd <sub>fa</sub> (%) | N fixed (kg/ha) |
|------------|--------------|-------|-----------------|----------|----------------------|-----------------|
| Control    | 1.75         | 2.18  | 38              | 2.43     | 65                   | 26              |
| Molybdenum | 3.08         | 2.28  | 71              | 1.37     | 80                   | 58              |
| Carbofuran | 2.58         | 2.19  | 57              | 2.04     | 71                   | 41              |
| Manure     | 2.42         | 2.44  | 60              | 0.79     | 89                   | 53              |
| Phosphorus | 3.58         | 2.01  | 65              | 1.56     | 78                   | 51              |
| Complete   | 3.75         | 2.66  | 100             | 0.80     | 89                   | 89              |
| SE         | ±0.47        | ±0.09 | ±10.39          | ±0.18    | ±2.56                | ±9.06           |
| CV (%)     | 28           | 6     | 27              | 20       | 6                    | 29              |

Source: Bationo (unpublished data).

**Figure 7. Effects of phosphorus and nitrogen on different cropping systems over four years, Sadoré, Niger.**

Source: Bationo (unpublished data).

The application of phosphorus, nitrogen, crop residue, ridging, and rotation of pearl millet with cowpea was evaluated to determine phosphorus-use efficiency. The results showed that soil productivity of the sandy Sahelian soils can be significantly increased with the adoption of improved crop and soil management technologies. Whereas the absolute control recorded 33 kg/ha of grain yield, 1829 kg was obtained when phosphorus, nitrogen, and crop residue were applied to plots that were ridged and in rotation



**Figure 8. Effects of phosphorus and cropping system on soil organic carbon, after four years of cultivation, Sadoré, Niger, 1995.**

Source: Bationo (unpublished data).

with cowpea. The plots without rotation yielded 1146 kg/ha. Results indicated that for grain yield, phosphorus-use efficiency increased from 46 kg/kg P with only phosphorus application, to 133 kg/kg phosphorus when phosphorus was combined with nitrogen and crop residue application and the crop was planted on ridges (Table 8).

## Conclusion and research opportunities

In the traditional cropping systems, cowpea is grown between cereals at very low density, as the farmers' primary goal is to produce cereal for family subsistence, cowpea being an additional benefit. This means that farmers need to be assured of sufficient cereal harvest to feed their families before integrating more cowpea in the cropping systems. Cowpea grain yield in the mixed systems is very low, varying between 50 and 300 kg/ha in marked contrast to over 2000 kg/ha on-station and by large-scale commercial enterprises in sole cropping. In addition to the low planting densities, pests and disease control, the inherent low fertility of the soil in the WASAT (particularly phosphorus) is one of the major constraints to cowpea production. Thus, soil fertility replenishment should be an integral part of any program aimed at reversing the downward trend in cowpea production and the conservation of the environment.

Phosphorus is the most limiting plant nutrient for cowpea production in the WASAT and there is ample evidence that indicates marked differences between cowpea genotypes for phosphorus uptake. Understanding the factors affecting phosphorus uptake such as the ability of plants to (i) solubilize soil P through acidification of the rhizosphere and the release of chelating agents and phosphate enzymes, (ii) explore a large soil volume, and (iii) absorb phosphorus from low phosphorus solution would help increase cowpea production and yield in the semiarid tropics.

The available and total phosphorus values are very low in the region. With these extreme low values of total phosphorus, selecting cultivars adapted to low phosphorus condition



**Table 8. Effect of mineral fertilizers, crop residue (CR), ridging, and crop rotation on pearl millet and phosphorus-use efficiency (PUE) wastes, Sadoré, Niger (1998 rainy season).**

| Treatment                      | Without CR, without N |                |                         |                | Without CR, without N |                |                         |                | Without CR, without N |                |                         |                | Without CR, without N |              |                         |                |
|--------------------------------|-----------------------|----------------|-------------------------|----------------|-----------------------|----------------|-------------------------|----------------|-----------------------|----------------|-------------------------|----------------|-----------------------|--------------|-------------------------|----------------|
|                                | TDM<br>Yield<br>kg/ha | PUE<br>kg/ha P | Grain<br>Yield<br>kg/ha | PUE<br>kg/ha P | TDM<br>Yield<br>kg/ha | PUE<br>kg/ha P | Grain<br>Yield<br>kg/ha | PUE<br>kg/ha P | TDM<br>Yield<br>kg/ha | PUE<br>kg/ha P | Grain<br>Yield<br>kg/ha | PUE<br>kg/ha P | TDM<br>Yield<br>kg/ha | PUE<br>kg/ha | Grain<br>Yield<br>kg/ha | PUE<br>kg/ha P |
| Control                        | 889                   |                | 33                      |                | 2037                  |                | 58                      |                | 995                   |                | 61                      |                | 1471                  |              | 98                      |                |
| 13 kg P/ha                     | 2704                  | 140            | 633                     | 46             | 4339                  | 177            | 1030                    | 75             | 4404                  | 185            | 726                     | 51             | 240                   | 4594         | 1212                    | 86             |
| 13 kg P/ha + ridge             | 2675                  | 137            | 448                     | 32             | 4057                  | 155            | 946                     | 68             | 3685                  | 210            | 785                     | 56             | 4530                  | 235          | 1146                    | 81             |
| 13 kg P/ha + rotation          | 5306                  | 340            | 1255                    | 94             | 6294                  | 327            | 1441                    | 106            | 5392                  | 338            | 1475                    | 109            | 6124                  | 358          | 1675                    | 121            |
| 13 kg/ P/ha + ridge + rotation | 5223                  | 333            | 1391                    | 104            | 5818                  | 291            | 1581                    | 117            | 6249                  | 404            | 1702                    | 126            | 7551                  | 468          | 1829                    | 133            |
| SE                             | 407                   |                | 407                     |                | 407                   |                | 407                     |                | 407                   |                | 407                     |                | 407                   |              | 407                     |                |

CR: Crop residue; N: Nitrogen; TDM: total dry matter; PUE: phosphorus-use efficiency (kg grain/kg P); yield: g/ha).

Source: Bationo (unpublished data).

would not be feasible as one cannot mine what is not there. Direct application of indigenous PR can be an economic alternative to the use of more expensive imported water-soluble P fertilizers. The effectiveness of mycorrhizal in utilizing soil P has been well documented (Silberbush and Barber 1983; Lee and Wani 1991; Daft 1991). An important future research opportunity is the selection of cowpea genotypes that can efficiently associate with vesicular-arbuscular mycorrhizal (VAM) for better utilization of P from applied PR.

Cereal-cowpea rotations have led to increased cereal yields at many locations in the WASAT. Factors such as mineral nitrogen (VAM) for P nutrition improvement and plant parasitic nematodes have been identified as mechanisms accelerating the enhanced yield of cereals in rotation with cowpea. Most of the research quantified the aboveground N fixed by different cowpea cultivars, but very little is known about the below-ground N fixed by cowpea. In the WASAT, most of the aboveground cowpea biomass is used for animal feed and not as green manure. Further research should focus more on on-farm quantification of the below-ground N fixed by cowpea in order to identify the best cultivar for soil N buildup.

The identification and alleviation of technical and socioeconomic constraints in order to increase cowpea in the present cropping systems needs attention in future. As a cash crop, farmers will increase their purchasing power to acquire external inputs such as fertilizers. The enhancement of cowpea in the present cropping systems will not only improve the soil conditions for the succeeding cereal crop, but will provide good quality livestock feed, and the manure produced will be of better quality for soil fertility improvement.

## References

- Aguilar, A.S. and A. Van Diest. 1981. Rock-phosphate mobilization included by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. *Plant and Soil* 61: 27–42.
- Arihara, J. and Y. Ohwaki. 1989. Estimation of available phosphorus in vertisol and alfisol in view of root effects on rhizosphere soil *in* XI Colloquium on plant nutrition-physiology and applications, 30 July to 4 August 1989. Wageningen, The Netherlands.
- Bagayoko, M., S.C. Mason, S. Traore, and K.M. Eskridge. 1996. Pearl millet/cowpea cropping systems yield and soil nutrient levels. *African Crop Science Journal* 4: 453–462.
- Bagayoko, M., A. Buerkert, G. Lung, A. Bationo, and V. Romheld. 2000. Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: soil mineral nitrogen, mycorrhizae, and nematodes. *Plant and Soil* 218: 103–116.
- Bationo, A. and A.U. Mokwunye. 1991. Alleviating soil fertility constraints to increased crop production in West Africa. The experience in the Sahel. *Fertilizer Research* 29: 95–115.
- Bationo, A., A. Buerkert, M.P. Sedogo, B.C. Christianson, and A.U. Mokwunye. 1995. A critical review of crop residue use as soil amendment in the West African semi-arid tropics. Pages 305–322 *in* Livestock and sustainable nutrient cycling in mixed farming systems of sub-Saharan Africa, edited by J.M. Powell, S. Fernandez Rivera, T.O. Williams, and C. Renard. Proceedings International Conference, ILCA, Addis Ababa, Ethiopia.
- Bationo, A., F. Lompo, and S. Koala. 1998. Research on nutrient flows and balances in West Africa: State-of-the-art. Pages 19–36 *in* Nutrient balances as indicators of production and sustainability in sub-Saharan African agriculture, edited by E.M.A. Smaling. *Agriculture, Ecosystems and Environment* 71: 1, 2, and 3.
- Bationo, A. and B.R. Ntare. 2000. Rotation and nitrogen fertilizer effects on pearl millet, cowpea, and groundnut yield and soil chemical properties in a sandy soil in the semi arid tropics, West Africa. *Journal of Agricultural Science, (Cambridge)* 134: 277–284.
- Bationo, A., S.P. Wani, C.L. Biielders, P.L.G. Vlek, and U. Mokwunye. 2000. Crop residue and fertilizer management to improve soil organic carbon content, soil quality, and productivity in the desert margins of West Africa. Pages 117–145 *in* Global climate change and tropical ecosystems, edited

- by R. Lal, J.M. Kimble, and B.A. Stewart. Advances in Soil Science. CRC Press, Washington DC, USA.
- Blumenthal, M.J., V.P. Quach, and P.G.E. Searle. 1982. Effect of soybean population density on soybean yield, nitrogen accumulation, and residual nitrogen. *Australian Journal of Experimental Agriculture* 28: 99–106.
- Breman, H. and C.T. de Wit. 1983. Rangeland productivity and exploitation in the Sahel. *Science* 221: 1341–1347.
- Buerkert, A., C. Haate, M. Ruckwied, and H. Marschner. 1998. Phosphorus application affects the nutritional quality of millet grain in the Sahel. *Field Crops Research* 57: 223–235.
- Chien, S.H. and L.L. Hammond. 1978. A simple chemical method for evaluating the agronomic potential of granulated phosphate rock. *Soil Science Society of America Journal* 42: 615–617.
- Curl, E.A. 1963. Control of plant diseases by rotation. *Botanical Review* 29: 413–479.
- Daft, M.J. 1991. Influences of genotypes, rock phosphate, and plant densities on mycorrhizal development and the growth responses of five different crops. *Agriculture, Ecosystems and Environment* 35: 151–169.
- Dwivedi, R.S., N.S. Randwawa, and R.L. Bansal. 1975. Phosphorus-zinc interaction. I. Sites of immobilization of zinc in maize at high levels of phosphorus. *Plant and Soil* 43: 639–648.
- Fussell, L.K. and P.G. Serafini. 1985. Associations de cultures dans les zones tropicales semi-arides d'Afrique de l'Ouest: strategies de recherche anterieures et futures. (In Fr.) Pages 254–278 in *Technologies appropriees pour les paysans des zones semi-arides de l'Afrique de l'Ouest*, edited by H.W. Ohm and J.G. Nagy. Purdue University, West Lafayette, Indiana, USA.
- Hafner, H., B.J. Ndunguru, A. Bationo, and H. Marshner. 1992. Effect of nitrogen, phosphorus, and molybdenum applications on growth and symbiotic N<sub>2</sub> fixation of groundnut in an acid sandy soil in Niger. *Fertilizer Research* 156: 164–176.
- Hedley, M.J., P.H. Nye, and R.E. White. 1982. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerad) seedlings. II. Origin of the pH change. *New Phytologist* 91: 31–44.
- Israel, D.N. 1987. Investigation of the role of phosphorus in symbiotic nitrogen fixation. *Plant Physiology* 84: 835–840.
- Khan, A.A. and G.K. Zende. 1977. The site for Zn-P interactions in plants. *Plant and Soil* 46: 259–262.
- Keeney, D.R. 1982. Nitrogen availability indices. Pages 711–730 in *Methods of soils analysis*, edited by A.L. Payne et al. American Society of Agronomy, Madison, Wisconsin, USA.
- Khasawneh, F.E. and E.C. Doll. 1978. The use of phosphate rock for direct application to soils. *Advanced Agronomy* 30: 155–206.
- Kirk, G.J.D. and P.H. Nye. 1986. A simple model for predicting the rate of dissolution of sparingly soluble calcium phosphate in soil. I. The basic model. *Journal of Soil Science* 37: 529–540.
- Klaij, M.C. and B.R. Ntare. 1995. Rotation and tillage effects on yield of pearl millet (*Pennisetum glaucum*) and cowpea (*Vigna unguiculata*), and aspects of crop water balance and soil fertility in semi-arid tropical environment. *Journal of Agriculture Science (Cambridge)* 124: 39–44.
- Lee, K.K. and S.P. Wani. 1991. Possibilities for manipulating mycorrhizal associations in crops. Pages 107–166 in *Phosphorus nutrition of grain legumes in the semi-arid tropics*, edited by C. Johansen, K.K. Lee, and K.L. Sahrawat. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, AP 502324, India.
- Lehr, J.R. and G.H. McClellan. 1972. A revised laboratory reactivity scale for evaluating phosphate rocks for direct application. *Bulletin* 43, Tennessee Valley Authority, Muscle Shoals, Alabama, USA.
- Mahamane, I., A. Bationo, F. Seyni, and Z. Hamidou. 1997. Acquis récents des recherches sur les phosphates naturels du Niger. Pages 73–78 in *Soil fertility management in West African land use systems*, edited by G. Renard, A. Neef, K. Becker, and M. von Oppen. Margraf Verlag, Weikersheim, Germany.

- Mokwunye, A.U., and P.L.G. Vlek (editors). 1986. Management of nitrogen and phosphorus fertilizers in sub-Saharan Africa. Martinus Nijhoff, Dordrecht, The Netherlands.
- Muleba, N., C. Dabire, J.B. Suh, I. Drabo, and J.T. Ouedraogo. 1997. Technologies for cowpea production based on genetic and environmental manipulations in the semi-arid tropics. Pages 195–206 *in* Technology options for sustainable agriculture in sub-Saharan Africa, edited by T. Bezuneh, A.M. Emechebe, J. Sedgo, and M. Ouedraogo. Publication of the Semi-Arid Food Grain Research and Development Agency (SAFGRAD) of the Scientific, Technical and Research Commission of OAU, Ouagadougou, Burkina Faso.
- Nicou, R.N. 1977. Le travail du sol dans les terres exondées du Sénégal. Motivations contraintes. ISRA–CNRA, Bombay, India. 51 pp.
- Norman, D.W. 1974. Rationalizing mixed cropping under indigenous conditions: the example of northern Nigeria. *Journal of Development Studies* 11: 3–21.
- Ntare, B.R. 1989. Intercropping morphologically different cowpea with pearl millet in a short season environment in the Sahel. *Experimental Agriculture* 26: 41–47.
- Ohwaki, Y. and H. Hirata. 1992. Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid levels in roots. *Soil Science and Plant Nutrition* 38: 235–243.
- Penning de Vries, F.W.T. and M.A. Djitéye. 1991. La productivité des pâturages sahéliens: une étude des sols, de la végétation et de l'exploitation de cette ressource naturelle. Center for Agricultural Publishing and Documentation (Pudoc-DLO), Wageningen, The Netherlands.
- Pieri, C. 1986. Fertilization des cultures vivrières et fertilité des sols en agriculture paysanne subsaharienne. *Agronomie Tropicale* 41: 1–20.
- Pieri, C. 1989. Fertilité des terres de savanes : Bilan de trente ans de recherches et de développement, Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) et le Ministère de la Coopération, Paris, France. 443 pp.
- Reddy, K.C., P. Visser, and P. Buekner. 1992. Pearl millet and cowpea yields in sole and intercrop system, and their after-effects on soil and crop productivity. *Field Crops Research* 28: 315–326.
- Rupela, O.P. and M.C. Saxena. 1987. Nodulation and nitrogen fixation in chickpea. Pages 191–206 *in* The Chickpea Farnham Royal UK, edited by M.C. Saxena and K.B. Singh. Commonwealth Agricultural Bureaux International and International Center for Agricultural Research in the Dry Areas.
- Sedogo, M.P. 1993. Evolution des sols ferrugineux lessivés sous culture: influence des modes de gestion sur la fertilité: Thèse de Doctorat Es-Sciences, Abidjan, Université Nationale de Côte d'Ivoire.
- Shetty, S.V.R., B.R. Ntare, A. Bationo, and C. Renard. 1995. Millet and cowpea in mixed farming of the Sahel. A review of strategies for increased productivity and sustainability. Pages 293–304 *in* Livestock and sustainable nutrient cycling in mixed farming systems of sub-Saharan Africa, edited by J.M. Powell, S. Fernandez Rivera, T.O. Williams, and C. Renard. Proceedings International Conference, ILCA, Addis Ababa, Ethiopia.
- Silberbush, M. and S.A. Barber. 1983. Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic-mathematical model. *Plant and Soil* 74: 93–100.
- Spurgeon, W.I., and P.H. Grimson. 1965. Influence of cropping systems on soil properties and crop production. Mississippi Agricultural and Forestry Experiment Station Bulletin No. 710.
- Steiner, K.G. 1984. Intercropping in tropical smallholder agriculture with special reference to West Africa. Stein, West Germany. 304 pp.

- Stoop W.A. and J.P.V. Staveren. 1981. Effects of cowpea in cereal rotations on subsequent crop yields under semi-arid conditions in Upper Volta. Pages 653–657 in *Biological nitrogen fixation technology for tropical agriculture*, edited by P.C. Graham and S.C. Harris. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- Stoorvogel, J.J. and E.M.A. Smaling. 1990. Assessment of soil nutrient depletion in sub-Saharan Africa 1983–2000. Report 28, The Winand Staring Centre for Integrated Land, Soil and Water Research (SC-DLO), Wageningen, The Netherlands.
- Stukenholtz, D.D., R.J. Olsen, G. Gogan, and R.A. Olsen. 1966. On the mechanism of phosphorus-zinc interaction in corn nutrition. *Soil Science Society of America Proceedings* 30: 759–763.
- Swinton, S.M., G. Numa, and L.A. Samba. 1984. Les cultures associées en milieu paysan dans deux régions du Niger: Filingue et Madarounfa. Pages 183–194 in *Proceedings of the Regional Workshop on Intercropping in the Sahelian and Sahelo-Sudanian zones of West Africa*, 7–10 November 1984, Institute du Sahel, Niamey, Niger.
- Takkar, P.N., M.S. Mann, R.L. Bansal, N.S. Randwawa, and H. Singh. 1976. Yield and uptake response of corn to zinc as influenced by phosphorus fertilization. *Agronomy Journal* 68: 942–946.
- Van der Heide, J. (editor). 1989. Nutrient management for food crop production in tropical farming systems. Institute for Soil Fertility Research (IB-DLO), Haren, The Netherlands.
- Van Reuler, H. and B.H. Jansen. 1989. Nutritional constraints in secondary vegetation and upland rice in southwest Ivory Coast. Pages 371–382 in *Mineral nutrients in tropical forest and savanna ecosystems*, edited by J.H. Proctor. Special Publication 9 of The British Ecological Society, Blackwell Scientific Publications, Oxford, England.
- Youngdhal, L.J., L.V. Svec, W.C. Liebhardt, and M.R. Tel. 1977. Changes in the zinc-65 distribution in corn root tissue with phosphorus variable. *Crop Science* 17: 66–69.

## Differential response of cowpea lines to application of P fertilizer

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### Abstract

Phosphorus is important for cowpea production in many tropical African soils with inherent low P fertility. Most farmers in Africa, however, do not have access to P fertilizer. Selection of cowpea lines that produce good yield under low soil P or those with high P-use efficiency can be a low input approach to solving this problem. Pot and field trials were conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, southwestern Nigeria, to assess the differential P responses of cowpea lines obtained from the germplasm collection at IITA. Thirty-five lines were assessed for P response in a pot trial using surface (0–15 cm) soil of a P-deficient Alfisol (Oxic Paleustalf). Seventeen lines (comprising of 12 lines selected from the pot trial and five not included in the pot trial) were further assessed in the field. In the pot trial, P fertilizer significantly enhanced shoot, root, and grain dry weights. More than 60% of the cowpea lines also had greater nodule weight with P. Response of some of the cowpea lines was more pronounced for shoots than roots. In the field trial, more than 50% of the cowpea lines showed significant response to P. Compared with the pot trial, there were considerable variations in the pattern of responses of the cowpea lines to P. The cowpea lines were classified on the basis of their dry grain weights in the pot trial into four groups. Based on our results, we recommend that lines IT 90K-284-2, IT 96D-724, and IT 93K-637-1 can be selected for further testing without P fertilizer. Lines IT 87D-941-1, IT 86D-719, and Dan Ila may perform very well without P fertilizer and give a high return when P is applied. When P fertilizer is available, line IT 87D-941-1 is recommended. These varieties should be tested at multiple sites to truly extend the results to breeding cowpea lines that could be targeted towards various soil P conditions.

### Introduction

Phosphorus (P) is among the most needed elements for crop production in many tropical soils. However, many tropical soils are P-deficient (Adetunji 1995). The deficiency can be so acute in some soils of the savanna zone of western Africa that plant growth ceases as soon as the P stored in the seed is exhausted (Mokwunye et al. 1986). Soil P-deficiencies primarily result from either inherent low levels of soil P or depletion of P through cultivation.

Phosphorus, although not required in large quantities, is critical to cowpea yield because of its multiple effects on nutrition (Muleba and Ezumah 1985). It not only increases seed yields but also nodulation (Luse et al. 1975; Kang and Nangju 1983) and thus N fixation. Phosphorus application influences the contents of other nutrients in cowpea leaves (Kang

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and Nangju 1983) and seed (Omuetti and Oyenuga 1970). Application of P is therefore recommended for cowpea production on soils low in P (Sellschop 1962; Rachie and Roberts 1974). However, inorganic P fertilizers are often expensive and not readily available to resource-poor farmers. Furthermore, fertilizer P can be fixed into forms unavailable to plants by Fe and Al oxides found in tropical soils (Sample et al. 1980). Application of inorganic P fertilizers can therefore not be relied upon to adequately alleviate P-deficiency for improved cowpea production. Genotypic differences in the effect of P on nodulation (Ankomah et al. 1995) and yield (Jain et al. 1986; Tenebe et al. 1995; Sanginga et al. 2000) of cowpea have been previously reported. However, mechanisms by which these cowpea varieties exhibit differential abilities to grow at low or high P supply are not completely understood. A better understanding of cowpea varietal differences in P nutrition may help in breeding new lines for areas where fertilizers are scarce and expensive. One of the options for overcoming the reliance on P fertilizers for improved cowpea production in P-deficient soils would be the selection of low soil P-tolerant cowpea lines that could access a greater proportion of the total soil P pool. There is, however, a paucity of information on variability in P responses among cowpea varieties. This paper reports the results of the responses of cowpea lines obtained from the germplasm collection at the International Institute of Tropical Agriculture (IITA) to P fertilizer.

## Materials and methods

### Pot trial

The trials were carried out at IITA, Ibadan, southwestern Nigeria. For the pot trial, surface (0–15 cm) soil of a P-deficient Alfisol (Oxic Paleustalf) that was collected from Fashola village, Oyo State, southwestern Nigeria was used. The soil has the following properties: pH-H<sub>2</sub>O 6.0 organic C; 6.5 g/kg total N; 0.5 g/kg extractable P; 7.5 mg/kg exchangeable (cmol (+)/kg soil) K 0.26; Ca 3.68, and Mg 0.96, respectively.

The experiment was a factorial combination of 35 cowpea lines and two P application rates in a randomized complete block design with three replications. Table 1 lists 35 cowpea lines from the germplasm collection at IITA grown in soil (3.5 kg/pot) with two levels of phosphorus (SSP): 0 (control) and 30 kg P<sub>2</sub>O<sub>5</sub>/ha. All the pots received basal dressing of 50 K (KCl); 50 Mg (MgSO<sub>4</sub>·7H<sub>2</sub>O); 5 Zn (ZnSO<sub>4</sub>); 10 Mn (MnCl<sub>2</sub>·4H<sub>2</sub>O); 5 Cu (CuSO<sub>4</sub>); 5 Mo [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O]; and 5 P (NaH<sub>2</sub>PO<sub>4</sub>·7H<sub>2</sub>O) in mg/kg soil.

Four seeds were sown in each pot on 5 October 1998. Two weeks after planting, the seedlings were thinned to two plants per pot. A mixture of Karate® 2.5 E.C. (a.i. 25 g lambda-cyhalothrin per liter; 4 ml in 1 liter of water) and Vertimec® (a.i. 1.8% w/v abamectin (18 g/liter; 1.5 ml in 1 liter of water) insecticides was sprayed twice to control insect pests during the experiment. The plants were grown to maturity. At maturity, pods were harvested from all pots. Dry pods were threshed by hand and grain weight determined. The plant shoots were cut at ground level. Roots were washed free of soil with water, using a screen with 1-mm openings. Nodules were collected from the roots and counted. Plant shoots, roots, and nodules were oven dried at 65 °C for 48 hours for dry weight determination. Litter was collected as part of the shoot biomass. Plant shoots were ground in a Wiley mill to pass through a 60-mesh size sieve and later analyzed for N and P concentrations using the procedure described by Okalebo et al. (1993). Data collected were subjected to analysis of variance using the SAS package (SAS 1985).

Table 1. Effect of P application on shoot, root, and grain dry weights (g/pot) of 35 cowpea lines.

| Lines          | Shoot |     |             | Root |      |             | Grain |     |                |
|----------------|-------|-----|-------------|------|------|-------------|-------|-----|----------------|
|                | -P    | +P  | Mean        | -P   | +P   | Mean        | -P    | +P  | Mean           |
| IT 90K-277-2   | 3.6   | 4.2 | 3.9 efg     | 0.57 | 0.78 | 0.68 defghi | 0.1   | 1.2 | 0.7 hijklmn    |
| IT 90K-284-2   | 2.1   | 6.5 | 4.3 cdefg   | 0.49 | 1.01 | 0.75 cdefg  | 0.2   | 2.6 | 1.4 abcd       |
| IT 90K-76      | 2.6   | 5.1 | 3.8 g       | 0.34 | 0.76 | 0.55 j      | 0.4   | 1.7 | 1.1 defghijklm |
| IT 90K-59-2    | 3.7   | 4.1 | 3.9 fg      | 0.68 | 0.78 | 0.73 cdefgh | 0.2   | 1.1 | 0.7 ghijklmn   |
| IT 93K-513-2   | 3.0   | 4.8 | 3.9 fg      | 0.45 | 0.73 | 0.59 ij     | 0.5   | 2.4 | 1.5 abcd       |
| IT 93K-693-2   | 2.8   | 5.5 | 4.2 defg    | 0.59 | 0.79 | 0.69 defghi | 1.0   | 2.6 | 1.8 a          |
| IT 93K-734     | 4.1   | 4.9 | 4.5 bcdefg  | 0.44 | 0.89 | 0.67 efghij | 0.3   | 1.2 | 0.8 fghijklmn  |
| IT 93K-452-1   | 2.5   | 5.9 | 4.2 cdefg   | 0.42 | 0.82 | 0.62 hij    | 0.4   | 2.2 | 1.3 abcdefg    |
| IT 93K-637-1   | 3.5   | 6.3 | 4.9 abcdefg | 0.67 | 1.10 | 0.89 ab     | 0.6   | 2.6 | 1.6 abc        |
| IT 94K-437-1   | 4.0   | 7.2 | 5.6 abc     | 0.57 | 0.70 | 0.64 ghij   | 0.6   | 1.8 | 1.2 bcdefghi   |
| IT 94K-440-3   | 4.3   | 6.8 | 5.5 abcd    | 0.44 | 0.86 | 0.65 fghij  | 0.6   | 2.5 | 1.5 abcd       |
| IT 95K-1406    | 2.8   | 6.1 | 4.5 bcdefg  | 0.55 | 0.75 | 0.65 fghij  | 0.1   | 1.3 | 0.7 ghijklmn   |
| IT 95K-1156-3  | 3.7   | 6.3 | 5.0 abcdefg | 0.53 | 0.87 | 0.70 defghi | 0.2   | 0.8 | 0.5 mn         |
| IT 95K-1090-12 | 2.8   | 5.8 | 4.3 cdefg   | 0.63 | 0.94 | 0.78 bcde   | 0.2   | 1.1 | 0.7 ghijklmn   |
| IT 95K-1096-7  | 4.0   | 6.5 | 5.2 abcdefg | 0.48 | 1.02 | 0.75 cdefg  | 0.1   | 0.8 | 0.5 mn         |
| IT 95K-1464    | 3.1   | 5.2 | 4.2 defg    | 0.63 | 0.75 | 0.69 defghi | 0.2   | 1.4 | 0.8 fghijklmn  |
| IT 95K-1090-1  | 3.3   | 7.3 | 5.3 abcdef  | 0.78 | 1.08 | 0.93 a      | 0.1   | 0.8 | 0.4 n          |
| IT 95K-105-2   | 3.5   | 7.0 | 5.3 abcdef  | 0.73 | 1.10 | 0.92 a      | 0.3   | 1.4 | 0.8 fghijklmn  |
| IT 95K-1091-3  | 3.2   | 6.3 | 4.8 abcdefg | 0.78 | 0.70 | 0.74 cdefg  | 0.1   | 1.4 | 0.8 fghijklmn  |
| IT 95K-1095-2  | 3.3   | 8.4 | 5.9 ab      | 0.63 | 0.95 | 0.79 bcd    | 0.02  | 1.1 | 0.5 mn         |

.....continued



Table 1. (continued)

| Lines          | Shoot |     |             | Root |      |             | Grain |     |                 |
|----------------|-------|-----|-------------|------|------|-------------|-------|-----|-----------------|
|                | -P    | +P  | Mean        | -P   | +P   | Mean        | -P    | +P  | Mean            |
| IT 95K-1088-4  | 3.1   | 7.1 | 5.1 abcdefg | 0.64 | 0.87 | 0.75 cdefg  | 0.03  | 1.3 | 0.7 ghijklmn    |
| IT 95K-1384    | 3.2   | 5.6 | 4.4 cdefg   | 0.46 | 0.83 | 0.65 fghij  | 0.1   | 1.3 | 0.7 ghijklmn    |
| IT 95K-1543    | 3.8   | 7.1 | 5.5 abcd    | 0.73 | 1.12 | 0.93 a      | 0.2   | 2.0 | 1.1 bcdefghijkl |
| IT 95K-1491    | 4.3   | 5.0 | 4.7 abcdefg | 0.70 | 0.85 | 0.78 bcde   | 0.4   | 2.4 | 1.4 abcde       |
| IT 96D-666     | 2.8   | 6.3 | 4.5 bcdefg  | 0.54 | 0.73 | 0.64 ghij   | 0.3   | 1.7 | 1.0 defghijklmn |
| IT 96D-740     | 3.2   | 6.1 | 4.7 abcdefg | 0.72 | 0.66 | 0.69 defghi | 0.2   | 1.5 | 0.8 efghijklmn  |
| IT 96D-748     | 2.5   | 6.1 | 4.3 cdefg   | 0.70 | 0.78 | 0.74 cdefg  | 0.1   | 2.3 | 1.2 bcdefghi    |
| IT 96D-757     | 3.2   | 6.2 | 4.7 abcdefg | 0.51 | 0.97 | 0.74 cdefg  | 0.3   | 1.9 | 1.1 bcdefghijkl |
| IT 96D-759     | 4.5   | 7.6 | 6.0 a       | 0.54 | 1.13 | 0.84 abc    | 0.1   | 1.1 | 0.6 klmn        |
| IT 89KD-374-57 | 3.5   | 4.5 | 4.0 efg     | 0.49 | 0.75 | 0.62 hij    | 0.3   | 1.1 | 0.7 ghijklmn    |
| IT 86D-719     | 3.7   | 5.5 | 4.6 abcdefg | 0.66 | 0.87 | 0.77 cdef   | 0.4   | 2.9 | 1.7 ab          |
| IT 86D-715     | 3.3   | 6.9 | 5.1 abcdefg | 0.56 | 0.85 | 0.71 defghi | 0.3   | 2.1 | 1.2 bcdefgh     |
| IT 89KD-288    | 3.0   | 7.1 | 5.1 abcdefg | 0.64 | 0.66 | 0.65 fghij  | 0.5   | 0.7 | 0.6 jklmn       |
| IT 87D-941-1   | 3.4   | 5.8 | 4.6 bcdefg  | 0.64 | 0.81 | 0.73 cdefgh | 0.4   | 1.9 | 1.1 bcdefghijkl |
| Dan IIa        | 2.5   | 5.2 | 3.8 g       | 0.62 | 0.73 | 0.68 defghi | 0.02  | 2.6 | 1.3 abcdef      |
| Mean           | 3.3   | 6.1 |             | 0.59 | 0.86 |             | 0.3   | 1.7 |                 |

Means followed by the same letter(s) within a column are not significantly different (DMRT) at 5% probability level.

## **Field trial**

Seventeen cowpea lines were selected from the pot trial as follows: six lines with high grain yield without P and high productivity with P, IT 86D-719, IT 94K-437-1, IT 87D-941-1, IT 94K-440-3, IT 93K-693-2, and IT 93K-637-1 (Category 1); five with low grain yield without P and high productivity with P, Dan Ila, IT 96D-748, IT 90K-284-2, IT 96D-757, and IT 86D-715 (Category 2); one line with high grain yield without P and low productivity with P, IT 89KD-288 (Category 3); and five lines not included in the pot trial, IT 96D-739, IT 96D-772, IT 89KD-349, IT 97K-820-18, and IT 96D-724 (Category 4) were sown in weed-free plots measuring 3 m × 3 m on 25 August 1999. Planting distance was 0.75 m between and 0.20 m within the row with two seeds per hole. There were two factors; 17 cowpea lines and two phosphorus levels, 0 and 30 kg P/ha laid out as a factorial in a randomized complete block design (RCBD) with three replicates. The fertilizer (SSP) was band applied along the planting row at planting. The seedlings were later thinned to one per stand at two weeks after planting (WAP). The plots were weeded twice at three and six WAP. Karate insecticide was sprayed twice to control insect attack. The plants were grown to maturity. At maturity, when the pods were dry, they were threshed by hand. The grain was then oven-dried for 24 hours at 65 °C and weighed. Data collected were analyzed as in the pot trial.

## **Results**

### ***Pot trial***

#### *Shoot, grain, and root dry weights*

The effect of P fertilizer on shoot, grain, and root dry weights of cowpea lines is presented in Table 1. Generally, application of phosphorus fertilizer had positive effects on shoot, grain, and root dry weights. Variability among cowpea lines in shoot, grain, and root dry weight response to P was pronounced and more so for shoots than roots. Lines IT 96D-759 and IT 95K-1095-2 produced the highest shoot weight while lines IT 95K-1090-1, IT 95K-1052-2, and IT 95K-1543 had the highest root dry weights. Interactions between cowpea lines and P levels on root and grain weights were significant (Table 2).

#### *Total aboveground dry matter (TDM)*

TDM consists of the total of shoot and pod (including grains) dry weights. Phosphorus fertilizer had positive effects on TDM and the cowpea lines did not show significant variations in their responses of TDM to P (Table 2).

#### *Nodulation*

Generally, P fertilizer significantly enhanced nodule dry weights of the cowpea lines, but nodule number was depressed by P (Table 3). There were variations among the cowpea lines in the responses of nodulation to P. Interactions between cowpea lines and P levels on nodulation were significant (Table 2). Line IT 94K-437-1 produced the highest nodule weight.

#### *Nutrient accumulation*

Phosphorus fertilizer had positive effects on N and P accumulation by the cowpea lines (Table 4) and there was variation in the P accumulation but not N between varieties (Table 2).

**Table 2. Probabilities (P 0.05) of the F test for the analysis of variance for biomass, nodulation, and N and P content variables of cowpea lines.**

| Source              | TDM    | Shoot dry weight | Root dry weight | Nodule number | Nodule weight | Grain (pot) | Grain (field) | N yield | P yield |
|---------------------|--------|------------------|-----------------|---------------|---------------|-------------|---------------|---------|---------|
| Cowpea lines (C)    | 0.3714 | 0.1137           | 0.0001          | 0.0026        | 0.0001        | 0.0001      | 0.0001        | 0.0239  | 0.6319  |
| Phosphorus rate (P) | 0.0001 | 0.0001           | 0.0001          | 0.0018        | 0.0001        | 0.0001      | 0.2611        | 0.0001  | 0.0001  |
| C × P               | 0.3860 | 0.2539           | 0.0001          | 0.0291        | 0.0001        | 0.0092      | 0.1792        | 0.1274  | 0.8098  |

**Table 3. Effect of P application on nodulation of 35 cowpea lines.**

| Lines          | Nodule number (no./pot) |    |            | Nodule weight (mg/pot) |      |             |
|----------------|-------------------------|----|------------|------------------------|------|-------------|
|                | -P                      | +P | Mean       | -P                     | +P   | Mean        |
| IT 90K-277-2   | 15                      | 20 | 18 b cdefg | 6.5                    | 15.7 | 11.1 jklm   |
| IT 90K-284-2   | 18                      | 12 | 15 defg    | 13.1                   | 9.5  | 11.3 ijklm  |
| IT 90K-76      | 18                      | 15 | 16 cdefg   | 8.7                    | 15.9 | 12.3 hijklm |
| IT 90K-59-2    | 18                      | 18 | 18 abcdef  | 7.9                    | 16.8 | 12.4 hijklm |
| IT 93K-513-2   | 14                      | 13 | 14 fg      | 11.2                   | 25.3 | 18.3 cdef   |
| IT 93K-693-2   | 16                      | 14 | 15 defg    | 10.5                   | 15.9 | 13.3 ghijkl |
| IT 93K-734     | 12                      | 21 | 17 bcdefg  | 5.6                    | 12.7 | 9.2 lm      |
| IT 93K-452-1   | 18                      | 14 | 16 cdefg   | 16.7                   | 12.6 | 14.7 efghij |
| IT 93K-637-1   | 12                      | 14 | 13 fg      | 7.2                    | 12.9 | 10.1 klm    |
| IT 94K-437-1   | 16                      | 12 | 14 efg     | 16.4                   | 38.8 | 27.6 a      |
| IT 94K-440-3   | 17                      | 18 | 17 bcdefg  | 17.2                   | 18.9 | 18.1 cdef   |
| IT 95K-1406    | 17                      | 22 | 20 abcd    | 22.5                   | 21.4 | 21.9 bc     |
| IT 95K-1156-3  | 22                      | 16 | 19 abcdef  | 12.4                   | 18.3 | 15.4 efghij |
| IT 95K-1090-12 | 19                      | 14 | 17 bcdefg  | 17.3                   | 13.9 | 15.6 efghij |
| IT 95K-1096-7  | 25                      | 17 | 21 abc     | 16.9                   | 25.3 | 21.1 bcd    |
| IT 95K-1464    | 15                      | 15 | 15 defg    | 12.2                   | 24.9 | 18.6 cde    |
| IT 95K-1090-1  | 19                      | 27 | 23 a       | 22.6                   | 12.1 | 17.3 defg   |
| IT 95K-105-2   | 17                      | 13 | 15 defg    | 17.1                   | 25.7 | 21.4 bcd    |
| IT 95K-1091-3  | 27                      | 16 | 22 ab      | 12.7                   | 15.4 | 14.0 fghijk |
| IT 95K-1095-2  | 17                      | 18 | 18 bcdefg  | 12.9                   | 18.9 | 15.9 efgh   |
| IT 95K-1088-4  | 16                      | 11 | 14 fg      | 7.2                    | 12.1 | 9.6 klm     |
| IT 95K-1384    | 20                      | 17 | 18 abcdef  | 12.3                   | 12.3 | 12.3 hijklm |
| IT 95K-1543    | 18                      | 17 | 18 abcdef  | 11.1                   | 20.4 | 15.8 efghi  |
| IT 95K-1491    | 16                      | 14 | 15 defg    | 12.0                   | 20.3 | 16.2 efgh   |
| IT 96D-666     | 15                      | 16 | 16 defg    | 14.6                   | 11.4 | 13.0 ghijkl |
| IT 96D-740     | 18                      | 21 | 20 abcd    | 22.1                   | 25.7 | 23.9 ab     |
| IT 96D-748     | 16                      | 11 | 13 fg      | 22.2                   | 15.5 | 18.8 cde    |
| IT 96D-757     | 13                      | 14 | 14 fg      | 5.7                    | 14.4 | 10.1 klm    |
| IT 96D-759     | 19                      | 16 | 18 abcdefg | 18.6                   | 13.0 | 15.8 efghi  |
| IT 89KD-374-57 | 23                      | 15 | 19 abcde   | 22.6                   | 12.2 | 17.4 cdefg  |
| IT 86D-719     | 19                      | 10 | 15 defg    | 17.8                   | 14.7 | 16.3 efgh   |
| IT 86D-715     | 18                      | 14 | 16 defg    | 12.1                   | 18.6 | 15.4 efghij |
| IT 89KD-288    | 12                      | 13 | 13 fg      | 9.1                    | 7.5  | 8.3 m       |
| IT 87D-941-1   | 13                      | 15 | 14 fg      | 10.3                   | 7.6  | 9.0 lm      |
| Dan Ila        | 26                      | 16 | 21 abc     | 11.8                   | 14.7 | 13.2 ghijkl |
| Mean           | 18                      | 16 |            | 10.4                   | 10.7 |             |

Means followed by the same letter(s) within a column are not significantly different (DMRT) at 5% probability level.

**Table 4. Effect of P fertilizer on N and P accumulation (mg/pot) of 35 cowpea lines.**

| Lines          | N   |     |            | P   |     |          |
|----------------|-----|-----|------------|-----|-----|----------|
|                | -P  | +P  | Mean       | -P  | +P  | Mean     |
| IT 90K-277-2   | 71  | 72  | 72 fgh     | 3.9 | 4.9 | 4.4 abcd |
| IT 90K-284-2   | 35  | 124 | 80 bcdefgh | 2.2 | 5.9 | 4.0 abcd |
| IT 90K-76      | 45  | 87  | 66 h       | 2.3 | 6.5 | 4.4 abcd |
| IT 90K-59-2    | 76  | 64  | 70 fgh     | 2.9 | 3.4 | 3.2 d    |
| IT 93K-513-2   | 69  | 71  | 70 fgh     | 3.2 | 4.2 | 3.7 cd   |
| IT 93K-693-2   | 53  | 98  | 76 cdefgh  | 2.7 | 5.7 | 4.2 abcd |
| IT 93K-734     | 78  | 105 | 91 bcdefgh | 3.5 | 4.7 | 4.1 abcd |
| IT 93K-452-1   | 47  | 101 | 74 efgh    | 2.2 | 5.5 | 3.9 bcd  |
| IT 93K-637-1   | 67  | 115 | 91 bcdefgh | 2.8 | 5.1 | 4.0 abcd |
| IT 94K-437-1   | 73  | 120 | 96 bcdefg  | 3.0 | 6.3 | 4.7 abcd |
| IT 94K-440-3   | 85  | 125 | 105 abc    | 3.3 | 6.6 | 4.9 abc  |
| IT 95K-1406    | 53  | 94  | 73 fgh     | 2.8 | 5.5 | 4.1 abcd |
| IT 95K-1156-3  | 76  | 132 | 104 abcde  | 2.9 | 6.7 | 4.8 abcd |
| IT 95K-1090-12 | 59  | 128 | 93 bcdefgh | 2.1 | 5.2 | 3.7 cd   |
| IT 95K-1096-7  | 78  | 121 | 99 bcdefg  | 3.7 | 5.5 | 4.6 abcd |
| IT 95K-1464    | 64  | 100 | 82 bcdefgh | 2.6 | 4.6 | 3.6 cd   |
| IT 95K-1090-1  | 63  | 154 | 109 ab     | 2.5 | 6.4 | 4.4 abcd |
| IT 95K-105-2   | 65  | 127 | 96 bcdefgh | 3.5 | 5.3 | 4.4 abcd |
| IT 95K-1091-3  | 67  | 123 | 95 bcdefgh | 2.8 | 4.8 | 3.8 cd   |
| IT 95K-1095-2  | 66  | 143 | 105 abcd   | 3.6 | 7.6 | 5.6 ab   |
| IT 95K-1088-4  | 65  | 118 | 91 bcdefgh | 2.5 | 5.8 | 4.1 abcd |
| IT 95K-1384    | 62  | 103 | 82 bcdefgh | 2.7 | 5.7 | 4.2 abcd |
| IT 95K-1543    | 72  | 104 | 88 bcdefgh | 3.2 | 6.8 | 5.0 abc  |
| IT 95K-1491    | 65  | 89  | 77 cdefgh  | 4.0 | 5.9 | 4.9 abc  |
| IT 96D-666     | 62  | 121 | 91 bcdefgh | 2.4 | 6.3 | 4.4 abcd |
| IT 96D-740     | 55  | 118 | 86 bcdefgh | 2.7 | 7.9 | 5.3 abc  |
| IT 96D-748     | 43  | 113 | 78 cdefgh  | 1.8 | 5.9 | 3.8 cd   |
| IT 96D-757     | 70  | 109 | 90 bcdefgh | 2.7 | 4.9 | 3.8 cd   |
| IT 96D-759     | 101 | 164 | 133 a      | 4.5 | 7.0 | 5.7 a    |
| IT 89KD-374-57 | 67  | 93  | 80 bcdefgh | 2.8 | 4.4 | 3.6 cd   |
| IT 86D-719     | 77  | 108 | 92 bcdefgh | 2.9 | 5.1 | 4.0 bcd  |
| IT 86D-715     | 55  | 139 | 97 bcdefg  | 2.4 | 6.1 | 4.2 abcd |
| IT 89KD-288    | 50  | 150 | 100 bcdef  | 2.9 | 6.6 | 4.8 abcd |
| IT 87D-941-1   | 75  | 107 | 91 bcdefgh | 2.4 | 5.3 | 3.9 cd   |
| Dan Ila        | 48  | 102 | 75 defgh   | 2.5 | 4.9 | 3.7 cd   |
| Mean           | 65  | 113 |            | 2.9 | 5.7 |          |

Means followed by the same letter(s) within a column are not significantly different (DMRT) at 5% probability level.

### *Classification of cowpea lines*

The cowpea lines were classified on the basis of their dry grain weights into four groups. Ten lines were classified as having high grain yield without P and high productivity with P application (Table 5).

### **Field trial**

In the field trial, more than 50% of the cowpea lines showed significant response to P. Compared with the pot trial, there were considerable variations in the pattern of response of grain yields of the cowpea lines to P (Table 6). Lines IT 87D-941-1 and IT 90K-284-2

**Table 5. Classification of cowpea lines on the basis of response of grain weights to P fertilizer.**

| 1            | 2           | 3            | 4              |
|--------------|-------------|--------------|----------------|
| IT 90K-76    | IT 89KD-288 | IT 90K-284-2 | IT 90K-277-2   |
| IT 93K-513-2 |             | IT 95K-1543  | IT 90K-59-2    |
| IT 93K-693-2 |             | IT 96D-666   | IT 93K-734     |
| IT 93K-452-1 |             | IT 96D-748   | IT 95K-1406    |
| IT 93K-637-1 |             | IT 96D-757   | IT 95K-1156-3  |
| IT 94K-437-1 |             | IT 86D-715   | IT 95K-1090-12 |
| IT 94K-440-3 |             | Dan Ila      | IT 95K-1096-7  |
| IT 95K-1491  |             |              | IT 95K-1464    |
| IT 86D-719   |             |              | IT 95K-1090-1  |
| IT 87D-941-1 |             |              | IT 95K-105-2   |
|              |             |              | IT 95K-1091-3  |
|              |             |              | IT 95K-1095-2  |
|              |             |              | IT 95K-1088-4  |
|              |             |              | IT 95K-1384    |
|              |             |              | IT 96D-740     |
|              |             |              | IT 96D-759     |
|              |             |              | IT 89KD-374-57 |

1. High yield without P (yield > 0.3 g/pot) and high productivity with P application (yield > 1.7 g/pot).

2. Low yield without P (yield < 0.3 g/pot) and high productivity with P application.

3. High yield without P and low productivity with P application (yield < 1.7 g/pot).

4. Low yield without P and low productivity with P application.

**Table 6. Effect of P fertilizer on grain yield (kg/ha) of cowpea lines in the field.**

| Lines         | -P  | +P  | Mean       |
|---------------|-----|-----|------------|
| IT 86D-719    | 263 | 346 | 305 cde    |
| IT 94K-437-1  | 123 | 225 | 174 fghij  |
| IT 87D-941-1  | 450 | 704 | 577 a      |
| IT 94K-440-3  | 60  | 65  | 63 j       |
| IT 93K-693-2  | 164 | 140 | 152 ghij   |
| IT 93K-637-1  | 344 | 226 | 285 cdef   |
| Dan Ila       | 303 | 358 | 331 cd     |
| IT 96D-748    | 62  | 168 | 115 hij    |
| IT 90K-284-2  | 523 | 405 | 464 ab     |
| IT 96D-757    | 95  | 104 | 99 ij      |
| IT 86D-715    | 140 | 289 | 215 defghi |
| IT 89KD-288   | 284 | 182 | 233 defgh  |
| IT 96D-739    | 219 | 177 | 198 efghi  |
| IT 96D-772    | 277 | 221 | 249 cdefg  |
| IT 89KD-349   | 179 | 309 | 244 cdefg  |
| IT 97K-820-18 | 185 | 148 | 167 fghij  |
| IT 96D-724    | 354 | 369 | 362 bc     |
| Mean          | 237 | 261 |            |

Means followed by the same letter(s) within a column are not significantly different (DMRT) at 5% probability level.

produced significantly highest grain yield. Only lines IT 86D-719, IT 87D-941-1, IT 86D-715, and IT 89KD-288 maintained their classification in conformity with the results obtained in the greenhouse. Some of the lines that performed well in the pot trial exhibited a dismal performance in the field, especially lines IT 94K-440-3 and IT 96D-757.

## **Discussion**

The clear response to P application observed in terms of shoot, root, grain weights, and nodule dry matter and N and P production of the cowpea lines confirms that P is an important nutrient element affecting the yields of cowpea (Anonymous 1977). There are, however, differential responses among the cowpea lines studied. Okeleye and Okelana (1997) also observed significantly increased nodulation, grain yield, and total dry matter for cowpea varieties in response to P application. The decreased nodule number with P addition observed in this study contradicts the findings of Luse et al. (1975), that reported increased nodule number in cowpea due to P application. The observed increased cowpea grain yield with P application agrees with the results of Luse et al. (1975) but contradicts the results obtained by Agboola and Obigbesan (1977), who observed that P application did not significantly increase cowpea yield but rather enhanced nodulation and P content of leaf and stem. Osiname (1978) also did not observe a significant effect on cowpea yield with P application at Ibadan. The observed differential performances of the cowpea lines under no P application could provide a basis for selecting lines with greater agronomic efficiency in P-deficient soils and so reduce fertilizer costs. The observed variations in the performance of some of the cowpea lines in the pot and field trials is a pointer to the fact that pot trial screening methodology (which does not represent the real-life situation) may not be a very good methodology for evaluating varieties for farmer release. However, it could be used for an initial assessment of large numbers of breeder lines. Sanginga et al. (2000), reported that about 42% of the cowpea breeding lines (18 out of 43 lines tested) screened for P-use efficiency and N balance had the same grouping for the field and pot experiments. Watanabe et al. (1997), observed a high correlation coefficient (0.666\*\*) of scores between field evaluation and pot evaluation of drought tolerance of cowpea in Nigeria. However, they stated that the highly significant correlation observed between scores evaluated by the two methods was beyond expectation and so suggested further testing of the methodologies.

Variability noted in response to P could be important for selecting lines suitable for a range of soil P conditions or farmer production systems. We recommend that lines IT 90K-284-2, IT 96D-724, and IT 93K-637-1 can be selected for further testing without P fertilizer. Lines IT 87D-941-1, IT 86D-719, and Dan Ila may perform very well without P fertilizer and give a higher return when P is applied. When P fertilizer is available, line IT 87D-941-1 is recommended. These varieties should be tested at multiple sites to truly extend the results to breeding cowpea lines that could be targeted towards various soil P conditions.

## **References**

- Adetunji, M.T. 1995. Equilibrium phosphate concentration as an estimate of phosphate needs of maize in some tropical Alfisols. *Tropical Agriculture* 72: 285–289.
- Agboola, A.A. and G.O. Obigbesan. 1977. Effect of different sources and levels of P on the performance and P uptake of Ife-Brown variety of cowpea. *Ghana Journal of Agricultural Science* 10 (1): 71–75.
- Ankomah, A.B., F. Zapata, G. Hardarson, and S.K.O. Danso. 1995. Yield, nodulation, and N<sub>2</sub> fixation by cowpea cultivars at different phosphorus levels. *Biology and Fertility of Soils* 22: 10–15.
- Anonymous. 1977. Notes on the cowpea and grain legume research program, cropping scheme meeting. Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

- Jain, V.K., Y.S. Chauhan, and P.C. Jain. 1986. Effect of different doses of phosphorus on growth, yield, and quality of cowpea (*Vigna unguiculata* [L.] Walp.). *Madras Agricultural Journal* 73 (4): 199–202.
- Kang, B.T. and D. Nangju. 1983. Phosphorus response of cowpea (*Vigna unguiculata* [L.] Walp.). *Tropical Grain Legume Bulletin* 27: 11–16.
- Luse, R.L., B.T. Kang, R.L. Fox, and D. Nangju. 1975. Protein quality in grain legumes grown in the lowland humid tropics, with special reference to West Africa. Pages 193–201 *in* *Fertilizer use and protein production*. XIth Colloquium, International Potash Institute, 1975. Ronne-Bornholm, Denmark.
- Mokwunye, A.U., S.H. Chien, and E. Rhodes. 1986. Phosphorus reaction with tropical African soils. Pages 253–281 *in* *Management of nitrogen and phosphorus fertilizers in sub-Saharan Africa*, edited by A.U. Mokwunye and P.L.G. Vlek. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
- Muleba, N. and H.C. Ezumah. 1985. Optimizing cultural practices for cowpea in Africa. Pages 289–295 *in* *Cowpea research, production, and utilization*, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons Ltd, Chichester, UK.
- Okalebo, J.R., K.W. Gathua, and P.L. Woomer. 1993. Laboratory method of soil and plant analysis: a working manual. *Tropical Soil Biology and Fertility Programme (TSBF)*, Nairobi, Kenya. 88p.
- Okeleye, K.A. and M.A.O. Okelana. 1997. Effect of phosphorus fertilizer on nodulation, growth, and yield of cowpea (*Vigna unguiculata*) varieties. *Indian Journal of Agricultural Science* 67(1): 10–12.
- Omuetti, J.O. and V.A. Oyenuga. 1970. Effect of phosphorus fertilizer on the protein and essential components of the ash of groundnut and cowpeas. *West African Biology and Applied Chemistry Journal* 13 (1): 299–305.
- Osiname, O.A. 1978. The fertilizer (NPK) requirement of Ife-Brown cowpea (*Vigna unguiculata* [L.] Walp.). *Tropical Grain Legume Bulletin* No. 11/12: 13–15.
- Rachie, K.O. and L.M. Roberts. 1974. Grain legumes of the lowland tropics. *Advances in Agronomy* 26: 44–61.
- Sample, E.C., R.J. Soper, and G.J. Racz. 1980. Reactions of phosphate fertilizers in soils. Pages 263–310 *in* *The role of phosphorus in agriculture*, edited by F.E. Khasawneh, E.C. Sample, and E.J. Kamprath. American Society of Agronomy, Madison, Wisconsin, USA.
- Sanginga, N., O. Lyasse, and B.B. Singh. 2000. Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. *Plant and Soil* 220: 119–128.
- SAS. 1985. SAS user's guide. Statistical Analysis System Institute, Cary, NC, USA.
- Sellschop, J.P.F. 1962. Cowpeas, *Vigna unguiculata* (L.) Walp. *Field Crops Abstracts* 15: 259–266.
- Tenebe, V.A., Y. Yusuf, B.K. Kaigama, and I.O.E. Aseime. 1995. The effects of sources and levels of phosphorus on the growth and yield of cowpea (*Vigna unguiculata* [L.] Walp.) varieties. *Tropical Science* 35: 223–228.
- Watanabe, I., S. Hakoyama, T. Terao, and B.B. Singh. 1997. Evaluation methods for drought tolerance of cowpea. Pages 141–146 *in* *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.

## Farmer participatory evaluation of newly developed components of cowpea and cotton intercropping technology

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### Abstract

A technology verification experiment was carried out in farmers' fields in eastern Tanzania in 1997 and 1998. An erect short-duration cowpea variety Vuli-1 was intercropped with the cotton variety IL 74. Cotton was planted in single rows alternating with either single or double rows of cowpea. In an alternating single row intercrop, cowpea was planted either two weeks or four weeks after cotton. In the intercrop where a single cotton row alternated with double rows of cowpea, cowpea was planted two weeks after cotton. After harvesting, farmers were asked to assess and rank the technology components using an open-ended questionnaire and pair-wise ranking. Statistical analysis showed cotton and cowpea yield differences between technology components. Farmers' assessment revealed variation in terms of technology component preferences and showed that farmers rejected the one : two cotton : cowpea row configuration. Farmers accepted the one : one row configuration, and cowpea planted two or four weeks after cotton. However, it was evident that the adoption of these acceptable technology components will depend on whether certain cotton production constraints are solved.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important grain legume in Tanzania where its tolerance to moisture stress makes it suitable for cultivation in semiarid areas. Its leaves and seeds are consumed as an important supplement to a staple cereal diet. In Tanzania, cowpea is grown in almost all the areas below 1500 m above sea level (Price et al. 1982). It is usually found intercropped with cereals or other crops, although it is sometimes grown as a monocrop. However, its productivity is limited by high infestation with insect pests so that spraying against such pests is important for good yield.

Cotton (*Gossypium hirsutum* L.) is an important cash crop for smallholder farmers in eastern and western Tanzania. It is currently rated third after cashew and coffee in terms of foreign exchange earnings. Like cowpea, insect pests limit its productivity. Thus, insecticide application is recommended for optimal yield. However, profit margins for cotton have recently been reduced as a result of the rising cost of insecticides. Consequently, some farmers opt not to apply insecticide, thereby reducing cotton yield and quality. Therefore, for both cotton and cowpea, technologies are needed to increase returns in order to make production more attractive.

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One way of optimizing profit margins would be to intercrop cotton and cowpea so that the cowpea benefits from insecticide sprays applied on cotton thus reducing production cost (Natarajan and Naick 1992; Myaka and Kabissa 1993; 1996). Willey (1979) outlined other advantages of intercropping including greater yield stability over different seasons and better use of growth resources.

Previous work on cotton and cowpea intercropping by Myaka and Kabissa (1996) showed that optimal yield depends on moisture regime, time of planting cowpea, and planting pattern. On this basis, it could be anticipated that the most appropriate time for planting cowpea should therefore be different in wet and dry areas. Furthermore, their results showed the possibility that cowpea yield could be raised by increasing plant density, especially in dry areas. However, these results were not tested on farmers' fields to verify such technology components. A technology verification experiment was therefore initiated in 1997 on farmers' fields in three districts in eastern Tanzania with the following objectives to:

- verify the on-station results on cotton and cowpea intercropping by Myaka and Kabissa (1996) on farmers' fields with the input of farmers.
- create farmers' awareness on the possibility of intercropping cotton and cowpea and on the use of the electrodyne sprayer as safe for the user and the environment.
- have farmers assess the technology to confirm its compatibility with the farming system.
- recommend acceptable cotton and cowpea technology components for wider adoption.

## **Materials and methods**

The experiment was conducted during 1997 and 1998 cropping seasons on farmers' fields in Mangae and Fulwe, Morogoro rural district; Magamba and Mzundu, Handeni district; and Kisiwani of same district. These locations were selected on the basis of prevailing moisture regimes and history of cotton cultivation; Mangae and Kisiwani were classified as dry while Fulwe, Mangae, and Mzundu were classified as wet locations.

Prior to field experimentation, baseline data were collected in the target areas in an informal survey involving participatory methods (Rhoades 1995). Data collected included the production system involving the two crops, whether farmers were practising cotton and cowpea intercropping, farmers' knowledge of insecticide applicators, and cotton/cowpea production constraints.

Fields where the trials were conducted lie between 500 and 750 m above sea level. Fulwe and Mangae have a monomodal to weak bimodal rainfall pattern. Fulwe experiences higher rainfall than Mangae. At these locations, rains usually fall between October/November and May with a dry spell from January to February. The trials at Fulwe and Mangae were conducted during the main rains. Mzundu and Magamba experience a bimodal rainfall pattern. The short rainy season is between October and December and the long rainy season from mid-February to June. Trials at Mzundu and Magamba were conducted during the long rainy season. Kisiwani experiences a monomodal rainfall pattern with rains falling between February and May. The trial at this location was conducted during this period.

In collaboration with staff from the extension service, farmers were selected as follows: Mangae, five farmers, Fulwe, two, Mzundu and Magamba, six, and Kisiwani eight. Criteria for selecting farmers were their willingness to participate and their accessibility to land.

In the 1997 season, the trial was laid out in a block of six plots of 10 m×10 m replicated twice in each farmer's field. Treatments were as follows:

1. Cotton and cowpea intercropped in alternate single rows and cowpea planted two weeks after cotton.
2. Cotton and cowpea intercropped in alternate single rows and cowpea planted four weeks after cotton.
3. Cotton and cowpea intercropped in one : two cotton to cowpea row ratio and cowpea planted two weeks after cotton.
4. Sole cropped cotton.
5. Sole cropped cowpea planted two weeks after cotton.
6. Sole cropped cowpea planted four weeks after cotton.

In the 1998 season, the trial was laid out in a single block (no replications) in response to farmers' observation that the replicated experiment in 1997 (two replications per farmer) was too complicated for them.

Cotton was sown in hills spaced 0.3 m apart within the row and thinned to one plant per hill three weeks after sowing, while the cowpea was sown in hills spaced 0.2 m apart with two plants per hill. Spacing between rows for each component crop was 0.9 m. This gave a target population of 37 000 plants/ha for cotton and 110 000 plants/ha for cowpea. In one : two row configuration, the target density for cowpea was 200 000 plants/ha.

Sole cotton was planted at the same density as in the intercrop. Sole cowpea was planted two or four weeks after cotton with a space of 0.5 m between rows and 0.2 m between plants within the row, and the plants were subsequently thinned to two plants per hill with the aim of achieving a population of 200 000 plants/ha.

Cotton variety IL 74 and cowpea variety Vuli-1 were used. IL 74 is an indeterminate, late-maturing (180 days) cotton cultivar. Vuli-1 is a determinate, erect, and early-maturing cowpea cultivar. The trial was farmer-managed. Table 1 shows the allocation of responsibilities for the main operations and management of nonexperimental variables.

After harvesting, yield data were recorded and subjected to analysis of variance using MSTATC statistical software package. In 1998, farmers were treated as replications. During both seasons, the analysis was done on a village basis. Farmers' assessment was done through individual farmer interviews and in groups using an open-ended questionnaire, and farmers used pair-wise ranking to rank the technology components.

**Table 1. Allocation of responsibilities for the main operations and management of nonexperimental variables of the field experiment.**

| Field operations        | Implementers           |
|-------------------------|------------------------|
| Land preparation        | Farmers                |
| Layout of experiment    | Researcher/VEO*/farmer |
| Planting                | VEO and farmer*        |
| Thinning                | VEO and farmer*        |
| Weeding                 | VEO and farmer*        |
| Insecticide application | Researcher/VEO/farmer* |
| Harvesting              | VEO and farmer*        |

\*Did the main job; VEO = village extension officer.

## Results and discussion

In 1997, rainfall was assessed as normal and within expectations in amount and distribution pattern, while in 1998, the rains started early in all locations (Table 2). In 1997, there was a clear difference between dry and wet locations in terms of rainfall amount and distribution. This conformed to the location classification, contrary to 1998 when all locations received similar rainfall in terms of amount and distribution (Table 2) except Magamba where that year's rainfall was not recorded. In 1998, rainfall was abnormally higher compared to long term averages. This abnormally high rainfall was probably due to the *El nino* phenomenon. Therefore, the hypothesis that a suitable cotton and cowpea intercropping pattern would depend on the moisture regime could not be tested during this season. Several seasons of evaluation will be needed to further investigate this.

The objective of the baseline data collection was to have information on farmers' knowledge on the technology and also to have some basis for future impact assessment. The baseline data collected revealed that, with the exception of Mangae, farmers were not aware of cotton and cowpea intercropping. Furthermore, it was apparent that prior to the present study, farmers had no knowledge of the electrodyne sprayer. Cotton production constraints mentioned and prioritized by farmers are listed in Table 3. Both men and women do most of the operations involving these two crops. However, only men sell the cotton. The same applies to cowpea but only when the harvest is large. When the cowpea harvest is small, the work is left for the women. For both crops, only men do the spraying. This shows that there is some gender balance in the execution of most of the field operations. However, only men control the income realized from these crops.

Sole-cropped cowpea yielded higher than intercropped cowpea during both years and at all locations except at Fulwe in 1998 (Tables 4 and 5). This indicates that intercropping affected cowpea yield. These results are in agreement with the on-station results reported

**Table 2. Rainfall totals (mm) and number of rainy days (in parentheses) over successive monthly periods at the experimental sites in eastern Tanzania in 1997.**

| Year | Month    | Magamba    | Mzundu     | Fulwe     | Mangae     | Kisiwani   |
|------|----------|------------|------------|-----------|------------|------------|
| 1997 |          |            |            |           |            |            |
|      | January  | 0.0 (0)    | 0.0 (0)    | —         | 0.0 (0)    | 0.0 (0)    |
|      | February | 0.0 (0)    | 8.0 (1)    | —         | 30.0 (1)   | 40.5 (2)   |
|      | March    | 72.0 (6)   | 49.5 (4)   | —         | 76.5 (5)   | 247.5 (6)  |
|      | April    | 144.1 (15) | 183.2 (14) | —         | 237.8 (12) | 52.9 (6)   |
|      | May      | 118.6 (10) | 100.1 (8)  | —         | 44.4 (4)   | 10 (4)     |
|      | June     | 21.4 (5)   | 99.2 (13)  | —         | 0.0 (0)    | 2 (1)      |
|      | July     | 0.0 (0)    | 0.0 (0)    | —         | 0.0 (0)    | 0.0 (0)    |
|      | August   | 0.0 (0)    | 0.0 (0)    | —         | 0.0 (0)    | 0.0 (0)    |
| 1998 |          |            |            |           |            |            |
|      | January  | —          | 196.5 (8)  | 208.4 (5) | 264.8 (8)  | 419.1 (21) |
|      | February | —          | 38.0 (3)   | 177.5 (5) | 107.1 (3)  | 135.3 (10) |
|      | March    | —          | 0.0 (0)    | 195.9 (6) | 170.1 (6)  | 90.0 (8)   |
|      | April    | —          | 171.0 (9)  | 225.0 (7) | 146.0 (11) | 169.7 (12) |
|      | May      | —          | 93.5 (6)   | 30.1 (1)  | 40.0 (5)   | 49.3 (5)   |
|      | June     | —          | 0.0 (0)    | 0.0 (0)   | 0.0 (0)    | 0.0 (0)    |
|      | July     | —          | 0.0 (0)    | 0.0 (0)   | 0.0 (0)    | 0.0 (0)    |
|      | August   | —          | 5.5 (2)    | 0.0 (0)   | 0.0 (0)    | 0.0 (0)    |

**Table 3. Cotton and cowpea production constraints in order of priority as prioritized by farmers using pair-wise ranking in eastern Tanzania in 1998.**

| Location       | Rank | Production constraints   |
|----------------|------|--|
| Handeni (A)    | 1    | Lack of market for cotton  |
|                | 2    | Cotton seed not available on time; when available has poor germination                 |
|                | 3    | Insecticides not available on time; when available are expensive and ineffective       |
|                | 4    | Cotton buyers come late  |
|                | 5    | Lack of harvesting and storage bags*   |
|                | 6    | Hunger stress during weeding time (people spend most of their time looking for food)   |
|                | 7    | Brown-seeded cowpea have no market   |
| Handeni (B)    | 1    | Lack of market for cotton  |
|                | 2    | Cotton seed not available on time  |
|                | 3    | Insecticides not available on time; when available are expensive and ineffective       |
|                | 4    | Low cotton price   |
|                | 5    | Lack of market for brown cowpea  |
|                | 6    | Lack of harvesting and storage bags*   |
| Morogoro (wet) | 1    | Lack of market for cotton  |
|                | 2    | Low cotton price   |
|                | 3    | Lack of credit facility  |
|                | 4    | Insecticides are brought too late and they are expensive                               |
|                | 5    | Tractors not enough, resulting in late land preparation                                |
|                | 6    | Vermin   |
| Morogoro (dry) | 1    | Cotton seed not available on time  |
|                | 2    | Insecticides not available on time; when available are expensive                       |
|                | 3    | Batteries for sprayers are unaffordable  |
|                | 4    | Cotton buyers come late  |
|                | 5    | Sprayers are not enough  |
|                | 6    | Tractors for hire are not available  |
| Same           | 1    | Insecticides not available on time; when available are expensive and ineffective       |
|                | 2    | Lack of market for cotton  |
|                | 3    | Aphids on both cotton and cowpea come early before the anticipated date of first spray |
|                | 4    | Lack of harvesting and storage bags*   |

\*The cooperatives used to supply bags but they have stopped.

by Myaka and Kabissa (1996). Cowpea intercropped with cotton in one : two row ratio yielded higher than that which was intercropped in one : one ratio with cotton. In 1997, higher cotton yields were observed from wet locations. In 1998, cotton was not affected by intercropping at all locations except Kisiwani.

Technology ranking was variable between locations. When ranks were pooled across moisture regime classifications, intercropping was scored high in wet locations while in dry locations, farmers preferred cowpea monocropping (Table 6). It is interesting to note that cotton monocropping was ranked low at all locations. Various comments on technology components from farmers are indicated in Table 7. When asked if they would continue intercropping these crops, farmers at all locations agreed to continue except at Mangae

**Table 4. Yield (kg/ha) of cotton and cowpea planted at four locations in eastern Tanzania in 1997.**

| Treatment               | Mangae |        | Kisiwani |        | Mzundu |         | Fulwe  |         |
|-------------------------|--------|--------|----------|--------|--------|---------|--------|---------|
|                         | Cotton | Cowpea | Cotton   | Cowpea | Cotton | Cowpea* | Cotton | Cowpea* |
| 1 : 1 intercrop 2 weeks | 1153   | 338    | 663      | 375    | 1517   | —       | 2000   | —       |
| 1 : 1 intercrop 4 weeks | 1017   | 283    | 850      | 267    | 1433   | —       | 1700   | —       |
| 1 : 2 intercrop 2 weeks | 1297   | 315    | 713      | 475    | 1455   | —       | 1900   | —       |
| Sole cotton             | 1097   | —      | 1250     | —      | 1300   | —       | 1200   | —       |
| Sole cowpea 2 weeks     | —      | 630    | —        | 467    | —      | —       | —      | —       |
| Sole cowpea 4 weeks     | —      | 480    | —        | 900    | —      | —       | —      | —       |
| CV                      | 33     | 31     | 37       | 67     | 17     | —       | 11     | —       |
| SE                      | 129    | 34     | 113      | 81     | 62     | —       | —      | —       |
| P                       | 0.63   | 0.007  | 0.007    | 0.03   | 0.49   | —       | 0.08   | —       |

\*Cowpea yield during this season wasnot recorded.

**Table 5. Yield (kg/ha) of cotton and cowpea planted at four locations in eastern Tanzania in 1998.**

| Treatment               | Mangae |        | Kisiwani |        | Mzundu |        | Fulwe  |        |
|-------------------------|--------|--------|----------|--------|--------|--------|--------|--------|
|                         | Cotton | Cowpea | Cotton   | Cowpea | Cotton | Cowpea | Cotton | Cowpea |
| 1 : 1 intercrop 2 weeks | 407    | 80     | 750      | 208    | 733    | —      | 450    | 550    |
| 1 : 1 intercrop 4 weeks | 347    | 90     | 863      | 291    | 567    | —      | 500    | 613    |
| 1 : 2 intercrop 2 weeks | 360    | 225    | 500      | 236    | 633    | —      | 450    | 700    |
| Sole cotton             | 420    | —      | 1200     | —      | 633    | —      | 325    | —      |
| Sole cowpea 2 weeks     | —      | 410    | —        | 597    | —      | —      | —      | 825    |
| Sole cowpea 4 weeks     | —      | 310    | —        | 500    | —      | —      | —      | 450    |
| CV                      | 10     | 33     | 30       | 35     | 15     | —      | 31     | 23     |
| SE                      | 13     | 46     | 92       | 58     | 160    | —      | 47     | 14     |
| P                       | 0.19   | 0.03   | 0.01     | 0.02   | 0.29   | —      | 0.64   | 0.28   |

**Table 6. Pair-wise ranking results of intercropping components and sole crops as ranked by farmers in eastern Tanzania in 1998.**

| Treatment               | Wet    |         |       | Dry    |          |
|-------------------------|--------|---------|-------|--------|----------|
|                         | Mzundu | Magamba | Fulwe | Mangae | Kisiwani |
| 1 : 1 intercrop 2 weeks | 1*     | 2 *     | 2*    | 4*     | 4 *      |
| 1 : 1 intercrop 4 weeks | 3      | 5       | 4     | 1      | 3        |
| 1 : 2 intercrop 2 weeks | 4      | 1       | 5     | 5      | 2        |
| Sole cotton             | 5      | 4       | 6     | 3      | 5        |
| Sole cowpea 2 weeks     | 2      | 3       | 1     | 2      | 1        |
| Sole cowpea 4 weeks     | –      | –       | 3     | –      | –        |

\*Technology component rank within a location.

Scale: 1 = very important, 6 = less important.

**Table 7. Summary of general comments given by farmers during the ranking exercise of technology components in eastern Tanzania in 1998.**

| Location | Comments on technology components   |
|----------|---|
| Magamba  | In one : one row intercrop two weeks, there is no competition<br>When cowpea is delayed to four weeks, cotton affects the cowpea; one : two row intercrop not preferred because it is difficult to weed and spray as rows are very close<br>Cowpea planted two weeks after cotton becomes vegetative                        |
| Mzundu   | When cowpea is delayed to four weeks, it is overshadowed by cotton<br>In one : two intercrop, cowpea yielded higher than in the other intercrops but is not preferred because of difficulty in weeding and spraying<br>All farmers preferred intercropping to monocropping because when they intercrop, they get both crops |
| Fulwe    | When cowpea is planted two weeks after cotton, there is good synchronization of weeding for both crops; one : two row intercrop not preferred because it is difficult to weed<br>Preferred intercrop to cotton sole-crop<br>Preferred sole cowpea to intercrop and sole cotton; there is no market for cotton               |
| Mangae   | Preferred to plant cowpea four weeks after cotton because both crops reach spraying time at the same time<br>one : two row intercrop not preferred because it is difficult to weed and there are too many insects<br>Preferred sole cowpea because it is marketable   |
| Same     | Preferred intercrop to sole crop  |

where they preferred to grow both crops as sole crops because they had observed that intercropping reduced the yield of both crops (Table 8).

Farmers cautioned that they would intercrop provided that there would be a cotton market and that insecticides would be provided on credit. Uncertain cotton markets and low cotton prices were priority constraints at all locations (Table 3). Farmers preferred alternate single rows and rejected the one : two cotton and cowpea row configuration (Table 6). This was in conformity with results reported by Myaka and Kabissa (1996). The latter reported 47% cotton yield reduction when intercropped with cowpea in one : two cotton and cowpea row configuration when compared to sole cropped cotton. In the present study, farmers rejected this pattern because it was difficult to walk through the rows during

**Table 8. Farmers' perception on whether to continue with cotton and cowpea intercropping or not in eastern Tanzania in 1998.**

| Situation             | Magamba (%)                 | Mzungu (%) | Fulwe (%) | Mangae (%) | Kisiwani (%) |
|-----------------------|-----------------------------|------------|-----------|------------|--------------|
| To continue           | 33                          | 100        | 100       | 0          | 95**         |
| Not to continue       | 67*                         | 0*         | 0**       | 100        | 5            |
| Number of farmers (n) | 3                           | 9          | 2         | 12         | 20           |
| Reason why no         | Yield of both crops reduced | —          | —         | —          | —            |
| Reason why yes        | You get two crops           | —          | —         | —          | —            |
|                       | Less production cost        | —          | —         | —          | —            |

\*Farmers are ready to divide the field into half; one half for cotton, the other for cowpea.

\*\*Farmers will continue on condition that market for cotton is assured and insecticide is obtained on credit.

spraying. They also complained that this configuration hampered weeding. The cowpea variety Vuli-1, which was used in the present study is brown-seeded. There is an indication that this type of seed does not fetch a good market price (Table 3). Research is needed to develop a short-duration cowpea suitable for intercropping with cotton like Vuli-1 but with acceptable cream color seed.

We conclude that the one : one row configuration and cowpea planted two or four weeks after cotton are acceptable to farmers and compatible with the existing farming system. Although farmers' preferences for these technology components were variable, their adoption will depend on the removal of production constraints for cotton which is the main crop in this intercropping system.

The use of cotton insecticide for cotton : cowpea intercrop does, however, need to be developed with caution. Inappropriate chemicals or timing of application could result in excessive contamination of cowpea food products.

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## References

- Myaka, F.A. and J.C.B. Kabissa. 1993. Cotton and cowpea relay intercropping: preliminary results on its economics and effects on some agronomic characters of both crops. Pages 60–67 *in* Trends in cowpea research. Proceedings of Cowpea Research Seminar, 25–26 September 1991, Harare, Zimbabwe.
- Myaka, F.A. and J.C.B. Kabissa. 1996. Fitting short duration cowpea into a cotton-based cropping system in Tanzania: Effect of planting pattern, time of planting cowpea and insecticide application to cotton. *Experimental Agriculture* 32: 225–230.

- Natarajan, M. and D.M. Naick. 1992. Competitive effect of short duration bush type cowpea when intercropped with cotton in Zimbabwe. *Experimental Agriculture* 28: 409–416.
- Price, M., F. Machange, and J.A. Assenga. 1982. Improved cultivation of cowpea (*Vigna unguiculata*) in Tanzania. Tanzania Ministry of Agriculture, Dar es Salam, Tanzania. 42p.
- Rhoades, R.E. 1995. The art of the informal agricultural survey. IITA Research Guide 36. International Institute of Tropical Agriculture, Ibadan, Nigeria. 57p.
- Willey, R. W. 1979. Intercropping: its importance and research needs. Part 1: Competition and yield advantages. *Field Crop Abstracts* 32: 1–10.



## Cowpea dissemination in West Africa using a collaborative technology transfer model

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### Abstract

Improved cowpea cultivars from the Bean–Cowpea Collaborative Research Support Program (CRSP) and from other sources were introduced into the cereal cropping system in Chad, Ghana, Mali, Niger, and Senegal to ameliorate the declining soil fertility in the Sudan–Sahelian zone of West Africa and contribute to food security. The project was implemented through the formation of country technology transfer teams involving CRSP, World Vision International (WVI), the national agricultural research systems (NARS), the national agricultural extension services, other nongovernmental organizations (NGOs), and leader farmers. Interventions involved the dissemination of appropriate improved cowpea cultivars grown in association or in rotation with cereal cultivars suitable for each environment. To minimize postharvest losses usually associated with cowpea in the region, appropriate storage technologies were introduced through the training of technicians and farmers. Indications are that those interventions have the potential to contribute to improvements in soil fertility and increased food security in the subregion.

### Introduction

Soils of most of West Africa are characterized by relatively low inherent fertility. This is due to the type of their parent material, high degree of weathering, lack of volcanic rejuvenation, and intensive leaching.

In Africa, 65% of the agricultural land, 31% of the permanent pasture land, and 19% of the forest and woodland are affected by human-induced soil degradation. It is estimated that about 332 million hectares of African drylands are subject to soil degradation, with nutrient depletion being a major factor influencing this degradation (Bationo and Lompo 1996).

Farmers traditionally practice shifting cultivation on these low fertility soils. In this agricultural practice, cropped lands are left fallow to restore fertility after they have been cultivated to a point where crop yields had declined to uneconomic levels. Increasing population pressure in the subregion has continued to reduce the fallow period, thereby limiting the effectiveness of shifting cultivation in restoring soil fertility. Compounding the problem is the fact that inorganic fertilizer is now beyond the reach of many small-holder farmers, either due to nonavailability or high prices.

The Collaborative Research Support Projects (CRSPs) working in West Africa have as their main thrust, technology development. For several years, these bilateral research

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teams have developed natural resource management (NRM) technologies for specific environments in individual countries. Many of these technologies have the potential of being adapted for use throughout West Africa. However, many are not yet widely adopted. While CRSPs' capacity for transfer of these technologies is limited, national and regional efforts are constrained by inadequate collaboration and linkages.

### **Project objectives and outputs**

The NRM InterCRSP initiative in West Africa has a specific charge to transfer NRM technologies in West Africa (Anonymous 1997). The major objectives of the project are:

- To develop a model for CRSP/NGO collaboration that will mobilize the existing knowledge, technologies, and capacity of CRSPs for major regional impact.
- To use this intervention model to improve natural resource management, reduce natural resource degradation, and improve farmers' food security and incomes in West Africa through regional adaptation and transfer of sustainable NRM technologies.

*The expected outputs are:*

- A model regional mechanism for collaborative adaptive research and transfer of CRSP NRM technologies in West Africa.
- Strengthened and mutually reinforced West African institutions and professional resources for NRM technology adaptation and transfer.
- The successful functioning of the model mechanism, leading to more productive exchanges among CRSPs, the national agricultural research system (NARS), the international agricultural research centers (IARCs), the national agricultural extension system (NAES), nongovernmental organizations (NGOs), and farmers in West Africa.
- Improved regional technology adaptation and transfer, leading to more sustainable yields and greater profitability for farmers.

### **Collaborating institutions**

The initial CRSPs are the Bean–Cowpea CRSP, which is the lead CRSP and the Sorghum–Millet CRSP (INTSORMIL). Scientists from both the USA and host countries collaborate in the project.

World Vision International (WVI) maintains programs in eight countries in West Africa, with a comparative competency in West African regional technology transfer. WVI maintains healthy collaborative relationships with the NARS and NAES in the countries in which it works.

Collaborative adaptive research and technology transfer teams, comprising CRSP, NARS, NAES, WVI, other NGOs, and farmer collaborators, were formed for each participating country. Each team, with a coordinator, prepared and implemented a work plan, setting targets for the adaptation and transfer of selected technologies. The CRSP and NARS team members implement adaptive research activities, while WVI, NAES, other NGOs, and farmer team members undertake transfer activities.

The Bean–Cowpea CRSP and WVI facilitate the exchange of NRM technologies among team members and between country teams. They complement internal and external linkages with additional regional collaborative relationships with the following networks in the region: the West and Central Africa Sorghum Research Network (WCASRN), the West and

Central Africa Millet Research Network (ROCAS), the West and Central Africa Cowpea Research Network (RENACO), and the cowpea protection network at IITA, Protection écologiquement durable du niébé (PEDUNE).

## Current activities

The project document stated that initially the NRM technologies adapted and extended would be confined to genetic resources (i.e., cowpea, sorghum, and millet varieties and storage technologies) developed by the Bean–Cowpea and INTSORMIL CRSPs. These activities are summarized in this paper. However, it should be noted that the mechanisms described, especially the interactions between multiple partners, have provided a means for subsequent transfer of additional technologies.

Technologies currently being promoted are mainly in the areas of dissemination of improved genetic materials of cowpea and sorghum, and millet and cowpea storage technologies. Cowpea is an important grain legume in West Africa, providing an inexpensive source of protein for both the urban and rural poor.

Incorporation of cowpea into the cropping system is crucial for sustainable crop production in sub-Saharan Africa. Two major cereals grown in the target areas of the project are sorghum and millet. It is hoped that incorporating cowpea in the cropping system, either as a sole crop or intercrop with sorghum and millet will go a long way to improve the fertility of those degraded soils and hence, contribute to NRM.

Cowpea improves the soil through the fixation of atmospheric nitrogen. Where soil degradation is a major constraint to crop production, inclusion of cowpea into the cropping system is crucial as it helps to replenish soil nitrogen. Cowpea rotation is an effective resource management technology in cereal-based systems, since part of the nitrogen requirement of cereal crops can be met by cowpea intercropping and/or rotation. Studies on cereal–cowpea rotation (Bationo et al. 2000) show that grain yields of cereals succeeding cowpea can, in some cases, double compared to continuous monoculture. The authors claimed that in an efficient soil fertility management system, cowpea can fix up to 88 kg N/ha and this results in an increase of nitrogen-use efficiency on the succeeding cereal crop from 20% in the continuous cereal monoculture to 28% when cereals are in rotation with cowpea. The authors also found that the use of soil nitrogen increased from 39 kg N/ha in the continuous cereal monoculture to 62 kg N/ha in the rotation systems. Similarly, cowpea, when intercropped with cereals, helps reduce the menace of *Striga hermonthica*, a major problem confronting smallholder farmers in the region.

The inclusion of cowpea in the cropping system will improve the nutrition of the people, increase the feed quantity and quality for livestock, and contribute to soil fertility maintenance. This will lead to increased food security and reduced environmental degradation.

The project also emphasizes the dissemination of cowpea storage technologies. A major deterrent to cowpea production is the problem of insect pests, which occur during post-flowering, preharvest, and in storage. Storage of cowpea seed is particularly problematic, due to high damage by storage insect pests. Indeed, this is often the major reason adduced by many smallholder farmers for not growing cowpea. Hence, along with the introduction of improved varieties, the project emphasizes disseminating cowpea storage technologies. These technologies were developed by the CRSP projects in Cameroon and Senegal, in

collaboration with the CRSP cowpea storage project at Purdue University, USA. (Kitch and Ntougam 1991; Kitch et al. 1992; Ntougam and Kitch 1991; Kitch et al. 1997).

Activities are currently in progress in Chad, Ghana, Mali, Niger, and Senegal. These participating countries cut across the Sudan–Sahelian zone where annual rainfall is between 200 and 1200 mm.

## Strategies and progress

1. *Collaboration.* The project has created a five-country network of over 50 collaborators from more than 15 different organizations, encouraging both national and international collaboration. Within each country, scientists and the entire country team members from different disciplines are working together to identify/develop, test, and disseminate technologies best suited to local conditions. There have been improved working relationships between WVI, NARS, NAES, and farmers. Apart from country team meetings to select appropriate technologies for testing, the team as a whole monitors progress on the field, participates in field days, and jointly analyzes and interprets the data collected. This type of interaction between scientists, extension agents, NGOs, farmers, and processors is a novelty in the subregion. Internationally, technologies are being shared throughout West Africa by NARS, CRSPs, IARCs, and the commodity networks.
2. *Mutual learning.* Exchange of expertise has been encouraged and facilitated by the project. Because farmers are directly involved in the project, scientists have learned a lot from the farmers. Among indigenous knowledge gained from farmers are the uses of shea butter (from *Vitellaria paradoxa* more commonly referred to as *Butyrospermum parkii*) in Ghana and the powder from the leaves of wild custard apple (*Anona senegalensis*) in Niger for local cowpea seed preservation. Even though the use of oils and botanicals has been documented by several authors (e.g., Murdock et al. 1997), the Ghana experience showed that a uniform layer of shea butter at the surface of the earthenware pot could be effective. The effective use of the leaf powder from the custard apple is worth further studies and refinement by scientists.
3. *Dissemination of technologies.* Several technologies have been disseminated by the project (Anonymous 1998, 1999, 2000). Some of these technologies are described in this paper.

## Storage technologies

Technicians and farmers from the five participating countries were trained on cowpea storage technologies (solar heater, triple bagging, drum storage, and improved ash storage) developed by CRSP scientists (Kitch and Ntougam 1991; Kitch et al. 1992; Ntougam and Kitch 1991; Kitch et al. 1997). These technologies consist of (1) using a solar heater to kill bruchids, (2) triple bagging in three layers of hermetically sealed plastic, and (3) mixing cowpea with wood ash for storage. Generally, it is recommended to use a solar heater to kill bruchids and then store by triple bagging or in wood ash. Even when used separately, each of the three techniques allows the storage of cowpea for six months or longer without bruchid infestation or damage. The farmer, therefore, has an option to keep the cowpea and seek higher prices long after harvest. In Senegal, a variation of these techniques combined the use of neem oil with either triple bagging or storage in sealed drums (Murdock et al. 1997).

The number of people trained in storage technologies between 1997 and 2000 per country are: Chad (2038), Ghana (578), Mali (65), Niger (86), Senegal (290), making a total of 3057.

These training activities targeted both men and women, with participants from several communities. Similarly, farmers were supplied with storage materials with which to train more farmers in their communities. Table 1 shows the number of participants in the storage training activity in Chad in 1998.

WVI also sent two staff members to Institut de Recherche Agricole pour le Développement (IRAD)/Bean-Cowpea CRSP project in Maroua, Cameroon, for in-depth training in cowpea storage technologies. These participants coordinate training activities in the subregion.

### **Farmer field schools**

Specific production training in the form of field schools was arranged for farmers in all the countries during on-farm trials, and for research technicians in Chad. Similarly, nonparticipating farmers were invited to visit the trial plots during field days. These interactions were of particular benefit to the participating and nonparticipating farmers, especially in making varietal preference decisions, based on phenotypic considerations.

### **Varietal and cropping system recommendations**

Improved cultivars were introduced to farmers through both adaptive (researcher-managed) and on-farm trials. The mix of cowpea, sorghum, and millet varieties tested was a combination of advanced breeding lines, improved varieties that have proven successful in different parts of the region, and local check varieties. Varieties with a range of maturation rates, seed colors, and yield potential were selected to match farmer preferences, and the range of weather patterns with which they must contend. Country teams determined the composition of the technology package best suited for each country. Farmers' preferred

**Table 1. Participants in the cowpea storage technology training workshops, Chad, 1998 cropping season.**

| Location        | Women | Men | Farmers who got storage technology materials for training other farmers | Total persons trained |
|-----------------|-------|-----|---|-----------------------|
| Laokassy        | 19    | 118 | 15  | 137                   |
| Souley          | 13    | 120 | 15  | 133                   |
| Mango           | 20    | 30  | 5   | 50                    |
| Maibombaye      | 25    | 25  | 5   | 50                    |
| Nangkesse       | 17    | 33  | 5   | 50                    |
| Nassian         | 23    | 27  | 5   | 50                    |
| Koro            | 30    | 40  | 11  | 70                    |
| Gama            | 70    | 80  | 11  | 150                   |
| Mouroum-Touloum | 100   | 150 | 22  | 250                   |
| Silambi         | 12    | 12  | 12  | 24                    |
| Rakena          | 15    | 15  | 15  | 30                    |
| Danamadji       | 26    | —   | 26  | 26                    |
| Total           | 370   | 650 | 147   | 1020                  |

varieties resulting from on-farm trials, field days, and palatability tests are enumerated and typified by data from participating countries (for on-farm trials, Table 2) and Ghana (for adaptive trials, Table 3).

Adaptive and on-farm trials in the participating countries were made up of replicated trials on research stations or researcher-managed in outstations (for adaptive trials) and in farmers' fields (for on-farm trials). The design and entries were decided by the country technology transfer teams. The entries were CRSP-developed materials for similar climatic environments, materials developed by the NARS and other NARS collaborators (e.g., the IARCs), and farmers' currently grown varieties. Though the number of participating farmers varied from year to year and from country to country, the average had been between 50 and 200 per year between 1997 and 2000. Varietal preferences are made by the farmers participating in the on-farm program, during field days in which other farmers participate, and during palatability tests conducted at the end of the season. During the end-of-year country team meetings (where field data are discussed), decisions are taken, with the help of the farmers in the team, on what farmers' preferred entries and technologies are. These decisions guide the NARS on materials and technologies to officially release. Table 2 shows some of the varieties released in some participating countries.

**Table 2. Cowpea, sorghum, and millet varieties extended by the InterCRSP project in participating countries (1997–2000).**

| Crop/variety                | Developed by                | Developed in           | Extended to                       |
|-----------------------------|-----------------------------|------------------------|-----------------------------------|
| <b>Cowpea</b>               |                             |                        |                                   |
| Mouride                     | Bean–Cowpea CRSP (HC & USA) | Senegal                | Senegal, Niger, Chad,             |
| Melakh                      | Bean–Cowpea CRSP (HC & USA) | Ghana, Mali<br>Senegal | Senegal, Niger, Chad, Ghana, Mali |
| C93W-24-130 (Lori Niébé)    | Bean–Cowpea CRSP (HC & USA) | Cameroon               | Senegal, Chad, Ghana              |
| C92S-12-58 (CRSP Niébé)     | Bean–Cowpea CRSP (HC & USA) | Cameroon               | Cameroon, Ghana                   |
| C93W-2-38                   | Bean–Cowpea CRSP (HC & USA) | Cameroon               | Cameroon, Ghana, Mali             |
| IT89KD-245                  | IITA                        | Nigeria                | Mali                              |
| IT89KD-374                  | IITA                        | Nigeria                | Mali                              |
| <b>Sorghum</b>              |                             |                        |                                   |
| NAD-1 (hybrid)              | INTSORMIL (HC & USA)        | Niger                  | Niger                             |
| Seguetana Cinzana           | IER                         | Mali                   | Mali                              |
| N'tenimissa                 | INTSORMIL (HC & USA)        | Mali                   | Mali                              |
| <b>Millet</b>               |                             |                        |                                   |
| HKP (Hainei-Khiere Precoce) | ICRISAT, INRAN              | Niger                  | Niger                             |

HC = Host country.

**Table 3. Grain yield (kg/ha) of elite cowpea lines tested across four locations in northern Ghana, 1999.**

| Entries         | Sites     |       |         |      | Average | Rank |
|-----------------|-----------|-------|---------|------|---------|------|
|                 | Nyankpala | Manga | Damongo | Wa   |         |      |
| IT87D-829-2     | 992       | 678   | 845     | 504  | 755     | 9    |
| Melakh          | 1141      | 573   | 1237    | 621  | 893     | 1    |
| IT93K-452-1     | 881       | 643   | 971     | 592  | 772     | 7    |
| IT95-1497       | 1191      | 539   | 857     | 692  | 820     | 5    |
| Bengpla (check) | 940       | 313   | 966     | 513  | 683     | 11   |
| ITP-148-1       | 1118      | 469   | 761     | 604  | 738     | 8    |
| SUL 518-2       | 1408      | 591   | 845     | 613  | 864     | 3    |
| IT87D-885       | 888       | 695   | 120     | 597  | 775     | 6    |
| IT87D-1951      | 1013      | 452   | 669     | 537  | 668     | 10   |
| IT86D-719       | 802       | 695   | 696     | 461  | 664     | 12   |
| 24-130          | 1135      | 608   | 1176    | 581  | 875     | 4    |
| SUL-87KD        | 1202      | 382   | 1121    | 670  | 844     | 2    |
| Mean            | 1059      | 553   | 922     | 582  | 779     |      |
| LSD (0.05)      | 484       | 339   | 330     | 434  | 397     |      |
| CV (%)          | 31.8      | 42.5  | 24.9    | 20.7 | 30.0    |      |

Some of the highlights of preferred technologies in the participating countries are:

### **Chad**

#### **Cowpea**

- IT8ID-994, C7-29, Melakh, and C93W-24-130 are top yielders.
- Farmers' preference: IT8ID-994 and IT89KD-288. Even though C93W-24-130 produced more haulms that could be of advantage as fodder, it was not preferred by the farmers.

#### **Sorghum**

- Identification of the sorghum variety GRW as promising. GRW was developed by the NARS in Chad.
- Local selections, such as GRW mentioned above, outperformed newly developed varieties on farmers' fields.
- Participatory approach in the selection of sorghum varieties best adapted for intercropping with cowpea, with the participation of about 160 farmers in all the 11 agricultural research centers.

#### **Sorghum–cowpea intercropping**

- Trials involving 86 participating farmers indicated that sorghum and cowpea grown in alternate hills on the same row or in alternate rows were most effective in reducing the menace of *Striga hermonthica* resulting in less *Striga* infestation on the field and higher sorghum yields. These two spatial arrangements showed superiority over the other two treatments: plots with sorghum only and plots where the local cultural practice was to plant sorghum and cowpea seeds were in the same hole.

## Ghana

### Cowpea

- Two CRSP cultivars (Melakh and C93W-24-130) are now being tested on-farm after being found promising in adaptive trials conducted by the project in collaboration with the Savanna Agricultural Research Institute at four locations (Nyankpala, Manga, Damongo, and Wa) for three years in Ghana. Table 3 shows the data of the third year adaptive trial in 1999.
- The three Cameroon–CRSP lines (C93W-2-38, C92S-12-58, and C93W-24-130) significantly outyielded all entries (12) in fodder production in the adaptive trials conducted at the above sites for the three years.

### Sorghum

- P 9407 (one of the four *Striga*-resistant cultivars obtained from Purdue University) was used as a source for genetic resistance to *Striga hermonthica* in the national program).
- Integrated *Striga* management, workshops, and demonstrations were held at four sites in northern Ghana (two sites each at Jirapa-Lambusie and Sissala districts) for three years. Agronomic practices and varietal resistance formed the major basis for an integrated management of the pest. Major components were:
  - Use of varietal resistance
  - Early weeding before the flowering of *Striga* plants
  - Intercropping with trap crops (cowpea and soybean)
  - Crop rotation
  - Uprooting *Striga* plants that emerged in between normal weeding times
  - Manuring/fertilization

### Sorghum–legume intercropping

- Cowpea intercropped with sorghum, irrespective of the pattern reduced *Striga hermonthica* infestation at the on-farm trials.
- Soybean intercropped with sorghum was more effective than cowpea in reducing *Striga* in the on-farm trials.

## Mali

### Cowpea

- Variety Korobalen (IT89KD-374) was early, high grain yielding, and preferred by most farmers.
- Farmers' specific preferences (with regards to traits) are: IT89KD-245 (high pulse yield, drought-tolerant, *Striga*-resistant, high haulm yield, and grain whiteness) IT89KD-374 (early maturity and palatability).
- Better performance (in terms of grain yield) over local varieties of the following CRSP cultivars: C93W-2-38, Mouride, Melakh, and Mame fama.

### Sorghum

- Seguetana Cinzana was preferred by most farmers during field days.
- Some farmers preferred N'tenimissa for the whiteness of its grain and the taste and consistency of its porridge, but complained of its weak stems and consequent lodging.



- With the exception of one of the sites, the local varieties outyielded the tested varieties (N'tenimissa, 96CZF498, and 96CZF499).

#### Medium-duration millet

- There was no significant difference in the average yield of the varieties (Guefore CMDT 16, Tontoro 21, and Indiana 05), although particular varieties topped at specific sites (Indiana 05 at Dakoumani and Tonto ADPs; Guefore CMDT 16 at N'Torosso-Sokourani; and the local variety at Parana-Boho).

#### Cereal-cowpea intercropping

- Intercropping of sorghum or millet with the improved cowpea cultivar IT89KD-245 in row intercropping or in alternate rows gave the best results in combating the menace of *Striga hermonthica*, compared to the farmers' traditional method of mixing cereal and cowpea and dibbling seeds from both crops. The farmers' traditional method gives very low cowpea stands, with resultant low grain yield. Farmers, however, complained about the laborious nature of interrow and alternate row intercropping.

#### Seed multiplication

- During the 1999 cropping season, the identified promising cultivars (millet: Guefoue, Tontoro 21; sorghum: N'tenimissa, Seguetana; cowpea: IT89KD-245, IT89KD-374) were multiplied by 97 farmers in three WVI ADPs: Diaramana, Bani Valley, and Yangasso.

The following quantities of seed were made available to the farmers: millet and sorghum 169 kg and cowpea: 84 kg. The total area planted was 13.75 ha, divided between sorghum: 8 ha; millet: 4 ha; and cowpea: 1.75 ha.

### Niger

#### Cowpea

- Top performance of Mouride (ISRA/CRSP), IT89KD-349, and IT89KD-374 for grain yield and the local variety (TN5-78) for fodder production (Table 4).

**Table 4. Grain and straw weight of cowpea varieties in on-farm trials at Zinder, Tera, and Maradi (Kornaka), Niger, 1999.**

| Varieties      | Origin    | Sites            |                  |                  |                  |                  |                  |
|----------------|-----------|------------------|------------------|------------------|------------------|------------------|------------------|
|                |           | Zinder           |                  | Tera             |                  | Kornaka          |                  |
|                |           | straw<br>(kg/ha) | grain<br>(kg/ha) | straw<br>(kg/ha) | grain<br>(kg/ha) | straw<br>(kg/ha) | grain<br>(kg/ha) |
| IT90KD-372-1-2 | IITA      | 1524             | 428              | 152              | 428              | 1523             | 426              |
| IT89KD-349     | IITA      | 2100             | 552              | 2100             | 555              | 2102             | 553              |
| Mouride        | ISRA/CRSP | 2200             | 703              | 2200             | 704              | 2202             | 315 <sup>†</sup> |
| Melakh         | ISRA/CRSP | 2050             | 480              | 2050             | 479              | 2051             | 306 <sup>†</sup> |
| Local (TN5-78) | INRAN     | 2700             | 436              | 2700             | 436              | 2500             | 434              |
| Mean           |           | 2114             | 520              | 2114             | 521              | 2134             | 408              |
| LSD            |           | 0191             | 72.1             | 0191             | 37.42            | 0186             | 31.04            |
| CV(%)          |           | 514              | 7.89             | 514              | 4.09             | 498              | 4.33             |

<sup>†</sup>Reduced yield of Mouride and Melakh due to overnight pilfering before the field day by farmers anxious to have seeds of both varieties.

#### Sorghum

- Top performance of the hybrid NAD-1.

#### Millet

- Top performance of the variety HKP.

#### **Senegal**

##### Millet

- Identification of GBS 8735 as an overall performer.

##### Cowpea

- Mouride and Melakh were top yielders preferred by farmers.

## **Conclusions**

Improved cultivars developed initially for specific countries stand a chance of adaptability and acceptability in other countries with similar environments. This is exemplified by the varieties Mouride and Melakh bred in Senegal, but now grown in Chad, Ghana, Mali, and Niger. Similarly, C93W-24-130 (Lori Niébé) bred in Cameroon is now widely grown in Chad and Ghana. Mouride and Melakh (bred in Senegal) and C92S-12-58 (CRSP Niébé), bred in Cameroon are now widely grown in Mali. These beyond-territory movements of CRSP cultivars would not have been possible without this intervention.

NRM can be improved in West Africa under the framework of current InterCRSP–WVI initiative that has involved the mobilization of existing capacities within the Bean/Cowpea CRSP and INTSORMIL. Specifically, the initiative has increased the cultivation and productivity of cowpea in participating countries, thus increasing effective resource management in cereal-based systems. Increased productivity of the introduced cultivars is likely to have increased the food security of our target communities.

An excellent demonstration of collaborative technology development and transfer is typified by the current West Africa NRM InterCRSP. The technology transfer aspect, which is the focus of this initiative, and which has come to be referred to as the “CRSP–NGO Model” appears to emerge as a model for the future. The model has reinforced the current state extension services with appropriate adaptive research projects, training, logistic, and evaluation tools.

## **Outlook**

There is the need to expand the number of NRM technologies included in the technology packages, particularly in the areas of integrated pest management and soil and water conservation. Two additional CRSPs being targeted as sources of such interventions are:

- SANREM (Sustainable Agriculture and Natural Resource Management) CRSP
- IPM (Integrated Pest Management) CRSP

However, relevant technologies from the IARCs and other advanced institutions will be included in a future expanded project.

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## References

- Anonymous. 1997. Adaptive Research with InterCRSP Natural Resource Management Technologies for Regional Transfer in West Africa. Project proposal submitted by Bean-Cowpea CRSP, INTSORMIL, and World Vision International, 14 February 1997.
- Anonymous. 1998. Adaptive Research Trial Results—First Year (March 1997–28 February 1998). Bean-Cowpea CRSP, Michigan State University, Michigan, USA.
- Anonymous. 1999. Adaptive Research Trial Results—Second Year (March 1998–28 February 1999). Bean-Cowpea CRSP, Michigan State University, Michigan USA.
- Anonymous. 2000. Adaptive Research Trial Results—Third Year (March 1999–28 February 2000). Bean-Cowpea CRSP, Michigan State University, Michigan, USA.
- Bationo, A., and F. Lompo. 1996. Technologies disponibles pour combattre la perte en éléments nutritifs des sols en Afrique de l'ouest. Proceedings organized by Centre International pour la Gestion de la Fertilité des Sols-Afrique, Lomé, Togo.
- Bationo, A., B.R. Ntare, S. Tarawali, and R. Tabo. 2000. Soil fertility management and cowpea production in the semiarid tropics of West Africa. Paper presented at the World Cowpea Research Conference III held at the International Institute of Tropical Agriculture, Ibadan, Nigeria, 4–7 September 2000.
- Kitch, L.W. and G. Ntoukam. 1991. Storage of cowpea in ash. Technical Bulletin 1. Bean-Cowpea CRSP, East Lansing, Michigan, USA.
- Kitch, L.W., G. Ntoukam, R.E. Shade, J.L. Wolfson, and L.L. Murdock. 1992. A solar heater for disinfecting stored cowpea on subsistence farms. *Journal of Stored Products* 28(4): 261–267.
- Kitch, L.W., H. Bottenberg, and J.L. Wolfson. 1997. Indigenous knowledge and cowpea pest management in sub-Saharan Africa. Pages 292–301 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Murdock, L.L., R.E. Shade, L.W. Kitch, G. Ntoukam, J. Lowenberg-DeBoer, J.E. Huesing, W. Moar, O.L. Chambliss, C. Endondo, and J.L. Wolfson. 1997. Postharvest storage of cowpea in sub-Saharan Africa. Pages 302–312 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Ntoukam, G. and L.W. Kitch. 1991. Solar heaters for improved cowpea storage. Technical Bulletin 2. Bean-Cowpea CRSP, East Lansing, Michigan, USA.

## **Section V**

# **Cowpea postharvest and socioeconomic studies**



## 5.1

# The economics of cowpea in West Africa

O. Coulibaly<sup>1</sup> and J. Lowenberg-DeBoer<sup>2</sup>

### Abstract

The contribution of cowpea to food security and poverty reduction can be substantial in West Africa if both biological and socioeconomic constraints are addressed. While some attention has been given to genetics, agronomy, and pest control, such economic issues as access to input, marketing, and consumer preferences are key research areas which contribute to the adoption and wide diffusion of improved cowpea technologies among small farmers. An area neglected in cowpea research but which is becoming important is consumer appreciation of improved cowpea grain. Results from the hedonic pricing analysis showed, for example, that consumers prefer larger grain size and seeds with low level of bruchid damage. Another area which needs to be investigated is the financial and economic profitability of chemical-intensive cowpea technologies. Cowpea is very sensitive to pests and chemical protection of the crop is financially profitable. However, this financial profitability may substantially decrease if hidden costs, such as the opportunity costs of capital, health hazards, and environmental costs are taken into consideration. This calls for the adoption of more environmentally sound and health conscious crop protection techniques such as the use of botanicals and an integrated pest management approach for cowpea research. The study also reviews the economic impact of cowpea research and concludes that the integration of biological and social science in cowpea research will lead to sustainable technology development for food security and poverty reduction.

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) has the potential to contribute to food security and to poverty reduction in West Africa. The demand for cowpea in this region is increasing because of high population growth, mainly from the urban areas, and also because of poverty and the demand for low-cost food. Moreover, cowpea yields can increase if technical and socioeconomic constraints are addressed. The high protein content of cowpea and its use as a staple in the diets of Sahelian and coastal populations make it also a crop with high potential for food security in these regions. Cowpea forage contributes significantly to animal feed mainly during the dry season when the demand for feed reaches its peak. The largest producer and consumer of cowpea in West Africa (and in the world) is Nigeria where a dense population creates an enormous demand for the crop. Niger is the largest cowpea exporter in West Africa (and in the world) with an estimated 215 000 MT exported annually, mainly to Nigeria. Substantial amounts of cowpea also come to Nigeria from other neighboring countries, especially Cameroon and Chad. A large proportion of cowpea

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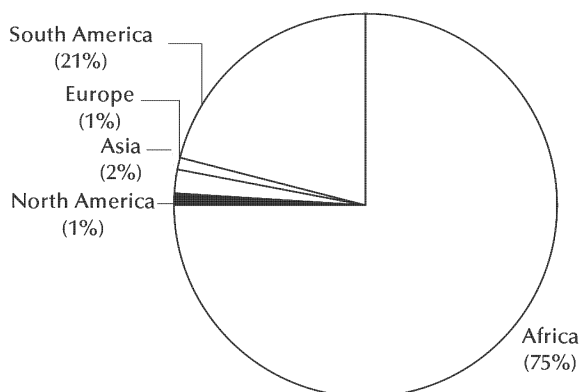
from Burkina Faso and Mali is imported into Côte d'Ivoire, and also Nigeria. Despite this importance of cowpea in food security, trade, and therefore poverty reduction, increased cowpea production, storage, and marketing face many constraints that need attention from research and development. The objective of this paper is to review past and ongoing studies to assess some of these key constraints and make recommendations for further research. The paper is divided into four sections. The first section deals with cowpea production, the second with marketing, the third analyzes cowpea pest management, and the fourth examines the impact of new cowpea technologies.

## **Cowpea production**

### ***Aggregate production***

World cowpea production was estimated at 3 319 375 MT and 75% of that production (Fig. 1) is from Africa (FAOSTAT 2000). West Africa is the key cowpea producing zone, mainly in the dry savanna and semiarid agroecological zones. The principal cowpea producing countries are Nigeria, Niger, Senegal, Ghana, Mali, and Burkina Faso (FAOSTAT 2000).

Among these countries, Nigeria and Niger are ahead with a production of 2 099 000 and 641 000 MT, respectively, in 1999 (FAOSTAT 2000). Nigeria, the largest cowpea producer in West Africa, also has the highest level of consumption (Table 1) with a population of 113.8 million and a per capita consumption of 23 kg per year (Table 1). The domestic supply of cowpea does not meet the demand, leading to a deficit of 518 400 MT per year. This is partly met by imports from neighboring countries, mainly Niger, where the estimated consumption of 78 200 MT in 1999 was far below the production figure of 641 020 MT (Table 1).



**Figure 1. Share of Africa in world cowpea production.**  
Source: Adapted from FAOSTAT (2000).

**Table 1. Cowpea production and consumption in West Africa.**

| Countries | Production<br>(t) | Consumption/<br>capita/year<br>(kg) | Population<br>(MI) | Consumption<br>(t) | Surplus/<br>deficit<br>(t) |
|-----------|-------------------|-------------------------------------|--------------------|--------------------|----------------------------|
| Burkina   | 10,000            | 5.2                                 | 11.6               | 60,320             | -50,320                    |
| Mali      | 110,060           | 7.4                                 | 11                 | 81,400             | 28,660                     |
| Niger     | 641,024           | 7.82                                | 10                 | 78,200             | 562,824                    |
| Nigeria   | 2,099,000         | 23.00                               | 113.8              | 2,617,400          | -518,400                   |

Source: Computed from FAO data, FAOSTAT (2000).

## Cropping systems and adoption of related improved technologies

### *Cowpea cropping system*

In West Africa, cowpea is grown mostly in subsistence farming systems and on a small scale in the lowland dry savanna and Sahelian regions. Traditionally, cowpea is grown in association or in relay cropping with cereals such as sorghum, millet, and maize mainly in the Sahelian regions (Pedune Mali 1999). However, cowpea cropping systems are moving towards monocropping as the crop's economic importance increases. For example, cowpea monocrop has taken off in central Mali, thanks to an integrated rural development project which supplied improved seed, fertilizer, and pesticides on credit (Coulibaly 1987). Cowpea monocrop is frequent in cotton producing zones and in inland valleys and the Lake Chad basin in Cameroon (Pedune Cameroon 1999). The increase in cowpea production is linked to the use of improved technologies including high yielding varieties and improved crop protection and production practices. A key issue behind the wide use of the improved cowpea technologies is their profitability. A few studies have been carried out on the profitability of cowpea technologies (Coulibaly 1995; Lowenberg-DeBoer et al. 1994; Aitchedji 2001) and a case study in Benin is reviewed in this paper.

### *Cost of production of cowpea with new technologies*

The profitability of the cowpea cropping system depends mainly on the varieties used (local or improved), the cropping practices and management (use of chemicals including fertilizers and pesticides), and the access to input and output markets. In this section, we review the financial profitability of cowpea production systems with improved production and protection technologies in Benin (Aitchedji 2001). The study was carried out in southern Benin on 35 farms with different combinations of improved technologies including improved variety, neem extract used as insecticide, chemical insecticide, and plastic bagging after solar drying (Table 2). The study used a Policy Analysis Matrix (PAM) developed by Monke and Pearson (1989) to assess the financial profitability of the improved cowpea technologies. Since access to capital has been reported by farmers as a key constraint (Aitchedji 2001), a sensitivity analysis was carried out with three scenarios linked to opportunity cost of the capital at 0, 25, and 50%. These rates are within those computed by Lowenberg-DeBoer et al. (1994) in the rural areas in West Africa which vary from 0 to 100%.

The results (Table 2) show that only improved cowpea technologies are profitable even under tight credit constraints, compared to local technologies mainly local cultivars and



**Table 2. Financial profitability of cowpea production systems in South Benin: application of policy analysis matrix (FCFA /ha): 3 scenarios with opportunity cost of capital (respectively 0%, 25%, and 50%).**

| Production systems   | Financial budget<br>(opportunity cost of capital 0%) |                         |                            |          | Financial budget<br>(opportunity cost of capital 25%) |                         |                            |          | Financial budget<br>(opportunity cost of capital 50%) |                         |                             |          |
|--|--|-------------------------|----------------------------|----------|---|-------------------------|----------------------------|----------|---|-------------------------|-----------------------------|----------|
|  | Gross revenue  | Costs of tradable input | Costs of nontradable input | Profits  | Gross revenue   | Costs of tradable input | Costs of nontradable input | Profits  | Gross revenue   | Costs of tradable input | Costs of non tradable input | Profits  |
| Technologies   |  |                         |                            |          |   |                         |                            |          |   |                         |                             |          |
| Improved variety IT 86 D-364 + botanical insecticide + local storage         | 212 600  | 29 975                  | 128 115                    | 54 510   | 212 600   | 37 483                  | 135 838                    | 39 279   | 212 600   | 44 972                  | 143 590                     | 24 038   |
| Local variety + botanical insecticide + chemical insecticide + local storage | 168 000  | 76 775                  | 128 965                    | -37 740  | 168 000   | 95 975                  | 136 918                    | -64 893  | 168 000   | 115 165                 | 144 870                     | -92 035  |
| Local variety + chemical insecticide + improved storage                      | 168 000  | 162 360                 | 122 965                    | -117 325 | 168 000   | 202 959                 | 130 918                    | -165 877 | 168 000   | 243 540                 | 138 870                     | -214 410 |
| Local variety + chemical insecticide + local storage                         | 140 000  | 52 095                  | 129 215                    | -41 310  | 140 000   | 65 124                  | 138 743                    | -63 867  | 140 000   | 78 146                  | 148 245                     | -86 391  |
| Local variety + botanical insecticide + local storage                        | 140 000  | 23 550                  | 129 115                    | -12 665  | 140 000   | 29 438                  | 137 107                    | -26 545  | 140 000   | 35 326                  | 145 095                     | -40 421  |
| Improved variety Vita 5 + chemical insecticide + local storage               | 200 000  | 68 130                  | 121 915                    | 9 955    | 200 000   | 85 168                  | 129 586                    | -14 754  | 200 000   | 102 196                 | 137 290                     | -39 486  |

Source: Aïtchedji et al. (2001).

Note: Physical quantity of inputs per hectare, tradable inputs: fertilizer (100 kg), chemical insecticide (5 l), storage bags (19, 5, 0, 7, 2, and 2 units), storage barrel or tank (0, 5, 8, 0, 0, and 3 units), Sofagrain (0, 10, 0, 0, 7, and 0 sachets), Gastoxin (19, 0, 0, 0, 0, and 0 tablets), Tarpaulin (0, 0, 15, 0, 0, and 0 units); Nontradable inputs: Seed (34, 44, 44, 44, 44, and 34 kg), Labor (243, 243, 228, 228, 243, and 228 man-day), Soap (4, 4, 0, 0, 4, and 0 units), Pepper (0, 0, 0, 25, 0, and 0 kg), Small equipment (990 FCFA), Land (18 675 FCFA); Output: average yields (1063, 1200, 1000, 1000, and 1000 kg/ha).

Profit = Gross revenue: (cost of tradable inputs + cost of nontradable inputs).

For more information about PAM model, see Monke and Pearson (1989), Adesina and Coulibaly (1998).

local storage techniques. The most profitable combination is improved varieties of cowpea sprayed with neem or papaya extract followed by improved varieties of cowpea sprayed with chemical insecticide. The difference in gross margins of cowpea production systems showed in Table 2 is explained by the difference in the type of varieties (more resistance to pests and diseases, drought and heat tolerance, and higher yield). Also, the type of pesticide used for treatment and the storage technology used can make a significant difference (Aitchedji 2001). This profit will be even higher if we include the costs of environment and public health. The misuse of pesticides in general has been causing deaths every year in the cotton and cowpea producing areas. In Benin, a study conducted by the Ministry of Health in the northern Borgou province in 1999 revealed that 37 people died due to endosulfan poisoning, while another 36 people experienced serious ill health (*Pesticides News* 2000). In view of the importance of the Borgou province to national cotton cultivation, it is felt that at least 70 people might in fact have died (Peter et al. 2000). Health hazards should be evaluated and discounted from the benefits of using pesticides. The loss in work days and the health costs can be substantial and are not considered by farmers in assessing the benefits of using pesticides.

## **Cowpea marketing**

During the past 20 years, the Bean–Cowpea Collaborative Research Support Program (CRSP) and international and national research institutions have made substantial contributions to cowpea production and protection technology. Beside new varieties, improved methods for controlling pests in the field and in storage have been developed. These technologies could dramatically increase cowpea production and grain quality in West Africa. The questions now are, Who will buy those cowpea? At what price? And what kind of cowpea would consumers prefer?

## ***Cowpea trade in West Africa***

Cowpea markets in West Africa are part of an ancient trade that links the humid coastal agroecological zones with the semiarid interior. This ancient trade is based on the comparative advantage in food production of each zone. In the humid coastal areas, it is relatively easy to produce carbohydrates (e.g., cassava, maize, rice), but because of pests and diseases, it is difficult to produce animal or vegetable protein. Lack of rainfall limits grain production in the interior, but creates good conditions for livestock, cowpea, and groundnut.

In the traditional cowpea growing countries of the Sudano-Sahelian zone, there is a well developed network of village buyers who assemble small quantities from farmers into 100 kg bags and merchants who transport and store the bags. These trade linkages can be illustrated with Ghana which though a major producer of cowpea imports about 10 000 MT annually (Langyintuo 1999). About 30% of the Ghanaian imports are from Burkina Faso (Table 3) and the rest from Niger. According to Langyintuo (1999), in Accra, the large, rough coated Nigerien cowpea (cowpea from Niger) sells for a premium, but they need to be marketed quickly because they do not store well in the humid coastal climate.

**Table 3. Official imports of cowpea into Ghana, 1992–1998.**

| Year | Total imports | Imports from Burkina-Faso |              | Imports from Niger |              |
|------|---------------|---------------------------|--------------|--------------------|--------------|
|      | (MT)          | (MT)                      | (% of total) | (MT)               | (% of total) |
| 1992 | 2055.34       | 592.00                    | 28.80        | 1463.34            | 71.20        |
| 1993 | 2640.80       | 637.92                    | 24.16        | 2002.88            | 75.84        |
| 1994 | 11798.98      | 2898.95                   | 24.57        | 8900.03            | 75.43        |
| 1995 | 13086.29      | 3295.95                   | 25.19        | 9790.34            | 74.81        |
| 1996 | 6816.80       | 3077.79                   | 45.15        | 3739.01            | 54.85        |
| 1997 | NA            | NA                        | NA           | NA                 | NA           |
| 1998 | 10167.18      | 3050.15                   | 30.00        | 7117.03            | 70.00        |

Source: Langyintuo 1999.

***Cowpea trade in Benin***

The traders interviewed indicated that there is an active trade between Benin, Niger, Togo, Nigeria, and Gabon. They noted that cowpea imported from Niger, Togo, and Nigeria are later exported to Gabon and Togo. Specifically, between 40 and 60 traders send cowpea totaling about 50 000 MT to Gabon and about half that quantity to Togo each year. Yet there were no cowpea trade statistics at the national level because the government of Benin considers cowpea trade a minor commercial activity. The traders consider this a favorable condition for their activities because they do not pay any tax on cowpea, unlike maize exporters who pay 100 FCFA on each bag exported as fiscal exit tax (*taxe de sortie*) (Langyintuo 2000).

***Cowpea trade in Togo***

Togo is very active in cowpea trade. Togolese traders frequently export cowpea to Gabon and sometimes to Congo. Traders from these countries also purchase cowpea directly from Togo. Exports to Ghana and Benin are mainly by Ghanaian and Beninois traders. It was estimated that on a given market day, between 20 and 40 Ghanaian traders purchase between 10 and 20 bags of cowpea each. Most of the traders from Ghana come from Akakyi, Agbozome, and Aflao in the Volta region, and Accra in the Greater Accra region. Gabonese and Beninois traders in the Akodesewa market number up to 20 from each country.

All Togolese traders exporting grain are expected to indicate the quantity being exported on their travel document (*laissez-passer*). Non-Togolese traders, on the other hand, are not obliged to do so. Consequently, the government does not keep track of grain shipped out of or into the country. In Ghana, on the other hand, the Ghana Plant Protection and Regulatory Service (PPRS) of the Ministry of Food and Agriculture (MoFA) subjects grain imported or exported to phytosanitary inspection. As a routine, the quantities of grain per trader are recorded, thus providing an opportunity for the tracking of grain movement in and out of the country.

Available export data show that between 1990 and 1998, Gabon was the only country importing cowpea from Togo annually (Table 4). Imports averaged 12.25 MT. Exports to Gabon increased dramatically from 20.18 MT in 1997 to 46.53 MT in 1998. Togo also imports cowpea from Senegal, Nigeria, Niger, Burkina Faso, Ghana, and Benin. In 1998,

**Table 4. Imports and exports of cowpea in and out of Togo (MT).**

| Year | Imports |       |         |                 |       | Exports |       |       |       |
|------|---------|-------|---------|-----------------|-------|---------|-------|-------|-------|
|      | Benin   | Ghana | Senegal | Burkina<br>Faso | Niger | Nigeria | Gabon | Congo | Ghana |
| 1990 | 10.70   | 0.14  | 78.08   | 6.20            | —     | —       | 5.48  | —     | —     |
| 1991 | —       | 0.14  | —       | 0.02            | —     | —       | 19.68 | —     | 210   |
| 1992 | 6.20    | 0.28  | —       | 73.80           | —     | 468.62  | 0.55  | —     | —     |
| 1993 | 1.20    | —     | —       | —               | —     | 5.70    | 1.05  | —     | 0.55  |
| 1994 | —       | —     | 30.30   | 0.72            | 0.55  | 7.00    | 2.00  | 149   | —     |
| 1995 | —       | —     | 1.00    | 8.00            | —     | —       | 8.00  | 2.00  | 400   |
| 1996 | —       | —     | 0.36    | —               | —     | —       | 1.80  | —     | 61    |
| 1997 | —       | —     | —       | —               | 14.07 | —       | 20.18 | —     | 720   |
| 1998 | 55.60   | 9.72  | —       | 35.10           | 1.41  | —       | 46.53 | —     | 334   |

Source: Langyintuo 2000.

Note: — = Zero or data unavailable.

for instance, Togo imported 101.830 MT of cowpea, 55% from Benin, 34% from Burkina Faso, 10% from Ghana, and 1% from Niger (Langyintuo 2000).

The hypothesis at the beginning of the cowpea marketing research was that most cowpea from northern Cameroon were marketed into Nigeria. Surveys in Cameroon showed that in fact most of the northern Cameroon production went to southern Cameroon, and that some was exported from there to Gabon and Congo.

In northern Senegal as the climate grew drier in the 1980s and the groundnut parastatal declined, cowpea increasingly replaced groundnut as the legume of choice. Some cowpea is exported to Mauritania and Gambia, but the transportation cost and lack of market links limit access of Senegalese cowpea to the large markets in Ghana, Nigeria, and elsewhere along the African coast. Senegal is the only country in the region with a substantial cowpea processing industry. Faye et al. (2000) identified five companies producing cowpea-based weaning food, cowpea flour, and cowpea-based crackers. All the products are made from recipes developed by the National Institute of Agriculture Research (ISRA) in Senegal's Food Technology Institute (ITA). In addition, there is a cracker manufacturer in Nouakchott, Mauritania, who uses primarily cowpea from Senegal.

### **Consumer preferences**

Knowledge of consumer preferences is essential to developing cowpea markets. Breeders need to know what characteristics consumers want. Integrated pest management specialists need an estimate of the level of grain damage acceptable to consumers. The Bean–Cowpea CRSP cowpea price and quality study was launched in Maroua, Cameroon, in September 1996, and later extended to four markets in northern Cameroon, three in Nigeria, two in Niger, three in northern Ghana, three in Mali, and six in Senegal using a common data collection protocol. Every month, CRSP researchers and technicians buy five samples per market from randomly selected sellers. They note the gender and other seller characteristics. In the laboratory, they record the 100 grain weight, average length and width of grains, number of bruchid holes per 100 grains, color, and texture of the testa, and eye color. The data are analyzed using a hedonic pricing regression model.

Initial results from a hedonic pricing analysis carried out by Langyintuo et al. 2000 and Faye et al. 2000, indicated that consumers in almost all areas prefer larger grain size. Consumers are more sensitive to bruchid damage than hypothesized. It was thought that West African consumers would tolerate a certain level of damage, but the data indicate that cowpea prices are discounted from the first appearance of damage. Results from the same study indicated that women in Cameroon appear to sell at a higher price than men, probably because women sell in small quantities for immediate consumption. In Senegal, consumers appear to pay a premium of 20 FCFA/kg for the traditional black speckled varieties.

### ***The Hedonic Model Framework: A review of literature and application***

The conceptual basis for estimating consumer demand for a good's quality is Lancaster's (1966) model of consumption theory which regards properties of the good and not the good itself as the direct object of utility. Using this concept, Ladd and Suvannunt (1976) developed the consumer goods characteristics model which describes the price of a good as a linear summation of the implicit value of its attributes.

For cowpea, the consumer goods characteristics model can be expressed mathematically as:

$$P_c = \sum_{j=1}^m X_{cj} P_{cj}$$

Where

$P_c$  = price of cowpea;  $X_{cj}$  = quantity of cowpea grain characteristic  $j$ , such as size of grain, testa texture, eye color, and damage by weevils.

$P_{cj}$  = implicit price of characteristic  $j$ .

Hedonic pricing models have received wide applicability in the scientific world. In his estimation of quality adjusted price index for computer processors in 1989, Dulberger concluded that hedonic prices could be useful in estimating quality adjusted indexes for the output of complex products manufactured in an industry characterized by technological change. The effects of milling and premilling operations on rice quality were examined by Bonifacio and Duff (1989) using a hedonic pricing model. The results indicated insignificant differences in paddy quality by mill type and confirmed that mill type affects milled rice quality and that millers attach economic significance to certain grain quality characteristics. Abansi et al. (1990) used the hedonic pricing model to evaluate consumer preferences for rice quality in the Philippines. They found that rice consumers attach economic significance to quality considerations. Walburger and Foster (1994) used data on boar performance traits from Purdue University Boar Test Station and auction sales data to estimate the implicit prices for back fat, loin eye area, average daily gain, and feed efficiency of boars in the US, using a hedonic pricing model. All of these variables have a

significant impact on the auction prices of boars. In 1995, Naik used the hedonic pricing theory to show that only 76% of the variations in silk price in India were explained by the quality characteristics, suggesting a poor linkage between quality and price.

***Hedonic relationship and implicit prices: A review of work by Langyintuo et al. 2000; Faye et al. 2000***

Langyintuo et al. (2000) and Faye et al. (2000) have undertaken a hedonic pricing study for each of three markets in Ghana (Table 5) and four markets in Cameroon (Table 6). The following hedonic equation was specified and estimated in a seemingly unrelated regression model:

$$P_{1t} = \alpha_{10} + \sum \gamma_{1r} Y_{1tr} + \sum \psi_{1l} M_{1tl} + \sum \beta_{1j} X_{1tj} + \varepsilon_{1t}$$

Where

|  |                      |
|--|----------------------|
| $P$ = price of cowpea                  | $i = 1, 2, \dots, n$ |
| $Y$ = yearly dummy                     | $r = 2, 3$           |
| $M$ = monthly dummy                    | $l = 1, 2, 3$        |
| $X$ = cowpea characteristics variables | $j = 1, 2, \dots, K$ |
| $\varepsilon$ = disturbance term       | $t = 2, 3, \dots, T$ |
| $\alpha$ = constant term               |                      |

$\beta, \psi$  and  $\gamma$  are parameters to be estimated

Cowpea price as the dependent variable was measured in FCFA/kg in Cameroon and Cedis/kg in Ghana. These were entered in the model as absolute values. Similarly, grain size measured as weight of 100 grains and number of insect holes as independent variables were also entered as absolute values. Other independent variables in the X-matrix including eye color, seed coat color, and gender of sellers (in the Cameroon models) were entered as dummy variables. Cowpea grain color and eye color are important when the intended use requires decortication. Where decortication is required, for example, in making *kosa*, poor pounding and winnowing may still leave some flecks for which consumers have a low tolerance level. Black flecks tend to be more conspicuous than other colors. A value of 1 was assigned to white grain color and zero otherwise. Similarly, black-eyed grain assumed a value of 1 and zero otherwise. The gender variable was entered as 1 for female and zero otherwise. To account for the effect of time in price variability, yearly and monthly dummies were used. For the yearly dummies, 1997 was used as the base year and each yearly dummy assumed a value of 1 for the year in question and zero otherwise. For the monthly dummies, November was used as the base year since prices in that month are the lowest in both countries. The monthly dummy assumed a value of 1 for the respective month and zero otherwise.

The estimated regression results indicate that seasonal supply, demand, cowpea size, color, and insect damage level explain between 53 and 72% of price variability in the seven markets studied (Tables 5 and 6). Across all markets, cowpea grain size was significant in explaining price variability. Table 5 indicates that besides grain size, color of eye, and number of insect holes are important in explaining cowpea price variability in Cameroon. Unlike grain size that influences price positively, there is an inverse relationship between price and grain eye color or insect holes. Consumers demand discounts (FCFA/kg) for black eye cowpea: 5.5 in Maroua, 4.86 in Mokolo, and 18.88 in Salak. An increase of one hole per every 100 grains leads to a discount of 0.29 FCFA/kg in Maroua and 0.29 FCFA/kg in Salak. Estimates for Mokolo and Banki show a discount for damage, but coefficients are not significant at conventional levels.

In Ghana, consumers are equally as sensitive to cowpea damaged by insects as Cameroonians. A discount of up to  $\text{¢}120/\text{kg}$  is demanded for a unit increase in number of damaged grains/100 grains in Tamale and Bolgatanga markets but less than a  $\text{¢}1/\text{kg}$  in Wa market (Table 5).

A contrasting feature of consumer demand for grain characteristic between Ghana and Cameroon is observed in the preference for grain eye color. In Ghana, consumers are willing to pay a premium of between  $\text{¢}109$  and  $\text{¢}226/\text{kg}$  for black-eyed cowpea, with traders in Wa receiving the highest premium. In contrast, consumers in Cameroon discount up to 14 FCFA for black-eyed cowpea. This result reflects the cultural roles of the grain. In Cameroon, one of the main dishes using cowpea

**Table 5. Estimated model coefficients for selected markets in Ghana.**

| Variables    | Tamale market |         | Bolgatanga market |         | Wa market |         |
|--------------|---------------|---------|-------------------|---------|-----------|---------|
|              | Coeff.        | T-ratio | Coeff.            | T-ratio | Coeff.    | T-ratio |
| Grain weight | 11.16         | 2.06**  | 11.78             | 2.15**  | 13.04     | 1.99**  |
| No. of holes | -50.98        | -1.34*  | -120.98           | -2.34** | -0.989    | -1.341* |
| Color of eye | 109.60        | 1.65**  | 226.68            | 1.77**  | 145.69    | 4.00*** |
| Grain color  | 21.22         | 2.29**  | 4.65              | 0.04    | 150.81    | 2.06**  |
| Constant     | 146.30        | 1.61    | 242.17            | 1.69    | 745.78    | 9.24    |
| R-Square     | 0.66          |         | 0.66              |         | 0.53      |         |

Source: Langyintuo et al. (2000).

Note: \*Significant at 10%; \*\*Significant at 5%; \*\*\*Significant at 1%.

**Table 6. Estimated model coefficients for selected markets in Cameroon (1997–2000).**

| Variables        | Banki market |         | Maroua market |         | Mokolo market |         | Salak market |         |
|------------------|--------------|---------|---------------|---------|---------------|---------|--------------|---------|
|                  | Coeff.       | T-ratio | Coeff.        | T-ratio | Coeff.        | T-ratio | Coeff.       | T-ratio |
| Grain size       | 3.73         | 3.42*** | 2.87          | 3.83*** | 2.07          | 1.35*   | 4.39         | 4.04*** |
| No. of holes     | -0.02        | -0.15   | -0.40         | -2.15** | -0.02         | -0.15   | -0.29        | -1.29*  |
| Color of eye     | -0.6         | -0.08   | 39.20         | -1.76** | 13.79         | 1.50*   | -35.67       | -1.64** |
| Grain color      | 8.48         | 1.06    | 1.14          | 0.18    | 1.13          | 0.13    | -5.97        | -0.76   |
| Gender of trader | 18.53        | 1.90**  | 13.52         | 1.99**  | -3.87         | -0.38   | 16.76        | 2.59*** |
| Constant         | 62.45        | 2.88*** | 123.22        | 4.68*** | 144.97        | 4.44*** | 107.28       | 3.19*** |
| R-Square         | 0.62         |         | 0.63          |         | 0.68          |         | 0.72         |         |

Source: Langyintuo et al. (2000).

Note: \*Significant at 10%; \*\*Significant at 5%; \*\*\*Significant at 1%.

is *kosa* which is preferred without black flecks. In Ghana, on the other hand, use of cowpea for *tubani* and a mixture of rice or *gari* (produced from fermented cassava dough) and beans is more important than *kosa*. Therefore, black flecks have little impact on demand. In Tamale and Bolgatanga in Ghana, white grain color does not appear to influence price. In Wa, on the other hand, white grain attracts a premium of up to ₦125/kg because of the role of *kosa* in the culture of this region. This seems to support the hypothesis that, as the cultural role of cowpea requires more decortication, color plays a significant role in price determination. In Cameroon, grain color is insignificant in explaining price variability.

The role of gender appears to be important in grain retail trade in Africa. In Cameroon, female vendors have a competitive edge over their male counterparts. This is reflected in the slightly higher premium of about 13–18 FCFA they receive in Maroua, Salak, and Banki. The hypothesis is that this is a premium for their service for selling in small quantities for immediate preparation. Female traders in Mokolo do not have a similar competitive edge over their male counterparts. Traders receive a premium for storage. Sales made beyond the fourth quarter of the year attract a premium, thus justifying the investment in storage materials.

## Farmers' pest management

It has been shown in most of the cowpea producing countries in West Africa that field pest problems are substantial, and insects such as flower thrips, mainly *Megalurothrips sjostedti* Tryb. (Thysanoptera: Pyralidae), and *Maruca* pod borer, *Maruca vitrata* Fabricius (syn. *M. testulalis*) (Lepidoptera: Pyralidae) are highly implicated in production losses (Jackai and Adalla 1997; Tamò et al. 1997; Bottenberg et al. 1997). Without chemical treatment at flowering, for instance, there can be total crop failure. Results from insecticide treatment on improved varieties have shown a substantial yield increase from 30 to 100% compared to nontreated cowpea (Pedune Nigeria 1999; Pedune Senegal 1999; Pedune Cameroon 1999; Pedune Ghana 1999; Pedune Mali). Most of the pest management research on cowpea in West Africa has focused on developing and testing field and storage pest control technologies. Among these technologies are improved genetic materials (pest and disease resistant and tolerant varieties), insecticide treatment, and plant extracts with insecticidal and fungicidal properties. Lately, the focus of research on plant extracts by such research networks as Pedune is primarily related to their low cost and very marginal disturbance of the environment. Also, botanical insecticides may represent a safe substitute for highly toxic pesticides such as cotton or cocoa insecticides, which are very often diverted onto cowpea. In Benin, for example, more than 294 000 farmers use banned insecticides such as organochlorides or organophosphates on cowpea (Pedune Benin 1999). Death and poisoning were reported from 16 villages in seven out of 12 districts in Benin. If poisoning occurred at the same rate throughout all cotton growing areas, at least 70 people might have died as a result of endosulfan (organochloride) use in just one cotton producing district in Benin (Pedune Benin 1999). Cotton insecticides are virtually the only pesticides available in the rural area of northern Benin and the only ones delivered on a credit basis. This may account for some of the hazardous uses of the insecticides, such as on food crops or in storage. In addition, farmers are not adequately informed about the hazards associated with these products. Such inappropriate uses of cotton pesticides in West Africa are well



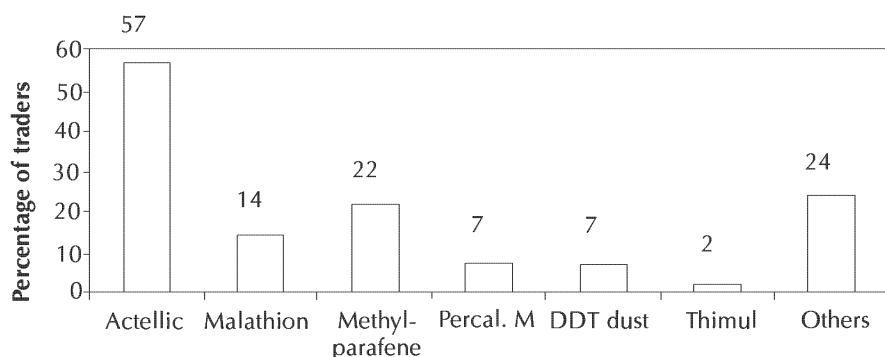
known to cotton research institutes and should have been considered when selecting insecticides for large-scale application.

In Cameroon, a Pedune survey in the Western midaltitude region showed that various chemicals are used for pest control in the field and in storage by farmers and traders (Nkamleu et al., unpublished). Synthetic chemical products are reported to be used by 46% of traders and 12% of farmers to protect cowpea in storage whereas 17% of the traders and 40% of the farmers reported using traditional methods of treatment (no chemicals). Among the traders using chemical control in storage (Fig. 2), 57% reported using Actellic® or Actellic® Super® (pirimiphos methyl, or pirimiphos methyl plus permethrin), and methyl-parafene (22%).

Other unidentified chemicals are used by 24% of chemical users for stored cowpea. Malathion® and prohibited DDT® are also fairly often used and are easily obtainable from local dealers.

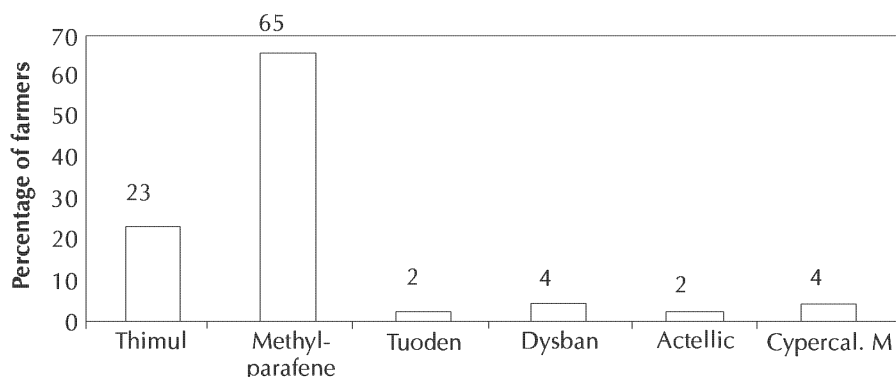
Among farmers using chemicals in storage, 65% reported the use of methyl parafene (Fig. 3) and 23% the use of Thimul. Ease of use (tablet or dust formulations) and effectiveness in controlling weevils were cited as the major reasons for the wide use of chemicals in storing cowpea.

Farmers also use nonchemical storage technologies that have been developed by both national and international research institutions (national agricultural research



**Figure 2. Storage chemical pest control by cowpea traders in West Cameroon.**

Source: Pedune, socioeconomic surveys in Cameroon, 1999.



**Figure 3. Storage chemical pest control by cowpea farmers in West Cameroon.**

Source: Pedune socioeconomic surveys in Cameroon, 1999.

systems, Purdue University, International Institute of Tropical Agriculture, cowpea research networks). The technologies include solar drying, triple bagging, ash storage, and the use of botanical extracts to store cowpea effectively and at low cost (Murdock et al. 1997). For example, the extracts of *Boscia senegalensis*, a common plant in the Sahel, is shown to cause 75–100% mortality among cowpea bruchids at very low concentration (0.67 g/l) in Senegal (Pedune Senegal 1999). While the botanicals need further testing for efficacy and adaptability to local agroecological and pest density conditions, solar drying and triple bagging are being largely disseminated. The storage pest management technologies are in high demand by both farmers and traders and would decrease losses and enhance the adoption of cowpea production technologies.

### **Economic impact assessment of cowpea technologies**

Impact assessment studies in Senegal, Cameroon, and Mali show that research on cowpea production and protection has reached a large number of people and is generating substantial economic benefits. In Senegal, over 80% of stored cowpea are stored with the CRSP drum storage technology (Faye and Lowenberg-DeBoer 1999). In northern Cameroon about 23% of the cowpea area are planted to Vya, BR1, and BR2, varieties that the CRSP helped develop and popularize (Diaz-Hermelo and Lowenberg-DeBoer 1999). About 10% of cowpea in northern Cameroon are stored with storage technologies developed by the IRAD/Purdue CRSP team. The CRSP storage technologies developed in Cameroon are now being extended to Nigeria, Niger, Burkina Faso, Mali, Senegal, Chad, Zimbabwe, and Mozambique.

Rates of return on cowpea research have varied widely. Schwartz et al. (1993) showed that returns to CRSP and USAID investments in Senegal in the early 1980s had a rate of return over 200% annually because of the large benefit from Operation Cowpea early in the life of the project. Sterns and Bernstein (1993) showed an annual rate of return of about 15% on cowpea varietal research in Cameroon in the 1980s and early 1990s. The annual rate of return on CRSP investment in the ISRA/University of California Riverside team in Senegal for the varietal development and storage after 1985 is about 16%. The rate of return to CRSP breeding and storage research in Cameroon alone is about 5%. In Cameroon and Senegal, the benefits are much higher when the use of the technology outside the country of origin is taken into account. The benefits to society resulting from the multicountry cowpea research and development range from US\$1.3 million to US\$12.3 million per year (Sanders et al. 1995).

### **Conclusion**

The contribution of social sciences to the development of the cowpea subsector for food security, income, and therefore poverty reduction is important but research is still far behind in this area compared to that in the biological sciences. The review showed that marketing studies are useful in indicating what varieties fit consumers' preferences and are widely adopted and sell for premium prices. Sub-Saharan consumers are more sensitive to bruchid damage than hypothesized, and grain color and size attract premiums according to countries and among consumer groups within countries. Seed production and dissemination will increase the diffusion rate of improved cowpea varieties. This information is useful in guiding entomologists, breeders, biotechnologists, and postharvest specialists in

developing new cowpea technologies to meet the demand. Marketing innovations should be promoted to reduce transactions and other costs that will enhance higher profits for producers and/or lower prices for consumers. The adoption studies carried out showed that farmers would adopt new cowpea technologies with substantial economic benefits. The key is to estimate the economic benefit after deducting all the costs, including transaction costs, opportunity cost of capital, and environmental and health costs associated with insecticide use. Biological scientists are challenged to produce low cost/environmentally sound cowpea to meet the increasing demand. The review showed also that major economic impact has been achieved from cowpea research in Senegal, Cameroon, Burkina Faso, and Mali with improved production and protection technologies. The next challenge is to measure the impact of these technologies on poverty reduction at the country and regional levels.

## References

- Adesina, A.A. and O.N. Coulibaly. 1998. Policy and competitiveness of agroforestry-based technologies for maize production in Cameroon. An application of policy analysis matrix. *Agricultural Economics* 19: 1–13.
- Abansi, C.L., F.A. Lantica, B. Duff, and I.G. Catedral. 1990. Hedonic model estimation: application to consumer demand for rice grain quality. *The Academy*.
- Aïtchedji, C.C. 2001. Etude de la rentabilité financière et économique des nouvelles technologies de la culture du niébé au Bénin: Cas du département du Couffo. Mémoire de Maîtrise es-Sciences Economiques, FASJEP, Université Nationale du Bénin (UNB).
- Bonifacio, E.P. and B. Duff. 1989. The impact of postharvest operations on paddy and milled rice quality. Proceedings of the twelfth ASEAN technical seminar on grain postharvest technology held at Surabaya, Indonesia, 29–31 August 1989. 25 p.
- Bottenberg, H., M. Tamò, D. Arodokoun, L.E.N. Jackai, B.B. Singh, and O. Youm. 1997. Population dynamics and migration of cowpea pests in northern Nigeria: implications for integrated pest management. Pages 271–284 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Coulibaly, O. 1987. Factors affecting the adoption of agricultural technologies in sub-Saharan Africa: the case of new varieties of cowpea around the agricultural research station of Cinzana, Mali. MSc thesis, Michigan State University, East Lansing, Michigan, USA.
- Coulibaly, O. 1995. Devaluation, new technologies, and agricultural policies in the Sudanian and Sudano-Guinean zones of Mali. PhD thesis, Purdue University, West Lafayette, IN, USA.
- Dulberger, E.R. 1989. The application of a hedonic model to quality-adjusted price index for computer processors. *In* Technology and capital formation, edited by D.W. Jorgenson and R. Landau. The MIT Press.
- Diaz-Hermelo, F. and J. Lowenberg-DeBoer. 1999. Estimating research benefits with both production and postharvest technology: the case of cowpea in Cameroon. Bean–Cowpea CRSP West Africa Regional Social Science Report # 2.
- FAO. Food and Agriculture Organization. 1998. FAO Production Yearbook, Rome, FAO.
- FAO. Food and Agriculture Organization. 1999. FAO Production Yearbook, Rome, FAO.
- FAOSTAT. 2000. Site internet : <http://www.Fao.org/statistics>
- Faye, M. and J. Lowenberg-DeBoer. 1999. Adoption of cowpea improved varieties and storage technology in the north central peanut basin of Senegal and economic impact implications. Bean–Cowpea CRSP West Africa Regional Report # 3.

- Faye, M., M. N'diaye, and J. Lowenberg-DeBoer. 2000. Identifying cowpea characteristics which command price premiums in Senegalese markets. Paper presented at the World Cowpea Conference, Ibadan, Nigeria.
- Jackai, L.E.N. and C.B. Adalla. 1997. Pest management practices in cowpea: a review. Pages 271–284 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Ladd, G. and V. Suvannunt. 1976. A model of consumer goods characteristics. *American Journal of Agricultural Economics* 58: 504–510.
- Lancaster, K. 1966. Change and innovation in the technology of consumption. *American Economics Review* 56: 132–157.
- Langyintuo, A. 1999. Summer research program in Ghana and Burkina Faso. Trip Report. Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA.
- Langyintuo, A. 2000. Summer Research Program in Ghana, Togo, Benin, and Burkina Faso, Trip Report. Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA.
- Langyintuo, A., G. Ntoukam, L. Murdock, and J. Lowenberg-DeBoer. 2000. The market value of cowpea characteristics in Cameroon and Ghana. Paper presented at the World Cowpea Conference, Ibadan, Nigeria.
- Lowenberg-DeBoer J., T. Abdoulaye, and D. Kaboré. 1994. The opportunity cost of capital for agriculture in the Sahel: case study evidence from Niger and Burkina Faso. Staff Paper #94–2, Department of Agricultural Economics, Purdue University, West Lafayette, IN, USA.
- Monke, E.A. and S.R. Pearson. 1989. The policy analysis matrix for agricultural development. Cornell University Press, Ithaca, USA.
- Murdock, L.L., R.E. Shade, L.W. Kitch, G. Ntoukam, J. Lowenberg-DeBoer, J.E. Huesing, W. Moar, O.L. Chambliss, C. Endondo, and J.L. Wolfson. 2000. A postharvest storage of cowpea in sub-Saharan Africa. Pages 302–312 *in* Advances in cowpea research, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Naik, G. 1995. Price-quality relationships in raw silk markets. *Indian Economics Review* 30(1): 101–119.
- Pedune-Benin. Production ecologiquement durable du niébé. 1999. Annual country report. Benin. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pedune-Ghana. Production ecologiquement durable du niébé. 1999. Annual country report. Ghana. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pedune-Cameroon. Production ecologiquement durable du niébé. 1999. Annual country report. Cameroon. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pedune-Mali. Production ecologiquement durable du niébé. 1999. Annual country report. Mali. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pedune-Senegal. Production ecologiquement durable du niébé. 1999. Annual country report. Senegal. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pedune. Production ecologiquement durable du niébé. 1999. Socioeconomic surveys in Cameroon. International Institute of Tropical Agriculture. Cotonou, Benin.
- Pedune-Nigeria. Production ecologiquement durable du niébé. 1999. Annual country report. Nigeria. International Institute of Tropical Agriculture, Cotonou, Benin.
- Pesticides News. 2000. Annual report. No. 47. March 2000. 24p.
- Peter, T., S. Tovignon, and S.D. Vodouhè. 2000. Endosulfan deaths and poisonings in Benin. Pesticides News 47. March 2000.

- Sanders, J., T. Bezuneh, and A. Schroeder. 1994. Impact assessment of the SAFGRAD commodity networks. USAID/AFR/ARTS/FARA, Washington, DC, USA.
- Sanders, J., B. Shapiro, and S. Ramaswamy. 1995. The economics of agricultural technology in semi-arid sub-Saharan Africa. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Schwartz, L., J.A. Sterns, and J.F. Oehmke. 1993. Economic returns to cowpea research, extension, and input distribution in Senegal. *Agricultural Economics* 8: 161–171.
- Sterns, J. and R. Bernstein. 1993. Assessing the impact of cowpea and sorghum research and extension in northern Cameroon. Michigan State University, International Development Working Paper No. 43.
- Tamò, M., H. Bottenberg, D.Y. Arodokoun, and R. Adeoti. 1997. The feasibility of classical biological control of two major cowpea insect pests. Pages 259–270 *in* *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Walburger, A. and K. Foster. 1994. Using censored data to estimate implicit values of swine breeding stock attributes. *Review of Agricultural Economics* 16(2): 259–268.

## 5.2

# Industrial potential of cowpea

C. Lambot<sup>1</sup>

### Abstract

In West Africa, cowpea is a popular legume occupying an important position in local food habits in such countries as Burkina Faso, Niger, and Nigeria where it is a staple food. This observation is, however, not valid in some other West African countries such as Côte d'Ivoire where people are traditionally high consumers of tubers and cereals. Cowpea is also important in some other regions of Africa (Kenya) and the world (India and Brazil), reinforcing its business potential for industrial food companies. The nutritional value of cowpea is mainly from its protein and carbohydrate content. Cowpea's high protein level represents its major advantage for use in nutritional products and compensates for the high proportion of carbohydrates often ingested in African diets. Cowpea is rich in lysine; consequently it can be used to balance cereals. Its deficiency in sulphurous amino acids is addressed when it is combined with milk protein and/or cereals known for their high methionine and cystine content. The antinutritional problems linked with the presence of tannins or trypsin inhibitors can be easily avoided with appropriate dehulling and heat treatment. The flatulent sugars are not a limiting factor; cowpea has a lower raffinose content than soybean. Taking into consideration the good nutritional value and the positive image of cowpea for African consumers, it can be concluded that cowpea could be a good source of protein for industrial product manufacturing. It can also be stated that its protein content can still be improved by breeding, using the existing natural diversity in cowpea. The major constraints to the industrial use of cowpea by food companies in Africa include the lack of reliable statistics on production, the strong price fluctuations during the year, the low quality of the raw material in terms of physical defects, and the lack of primary processors. Again, the comparative price with soya, which is influenced by world market price, is sometimes not in favor of cowpea. The low quality of cowpea available in the open market is due mainly to the high level of physical defects, but the problem of pesticide residues is also critical for this raw material, which is highly susceptible to insect damage. Processed ingredients based on cowpea are not readily available, forcing food companies to invest in primary processing. There are clear opportunities to develop industrial products using cowpea as a source of protein, but strong support from governments is necessary to promote and to organize the supply chain and the primary processing. The support from research institutions for programs aiming to increase the protein content of cowpea is also required.

### Introduction

Food habits in West Africa are mainly based on tuber crops (cassava, yam) and cereals (maize, rice). Although they have a high nutritional value, grain legumes are a minor component of food diets. Tentative efforts have been made to introduce soybean in African food habits and farmer activities, but with little success. Even in Nigeria, where the annual

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national production of soybean is around 326 000 t, it is still considered an industrial crop, difficult to cook, and with an undesirable taste. Unlike soybean, cowpea is appreciated and is gradually assuming an important position in the food habits of West Africans. Different traditional African meals, foods, and seasonings are prepared from cowpea, among them homemade weaning foods. Cowpea is bought in the market, and processing (soaking, dehulling, milling, etc.) is done at home.

In Africa, cowpea is the most popular legume and the largest part of world production originates from this continent. Cowpea is adapted to stressful environments where other crops either fail or do not perform well. It is a food security crop in the semiarid zone of West and Central Africa (WCA) which ensures farm household subsistence food supply even in dry years. Recently, FAO (1996) estimated the world production area as 5.6 million ha, of which at least 90% is in West and Central Africa, and the annual world grain production is estimated at 2.7 million tonnes. There are some indications that recent FAO statistics underestimate the production.

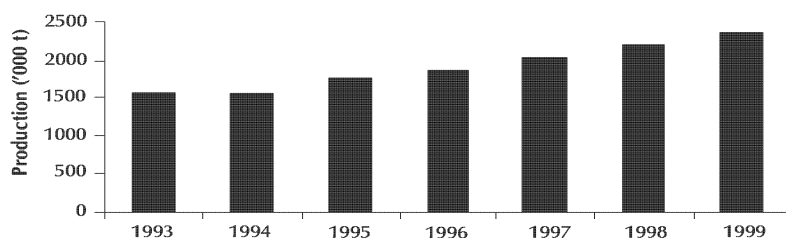
Cowpea scientists indicate a much larger production area of 12.5 million ha, with 8 million ha (64%) in WCA and an annual world grain production of 3 million tonnes (Singh et al. 1997). If the production area is nearly 12 million ha, this is about 45% of the area recorded for dry beans. Production areas are also spread all around the world, making cowpea a global crop.

The important cowpea growing countries in WCA are Benin, Burkina Faso, Cameroon, Chad, Ghana, Mali, Niger Republic, Nigeria, Senegal, and Togo. Some countries in East Africa (e.g., Kenya which produces around 250 000 t/year) are also important for cowpea production.

National production increased rapidly in Nigeria from 1 576 000 t in 1993 to 2 181 000 t in 1999. The same trend was noticed in Burkina Faso (79 797 t in 1994 to 327 000 t in 1998) (Figs. 1 and 2). This reflects consumer appreciation and national support for cowpea.

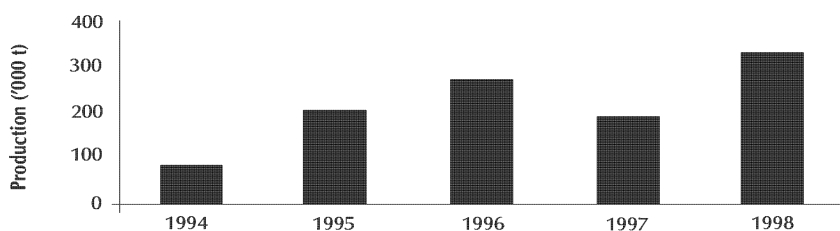
## **Nutritive value of cowpea**

The chemical composition of cowpea is similar to that of most edible legumes. It contains about 24% protein, 62% soluble carbohydrates, and small amounts of other nutrients. Thus, most of its nutritional value is provided by proteins and carbohydrates. Many references are available on cowpea nutrient content (Table 1). The high protein content represents a major advantage in the use of cowpea in nutritional products, for infant and children's food, and to compensate for the large proportion of carbohydrates often ingested in African diets.



**Figure 1. National production of cowpea in Nigeria. Estimated annual production for 1998 and 1999.**

Source: Central Bank of Nigeria 1997 and 1998.



**Figure 2. National production of cowpea in Burkina Faso.**

Source: SAFGRAD unpublished data 1999.

**Table 1. Cowpea grain composition (g/100 g).**

|                              | (Bliss 1975) | (Omuetti and Singh 1987) | (Nielsen et al. 1993) |
|------------------------------|--------------|--------------------------|-----------------------|
| Number of cultivars analyzed | 8            | 37                       | 100                   |
| Protein                      | 24.1–25.4    | 20.30–29.05              | 22.9–32.5             |
| Crude fiber                  | 5.0–6.9      | 2.7–5.8                  | –                     |
| Carbohydrate                 | 60.8–66.4    | –                        | 59.7–71.6             |
| Soluble sugar                | –            | 5.9–8.3                  | –                     |
| Starch                       | –            | 39.1–54.9                | –                     |
| Ash                          | 3.4–3.9      | –                        | 2.9–3.9               |
| Fat (g/100 g)                | 1.1–3.0      | 1.66–2.82                | 1.4–2.7               |

Since cowpea is a major source of protein in the diet of many people in sub-Saharan Africa, any effort made to increase the level of protein in the seeds would improve the quality of the diet of the population. There are indications that progress can be made through appropriate selection of parental lines for crossing. Considering the existing variability mentioned in literature, the protein content in cowpea can probably be increased by up to 33%.

Cowpea is especially rich in lysine, but it is deficient in sulfurous amino acids. Compared to other legumes, methionine and tryptophan levels are high. Except for total sulfurous amino acids, and to a lesser extent isoleucine, levels of essential amino acids are at least as high as those in soybean (Table 2). The protein quality of cowpea products can be increased significantly by combining it with milk protein and/or cereals known for their high methionine and cystine content (Boulter et al. 1975).

Cowpea contains a higher level of flatulent sugars than that found in soybean but its raffinose content (most flatulent sugar) is lower than that in soybean (Table 3). Grain soaking before dehulling and milling decreases levels of the flatulent sugars. Therefore, their presence in cowpea should not limit its use.

Levels of trypsin inhibitors are about half the values observed on soybean and are inactivated by a heating process. Also, the phytate content of cowpea is half that of soybean.

### Cowpea procurement

Raw materials procurement is crucial in Africa since supply chains for agricultural raw materials are not adapted to industrial needs and specifications. The problems faced are the



**Table 2. Essential amino acids in cowpea, food legumes, and cereals.**

|              | Protein    | Lysine    | Met.      | Met.+cys. | Tryptophan | Leucine   | Isoleucine | Phenyl. +Tyr | Histidine | Threonine | Valine    |
|--------------|------------|-----------|-----------|-----------|------------|-----------|------------|--------------|-----------|-----------|-----------|
|              | (N × 6.25) | (g/16 gN) | (g/16 gN) | (g/16 gN) | (g/16 gN)  | (g/16 gN) | (g/16 gN)  | (g/16 gN)    | (g/16 gN) | (g/16 gN) | (g/16 gN) |
| Flour        | (N × 6.25) | (g/16 gN) | (g/16 gN) | (g/16 gN) | (g/16 gN)  | (g/16 gN) | (g/16 gN)  | (g/16 gN)    | (g/16 gN) | (g/16 gN) | (g/16 gN) |
| Cowpea       | 21.6       | 6.9       | 1.4       | 2.3       | 1.2        | 8.0       | 4.1        | 9.0          | 3.2       | 3.9       | 5.0       |
| Lentil seeds | 19.5       | 8.0       | 0.94      | 2.0       | 1.1        | 9.0       | 5.1        | 9.5          | 3.0       | 4.8       | 5.9       |
| Mung bean    | 24.9       | 8.1       | 1.6       | 2.7       | 1.6        | 9.25      | 5.3        | 10.2         | 3.5       | 4.2       | 6.0       |
| Chickpea     | 22.4       | 6.9       | 1.3       | 2.7       | 0.8        | 7.4       | 5.8        | 8.2          | 2.7       | 3.5       | 4.95      |
| Millet       | 10.0       | 2.8       | 2.5       | 4.0       | 1.8        | 13.5      | 5.5        | 7.2          | 1.9       | 4.2       | 6.1       |
| Soybean      | 40         | 6.6       | 1.3       | 3.0       | 1.4        | 8.1       | 4.8        | 9.0          | 2.7       | 4.3       | 5.0       |
| Wheat        | 10.5       | 2.5       | 1.7       | 4.4       | 1.4        | 8.1       | 4.6        | 8.4          | 2.3       | 3.3       | 5.0       |
| Milk         | 34.3       | 7.8       | 2.5       | 3.3       | 1.4        | 9.8       | 6.4        | 9.9          | 2.6       | 4.6       | 6.9       |
| FAO infant   |            | 6.6       |           | 4.2       | 1.7        | 9.3       | 4.6        | 7.2          | 2.6       | 4.3       | 5.5       |
| FAO 2-5y     |            | 5.8       |           | 2.5       | 1.1        | 6.6       | 2.8        | 6.3          |           | 3.4       | 3.5       |

Source: Gaudard de Weck et al. 1999.

Note:

Met = Methionine

Cys = Cystine

Try = Tryptophan

Phenyl = Phenylalanine

**Table 3. Antinutritional factors and flatulent sugars in cowpea and soybean.**

|                                   | Cowpea<br>Dehulled seeds<br>(in-house analyses<br>results) | Literature<br>value | Soybean  |
|-----------------------------------|--|---------------------|----------|
| Flatulent sugars <sup>†</sup> (%) | 5.5–6.6  | 3.0–7.8             | 2.9–5.5  |
| Raffinose (%)                     | 0.36–0.48  | 0.4–1.2–2.5         | 0.7–1.0  |
| Trypsin inhibitors<br>(TIU/mg N)  | 228–646  | 122–440             | 390–1030 |
| Phytates (%)                      | 0.37–0.54  | 0.44–1.7            | 1–1.5    |

<sup>†</sup>raffinose + stachyose + verbascose.

Source: Gaudard de Weck, personal communication.

high fluctuations in price and quality, the difficulty in identifying reliable intermediaries, and the absence or low development of local raw material primary processors. The informal sector is a major force in the food crops market in Africa and, usually, the proportion of the food crop production, which is commercialized, is estimated at 15%.

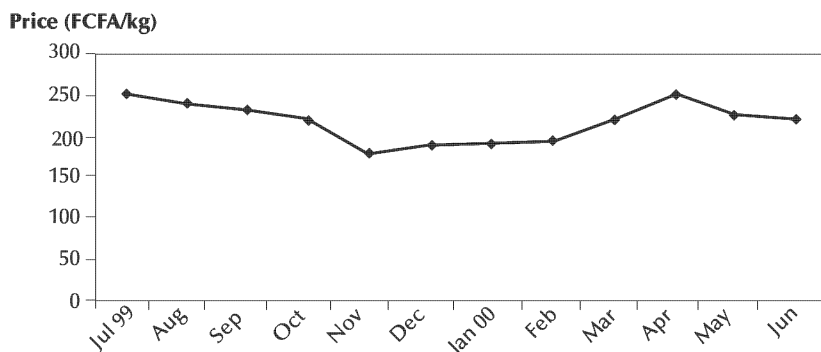
### Cowpea procurement strategies

The absence of primary processors of grain in the supply chain forces food industries interested in cowpea to look for grain procurement. Different approaches could be considered ranging from purchasing on the market to direct procurement from farmers to contract growing. Studies carried out in Côte d'Ivoire and Burkina Faso in a collaborative program between Nestlé R&D Center, Abidjan, and SAFGRAD (Semi-Arid Food Grains Research and Development) demonstrated the feasibility of the contract growing option. The profitability of cowpea for farmers is guaranteed when they grow improved varieties using fertilizer and insecticide. The break-even point was estimated at 300 kg/ha. Furthermore, the daily income generated by growing cowpea is comparable to the average local daily wage. Hence, it was concluded that contract growing is interesting if a specific variety is required for product manufacture, giving also the advantage of a better quality compared to that of the open market option.

Nevertheless, cowpea is available in large quantities on the open market of the different West African countries, a good argument in favor of this procurement strategy. But price and quality fluctuations during the year are important constraints requiring investigations to adapt the strategy accordingly. The main varieties available on the open markets in West Africa are white with black eye. One of them in Nigeria is named Kanannado.

### Cowpea price fluctuations

Close market monitoring for cowpea price registration was carried out in Côte d'Ivoire and Nigeria. During the 1995/1996 season in Côte d'Ivoire, it appeared that cowpea price would double within a year with a maximum in September and a minimum just after the harvesting period during November and December. These seasonal fluctuations reduced recently, probably as a result of the rapid increase in national cowpea production in Burkina Faso which is a natural supplier for Côte d'Ivoire. Cowpea price in November at wholesale level in Abidjan decreased from 250 FCFA/kg in 1995 to 180 FCFA/kg in 1999 (Fig. 3).



**Figure 3.** Cowpea price fluctuation during the 1999/2000 season at the Abidjan wholesale market.

For Nigeria, in 1999/2000, cowpea price fluctuated in six different markets. The average of delivery price to Lagos is indicated in Figure 4. Prices were lower during the harvest period (October–December) and higher from April to June in 1999. Prices steadily decreased from September 1999 until December 1999, and then increased in January and February 2000.

### Quality evaluation

Kanannado samples were collected from different markets in Nigeria and at different periods during the 1999/2000 season (Table 4, Fig. 5). Laboratory results of samples collected monthly from each location indicated that the average protein content of white cowpea is 23.6%. Humidity levels ranged between 6.6% and 13.5% depending on the period of the year. However, high defect counts, 12.9% on the average, were noticed in the samples collected.

The poor quality of cowpea available on the markets is a major constraint for industrial use considering that 3.7% will be lost (stones and waste) and that 9.2% are defective grains. It was estimated that only 40% of cowpea available on the open market is acceptable for industrial use in relation to specifications for physical defects.

Considering that cowpea grain is highly susceptible to weevils (*Callosobruchus* sp.), it was also important to evaluate the level of pesticide residues in the raw material. Cowpea purchased in April or May 1999 showed higher levels of the pesticide residues than normal, especially for chlorpyrifosethyl and pirimiphosmethyl (Table 5). The concentrations of these two organophosphates are such that will result in a calculated exposure exceeding safety standards. The case of chlorpyrifosethyl is unclear, as this pesticide is usually not used for grain storage but for soil treatment and building maintenance. Pirimiphosmethyl is a common pesticide used for grain storage with well known commercial forms (Actellic).

The level of contamination with pesticides was higher for samples purchased in April–May than for samples purchased during the period following harvesting (Nov–Jan). This indicates that the pesticide residues could be a consequence of long storage in non-adapted conditions. It can be suspected that postharvest treatments applied to cowpea are not performed correctly and this leads to variation in the quality of the raw material.

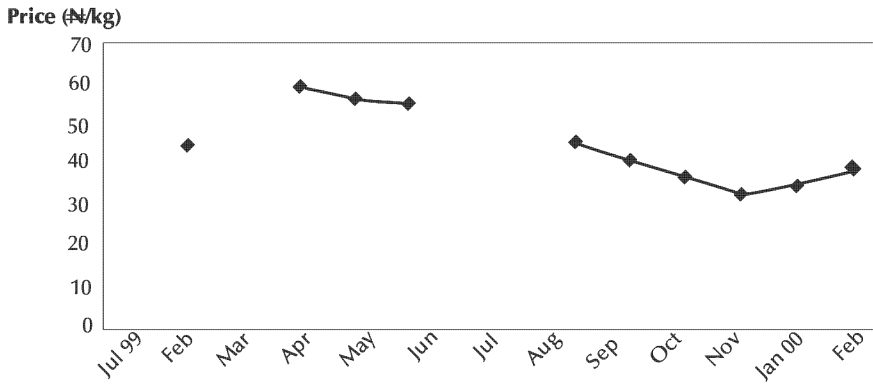


Figure 4. Cowpea price fluctuation—Average of delivery price to factory in Lagos.

Table 4. Cowpea quality evaluation (%) overall.

|                   | Average | Minimum | Maximum |
|-------------------|---------|---------|---------|
| Protein (DM)      | 23.64   | 22.3    | 26.1    |
| Moisture          | 8.74    | 6.6     | 13.5    |
| Total defects     | 12.89   | 5.0     | 20.9    |
| Broken            | 2.08    | 0.0     | 6.2     |
| Holes             | 5.75    | 0.0     | 17.1    |
| Stones            | 0.16    | 0.0     | 1.2     |
| Colored           | 0.78    | 0.0     | 6.7     |
| Foreign varieties | 0.60    | 0.0     | 2.9     |
| Waste             | 3.52    | 0.3     | 8.6     |

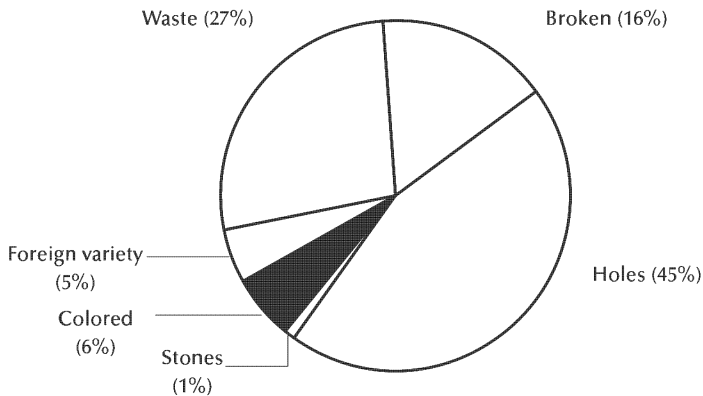


Figure 5. Defects category in Kanannado variety (overall mean).

**Table 5. Aluminum and pesticide residues in cowpea—different periods and markets in Nigeria.**

| Period of the year        | Norms codex <sup>†</sup> | May 1999  | April 1999 | November 1999 |                            | January 2000 |                            |
|---------------------------|--------------------------|-----------|------------|---------------|----------------------------|--------------|----------------------------|
| Markets                   |                          |           |            | Katsina       | Five other Nigeria markets | Katsina      | Five other Nigeria markets |
| Aluminum                  | Ppm                      | 10        | 17         | 9.2           | 8.2                        | 12.0         | 11.3                       |
| alpha-chlordane           | Ppb                      |           | 19         |               |                            |              |                            |
| Gamma HCH                 | Ppb                      | 1000 (db) | 210        | 350           | 13.7                       |              |                            |
|                           |                          |           |            | 93            |                            |              |                            |
| Chlorpyrifosethyl         | Ppb                      | 400 (fb)  | 1260       | 940           |                            |              |                            |
| Diazinon                  | Ppb                      | 100 (g)   | 98         | 99            |                            |              |                            |
| Pirimi-phosmethyl         | Ppb                      | 1000 (fb) | 1560       | 910           | 485                        | 168          | 11                         |
| Triazophos                | Ppb                      |           |            | 290           |                            |              |                            |
| Profenofos                | Ppb                      |           | 770        |               |                            |              |                            |
| Cypermethrin              | Ppb                      | 100 (fb)  | 910        | 58            |                            |              |                            |
| Deltamethrin, Decamethrin | Ppb                      | 1000 (db) | 19         | 31            |                            |              |                            |
| Permethrin                | Ppb                      | 100 (db)  | 200        | 61            |                            |              |                            |

<sup>†</sup>db = dry bean; fb = fresh bean; g = groundnut.

## Conclusions and recommendations

Cowpea is a source of good quality protein appreciated by Africans. The rapid increase in volume of production is a proof of the acceptance of this legume. However, increased production is affecting the prices of cowpea.

The industrial use of cowpea is facing some major constraints: primary processors do not exist, forcing food industries to process the grain; the quality of the grain available on the open market is poor, with a high percentage of physical defects and a risk of pesticide residue contamination; strong price fluctuations along the year are forcing procurement during a short period; the protein content of available cowpea is low compared to that of soybean.

Considering these constraints, it can be suggested that national and international research programs on cowpea should be encouraged to promote and support the development of primary processing of cowpea for food industries.

Training and technical support to farmers and wholesalers on the proper application of pesticides is also a priority in order to ensure the safety of the product. The development of varieties resistant to weevil infestation would ensure high quality grain with low levels of pesticide contamination.

Increased protein content would make cowpea more attractive for the African food industry as it would then compete with soybean. Furthermore it would have a positive impact on consumers' health through improvement of their diet.

- Lowenberg-DeBoer, J. 1999. Trip report: Zimbabwe, Mozambique, Benin, 15 February to 10 March 1999. Bean–Cowpea CRSP, East Lansing, MI, USA.
- Masters, W., B. Coulibaly, D. Sanogo, M. Sidibe, and A. Williams. 1996. The economic impact of agricultural research: a practical guide. Purdue University, West Lafayette, IN, USA.
- Murdock, L. and R. Shade. 1988. 1988 Annual Report, Bean–Cowpea CRSP, Purdue University/IRA–Cameroon Project.
- Murdock, L., R. Shade, and Z. Boli. 1989. 1989 Annual Report, Bean–Cowpea CRSP, Purdue University/IRA–Cameroon Project.
- Murdock, L.L., R.E. Shade, L.W. Kitch, G. Ntougkam, J. Lowenberg-DeBoer, J.E. Huesing, W. Moar, O.L. Chambliss, C. Endondo, and J.L. Wolfson. 1997. Postharvest storage of cowpea in sub-Saharan Africa. Pages 302–312 in *Advances in cowpea research*, edited by B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria.
- Muth, R.F. 1964. The derived demand curve for productive factor and the industry supply curve. *Oxford Economic Papers* 16: 221–234.
- Ntougkam, G. and L. Kitch. Solar heaters for improved cowpea storage. Technical Bulletin. Bean–Cowpea CRSP, East Lansing, MI, USA.
- National Cereals Research and Extension (NCRE) Project, Plan of Work, Republic of Cameroon, Ministry of Higher Education and Scientific Research (MESIRES), Institute of Agricultural Research (IRA), 1988, 1991, 1992, 1993, 1994.
- Oumarou, J.-P. 1999. Etude sur le circuit commercial du niébé dans la Province de l'Extrême Nord du Cameroun. Undergraduate thesis, University of Ngaoundéré, Ngaoundéré, Cameroon.
- Purdue University/Institut de la Recherche Agronomique (Purdue/IRA). 1987. Detailed Project Annual Report, Bean–Cowpea CRSP, Purdue University/IRA–Cameroon Project.
- Purdue University/Institut de la Recherche Agronomique pour le Développement (Purdue/IRA). 1992. Detailed Project Annual Report, Bean–Cowpea CRSP, Purdue University/IRA–Cameroon Project.
- Purdue University/Institut de la Recherche Agronomique pour le Développement (Purdue/IRA). 1993. 1993 Detailed Project Annual Report, Bean–Cowpea CRSP, Purdue University/IRA–Cameroon Project.
- Purdue University/Institut de la Recherche Agronomique pour le Développement (Purdue/IRA). 1997. 1996 Detailed Project Annual Report, Bean–Cowpea CRSP, Purdue University/IRAD - Cameroon Project.
- Purdue University/Institut de la Recherche Agronomique pour le Développement (Purdue/IRA) and the National Cereals Research and Extension Project Testing and Liason Unit (NCRE/TLU). 1990. On-farm tests of storage technologies: 1990–1991. In *Supplement to the 1990 Detailed Project Annual Report, Bean–Cowpea CRSP, Purdue/IRA–Cameroon Project*.
- Rose, F. 1980. Supply shifts and the size of research benefits: comment. *American Journal of Agricultural Economics* 62: 834–837.
- Schultz, M.A. 1993. Economic assessment of cowpea grain storage techniques: a case study of North Cameroon. Unpublished MSc Thesis, Dept. of Agricultural Economics, Michigan State University, East Lansing, MI, USA.
- Sterns, J. and R. Bernstein. 1993. Assessing the impact of cowpea and sorghum research and extension in northern Cameroon. Michigan State University, International Development Working Paper No. 43. Michigan State University, East Lansing, MI, USA.
- Vijverberg, W.P. 1991. Profits from self-employment: the case of Côte d'Ivoire. *World Development* 19(6): 683–696.
- Wolfson, J. 1990. Analysis of cowpea production and storage methodologies used by small farmers in northern Cameroon. Purdue/IRA Cowpea Storage Project, January 1990.

## 5.6

# Identifying cowpea characteristics which command price premiums in Senegalese markets

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### Abstract

Since the 1980s cowpea has become an alternative cash crop in northern Senegal. However, in 1997, surveys showed that farmers often sold their crop at unprofitable prices. The hypothesis was that farmers could improve selling prices if they produced cowpea with the characteristics demanded by consumers. To identify those characteristics, data were collected in six markets from January 1998 to December 1999. Regression analysis was used to estimate a linear relationship between price and grain characteristics. The results showed that consumers were willing to pay premiums for grain size (119 FCFA/100 g) but discount price for number of bruchid holes (0.62 FCFA/hole), red eye (40 FCFA/kg), red skin (30 FCFA/kg), and smooth skin (21 FCFA/kg).

### Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) commonly referred to as black eyed peas in the US, is an important source of nutrition in West Africa. This crop serves to bridge the hunger gap between the planting and harvesting periods of the main food crops. With 10% of the area cultivated, cowpea is the third most important crop in Senegal after millet, the main food crop, and peanuts, a major cash crop. Cowpea was traditionally grown in Senegal for food (Tall 1991). However, since 1985, following several years of poor peanut harvests, cowpea has become increasingly viewed as an alternative cash crop. This is particularly true in the northern part of the country where a short rainy season and an annual rainfall of less than 300 mm does not favor peanut production.

For this reason, the Senegalese Agricultural Research Institute (ISRA) is currently engaged in a research program focusing on the breeding and dissemination of early maturing (less than 45 days) and high yielding varieties of cowpea. The purpose of this program is to improve production and to promote cowpea marketing by providing earlier varieties, which could be marketed before the traditional varieties and receive a higher price.

In 1997, as part of the research program, surveys were done to assess the impact of the new varieties on production and cowpea marketing. The results of the survey revealed that the area planted to cowpea had not expanded as expected. In addition, the farmers were found to still be selling their production surplus at prices below their cost of production.

### Problem statement

Farmers face difficulty in selling their production surplus at profitable market prices. In order to address this problem, it is necessary to know how the buyers value the different

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cowpea qualities. Nongovernmental organizations (NGOs), which are involved in new cowpea variety extension, ISRA, and cowpea producers and authorities in Senegal are all interested in this problem as they require more information on cowpea market price and grain characteristics. The specific objective of this paper is to measure the relationships between price and grain characteristics in order to identify priority areas for the breeding program.

## **Literature review and theoretical model**

Based on the economic principle that product demand stems from the utility provided as a function of its quality characteristics (Berndt 1991), a hedonic pricing model was used to analyze the data. Since its introduction, numerous economists have employed hedonic pricing models as a tool for estimating the price–quality relationships of commodities over time or through cross-sectional data analysis. One of the earliest examples of this methodology dates back to 1974 when Sherwin Rosen (1974) first sketched on scratch paper a model of product differentiation based on the hedonic hypothesis that goods are valued for their utility-bearing attributes. In his model, Rosen used observed product prices and the specific number of characteristics associated with each good to define a set of implicit or hedonic prices.

Brorsen et al. (1984) further contributed to the acceptance of this analytical tool by studying market acceptance of rough rice. The Brorsen study revealed that several factors are involved in the distinction of rough rice. He evaluated the ability of federal grain inspectors to explain the factors that led to the grade classification and estimated the discount associated with each factor using a hedonic price model.

Espinosa and Goodwin (1991), with the same motivation as those authors cited earlier, employed a profit maximization framework and hedonic pricing model to assess the impact of wheat characteristics on market price.

This paper follows the framework outlined in the Espinosa study with one notable exception: the hedonic-pricing model used here on cowpea does not incorporate the possibility of processing cowpea since data based on these characteristics are unavailable.

Despite the absence of processing attributes, the general theory of hedonic pricing as developed by Espinosa closely relates to the current study in one important way: it follows a consumer goods approach and considers individual characteristics as utility providing attributes in a utility maximizing problem. Under this approach, an agricultural product is desired because of its particular quality characteristics. From this, it is assumed that cowpea consumers behave as utility maximizing agents.

From the first order condition of the utility maximization problem can be derived the general form of the hedonic price model. This function would be expressed as a regression of the following form:

$$P_{it} = \alpha_0 + \sum_{k=1} \beta_k Z_{itk}$$

Where  $P_{it}$  = Per unit price of cowpea

$\alpha_0$  = Intercept

$\beta_k$  = Marginal value of characteristic k

$Z_{itk}$  = Amount of characteristic k in good i at time t

Some authors use the semi-logarithm functional form or combine linear and quadratic time trends and dummy variables. In this paper, the linear model is used because of its



theoretical interpretation (discounts or premiums) and also because it is easier to explain to market participants.

### Source of data

Data were collected from six markets (Fig. 1) chosen according to their location and volume of cowpea sales.

In each market, samples were bought from five different vendors every month from January 1998 to December 1999. The choice of the vendors at a given market was done randomly. For each sample, the following variables were noted: market price, skin texture, skin color, eye color, weight of 100 grains, length, width, and number of bruchid holes per 100 grains. Also observed were the locations (markets), the gender of the sellers, and the selling period (month).

### Econometric method

Regression analysis was employed to estimate a linear relationship between price and grain characteristics. Generalized least square (GLS) was used to correct for temporal error correlation across the cross sectional observation. The dependent variable was price (P) in franc CFA per kg. The independent variables were: average weight (W) of 10 grains in mg, number of bruchid holes (NH), skin color, skin texture, eye color, and grain size (refers to average length of 10 grains multiplied by average width of 10 grains).

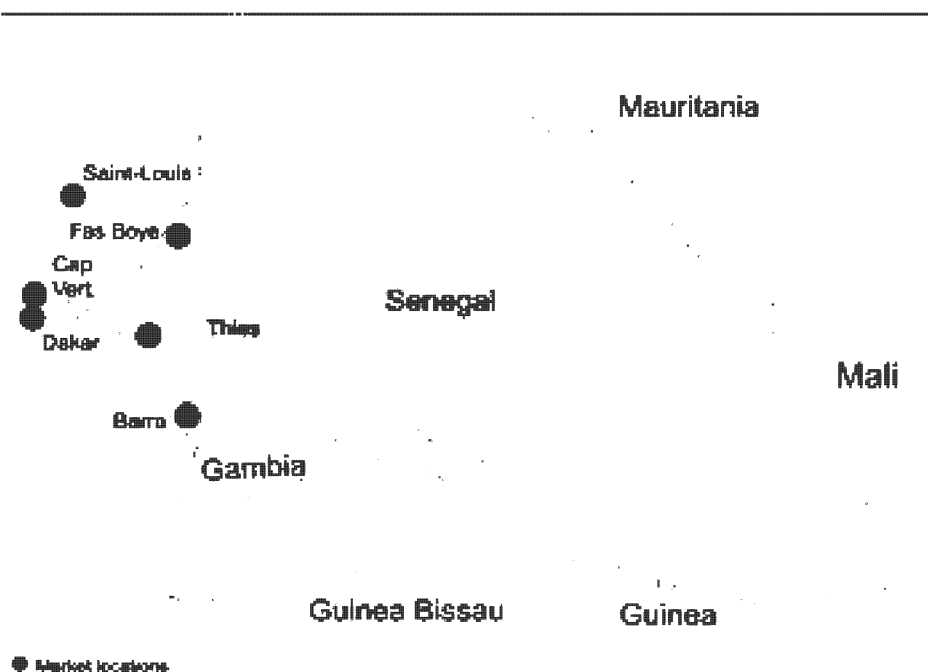


Figure 1. Market locations.

The variables (month, market [location] and gender of sellers were handled as dummy variables as well as all the other qualitative variables. A base variable was defined for each group of dummy variables (Table 1).

## **Expected signs for estimated parameters**

The common characteristics were those which could be taken into account in breeding programs, or were generally used to determine the value of cowpea grain. These variables included: number of holes per 100 grains, grain skin and eye color, grain skin texture, and grain size. The number of holes (NH) refers to the level of insect damage and is expected to have a negative sign. The signs for white skin color and rough skin texture are expected to be positive.

Grain size (Gsize) would have a positive sign because consumers prefer large seeds for their sauce or rice. Also because grain size refers to the quantity of flour, processors would be willing to pay a premium for it.

## **Results**

This part will begin with an overview of the cowpea marketing and market structure in Senegal. Then it will discuss, respectively, the type of sellers and the distribution of skin color, eye color, and skin before reporting the results on hedonic price estimations.

### **Market structure**

Compared to peanut for which the market is supervised by the government, cowpea has a competitive market without any government intervention.

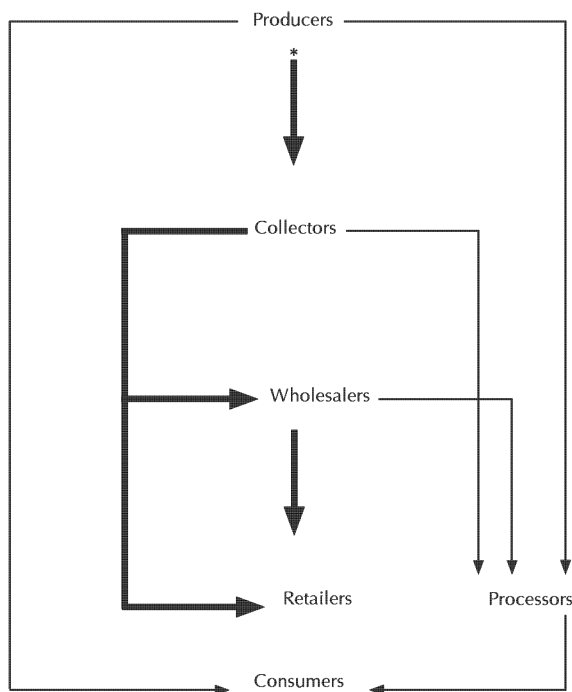
Relationships among market participants are based on informal agreement and on some ethics they define. In moving cowpea from farm gate to urban consumers, different linked steps are identified in terms of market participants (Fig. 2).

Producers are the first cowpea suppliers on the market channel. They supply on average 28 000 tonnes per year, 71% of which is supplied from northern Senegal (DSA1998). These producers sell their produce directly to the collectors, wholesalers, processors, retailers, or to the consumers.

Collectors, on the other hand, are individual entrepreneurs. They buy cowpea from market to market in the production area, and in return supply wholesalers, retailers, and processors. But to avoid any competition with the retailers (who buy from them) they don't sell to consumers. Most of the time, collectors use their own money to make their transactions. They can also get money from wholesalers based in towns depending on some specific relationships (relative or close friend). Collectors don't usually have a specific storage space except if their home village or town is close to the collecting area. In this

**Table 1. Base variables.**

| Variables    | Base    |
|--------------|---------|
| Skin color   | White   |
| Skin texture | Rough   |
| Eye color    | White   |
| Market       | Sagatta |
| Month        | October |
| Gender       | Female  |



**Figure 2. Market participants and relationships.**

\*The size of the arrows indicates the importance of the quantities of cowpea exchanged between two groups of participants.

case they use one room in the house as a storage area and keep their cowpea in metallic drums or in plastic bags.

The processors own small units where they process cowpea into flour or other cowpea-based products. Depending on their location, they buy their input from collectors, wholesalers, or sometimes directly from producers.

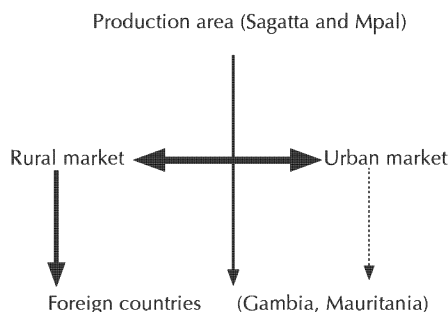
Wholesalers and retailers are shopkeepers with the difference that wholesalers specialize in one or two agricultural products.

Consumers are the last users and account for all cowpea buyers whose objective is to consume cowpea as food.

As shown in Figure 3, cowpea traders go from the production area to the rural or urban markets from where the product can be exported to neighboring countries like Gambia and Mauritania.

Any movement in cowpea leads to financial charges, the most important being those for transportation and storage. In Senegal, loading cost is 25 FCFA per 50 kg bag in the rural market. Producers use carts and trucks to transport cowpea from their area to the rural market. They often pay between 50 and 100 FCFA per 50 kg bag. The same price is charged from rural to urban markets, no matter the distance.

To transport cowpea outside Senegal, importers from Banjul and Mauritania use trucks. The transportation cost is fixed per load and varies between 50 000 and 125 000 FCFA depending on the volume of cowpea transported. The bags used to store the cowpea cost



**Figure 3. Spatial pattern of cowpea marketing.**

100 FCFA per unit and can contain 50 kg. As shown in Figure 4, farmers face variable prices (from 46 to 780 FCFA). Prices are lowest between October and December, which corresponds to the harvest period.

## Data overview

### *Types of sellers*

Male sellers play an important role in selling cowpea in Dakar and Nioro. More than 91% of the sellers in Dakar are men against 100% for Nioro (Table 2). In Bambey and Mpal on the other hand, females represent most of the sellers, with 76 in Bambey and 74% in Mpal of the population interviewed. It is only in Sagatta that equity between men and women selling cowpea was observed. Wholesalers are present only in Dakar (Tilene) and in Mpal.

### *Skin texture*

Two types of skin texture, smooth and rough, were observed. Except for Castors where the percentage of cowpea with rough skin was 39%, Figure 5 shows that 50–65% of the grains had rough skin.

### *Skin color*

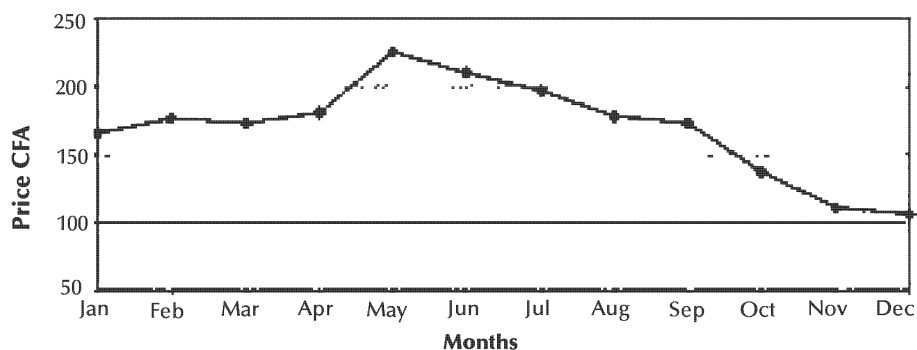
In Mpal, 52% of the cowpea sold was white while in Bambey, Nioro, and Sagatta, the proportion of cowpea with mixed color was above 50% of the samples observed (Fig. 6).

That the white-fleshed variety is the dominant in Mpal seems to confirm that buyers from Mauritania prefer the variety. This assumption will be tested in later studies.

### *Eye color*

Black and red eye colors were observed with the black-eyed color representing more than 50% of the sample. In Bambey, Castors, and Tilene, 70–72% of the cowpea sold had black eyes (Fig. 7). According to the sellers, some consumers in Dakar prefer the black cowpea and particularly one local variety called Baye Ngagne because of its taste.

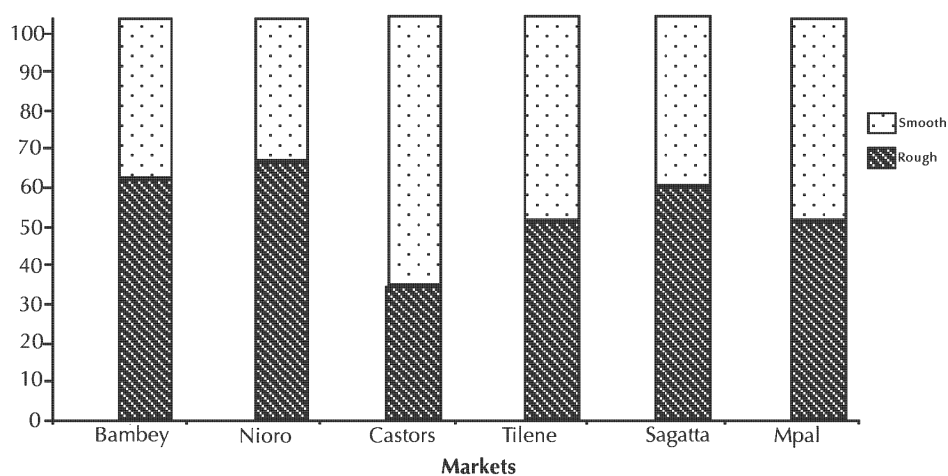
Table 3 shows that price varies between 46 and 780 FCFA, with a mean of 260 FCFA. The highest level of damage observed was 100 holes for a sample of 100 grains due to the high rate of use of metallic drums to store cowpea. Throughout the survey, this level was observed only once in Dakar. The factors weight (W) and grain size refers to the quantity of flour of a seed sample. They, respectively, have a mean of 18 g and 54 mm<sup>2</sup>.



**Figure 4.** Cowpea price variations in 1999.

**Table 2.** Types of sellers interviewed (%).

|                      | Castors | Tilene | Bambey | Nioro | Sagatta | Mpal |
|----------------------|---------|--------|--------|-------|---------|------|
| Female producer      | 0       | 0      | 48     | 0     | 13.5    | 38   |
| Female retailer      | 0       | 0      | 28     | 0     | 36.5    | 26   |
| Total female sellers | 0       | 0      | 76     | 0     | 50      | 64   |
| Male producer        | 0       | 0      | 24     | 3     | 36.5    | 13   |
| Male retailer        | 100     | 83     | 0      | 97    | 13.5    | 19   |
| Total male sellers   | 100     | 83     | 24     | 100   | 50      | 32   |
| Wholesaler           | 0       | 17     | 0      | 0     | 0       | 4    |



**Figure 5.** Skin texture.

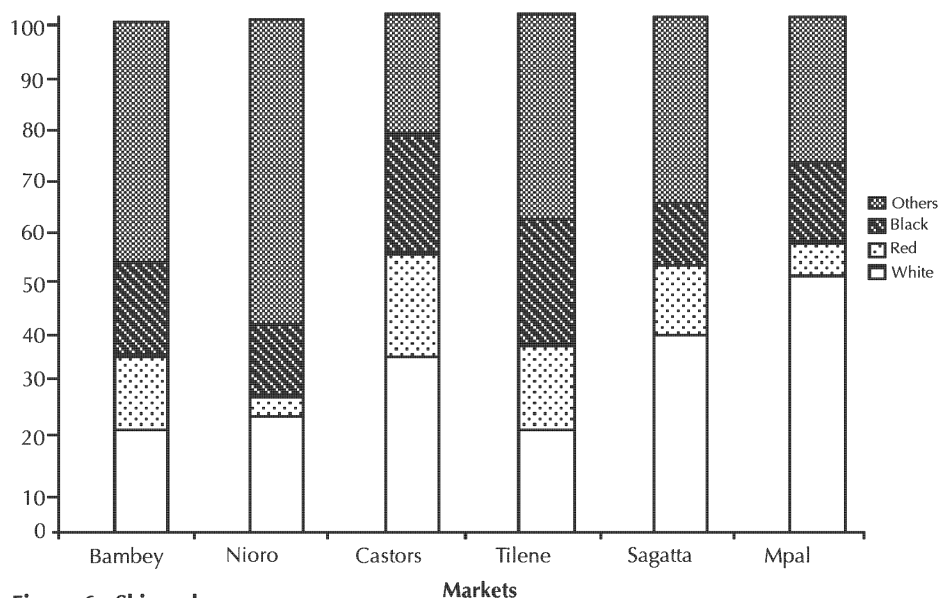


Figure 6. Skin color.

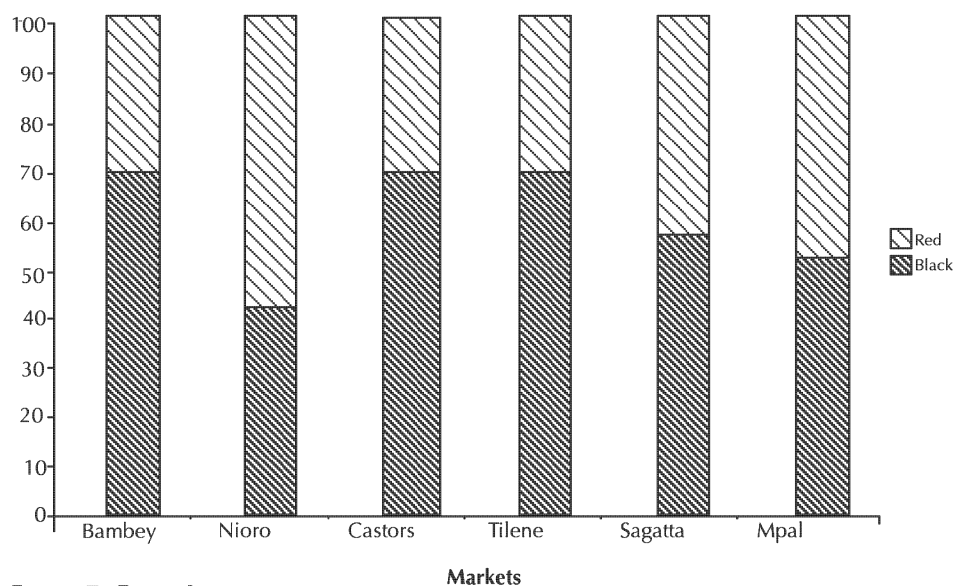


Figure 7. Eye color.

The regression model: This model measures the explanatory power associated with all the variables listed as grain characteristics. The hypothesis tested is whether or not the information conveyed by dummy variables and all the quantitative variables jointly can explain the observed price variation. The output of the regression model is in Table 4. These results suggest that the model explains the weighted variation in price. The F-test rejects the hypothesis that there is no relationship between price and

variables in the model. The model shows that the standard characteristics including two quantitative (NH, GSIZE) and five dummy variables representing the main grain characteristics (REDSKIN, BLSKIN, OTHERSKINC, SMOOTH, and REDEYE) are meaningful for buyers and therefore should be important for cowpea breeders. However, from the individual T-test, the relationship between number of holes (NH) and price is not significant.

The model also shows that the selling period has an impact on the market price. October (MO10), which corresponds to harvesting time, witnesses a price collapse. Selling between January and September would lead to an increase in price with respect to the reference period. The best periods to sell cowpea appears to be from June to September. This can be explained by the fact that June and July correspond to the planting time when the demand for seeds becomes very high while in August and September most of the reserves would have been consumed and farmers are yet to harvest. Selling in November (MO11) or December (MO12) would, respectively, decrease price received by about 25 and 40 FCFA. However from the individual t-test, November (MO11) does not have a significant relationship with price.

The impact of factor location (market) on price is significant and positive for all markets. For example, in Bambey, selling price increased by 20 FCFA compared to the price in Sagatta market. This is expected because when you move from the production area to the consumption area, price should increase. The positive effects of location on market price would likely reflect the difference in cost of transportation from the base market, Sagatta in the production area, to the other locations.

## Conclusion

This analysis has considered hedonic price models using the main cowpea physical characteristics and other variables that can influence the market price of cowpea such as location (market) and selling period (month). The results show that buyers are willing to pay a premium for grain size and white skin color but discount price for any other skin color and number of holes. It shows also that selling cowpea between January and September

**Table 3. Univariate statistics.**

| Variable | Mean     | Std Dev  | Minimum | Maximum  |
|----------|----------|----------|---------|----------|
| P        | 260.7978 | 141.7557 | 46.0000 | 780.0000 |
| W        | 18.6207  | 4.3331   | 10.0000 | 94.0000  |
| NH       | 6.3260   | 9.5609   | 0.0000  | 100.0000 |
| GSIZE    | 54.0288  | 9.7536   | 4.7000  | 93.8000  |

P = Price/kg in FCFA.

W = Average weight of 10 grains.

NH = Number of holes/100 grains.

GSIZE = Grain size.

would increase the price received by farmers but that November and December would be bad periods to market cowpea. Bambey had the greatest positive effect on price even though the impact of the variable market was not significant. These results are useful for breeders, policymakers, and farmers for addressing cowpea price variation issues. The implications for cowpea breeders would be to focus on a new breeding program incorporating white skin and large grain size as main characteristics since buyers are

**Table 4. Parameter estimates.**

| Variable                | DF | Estimate | Std Error | T Stat  | Prob > T |
|-------------------------|----|----------|-----------|---------|----------|
| INTERCEPT               | 1  | 224.7531 | 25.0001   | 8.9901  | 0.0001   |
| NH                      | 1  | -0.6222  | 0.3484    | -1.7856 | 0.0747   |
| GSize                   | 1  | 1.1910   | 0.3382    | 3.5213  | 0.0005   |
| MSeller                 | 1  | 74.8358  | 11.3778   | 6.5774  | 0.0001   |
| REDSKIN                 | 1  | -31.8342 | 9.1192    | -3.4909 | 0.0005   |
| BLSKIN                  | 1  | -4.7575  | 10.6163   | -0.4481 | 0.6542   |
| OTHERSKC                | 1  | -17.7121 | 9.7742    | -1.8121 | 0.0705   |
| SMOOTH                  | 1  | -21.2304 | 8.4291    | -2.5187 | 0.0120   |
| REDEYE                  | 1  | -40.5880 | 29.8127   | -8.0700 | 0.0001   |
| BLEYE                   | 1  | -3.2755  | 9.4980    | -0.3449 | 0.7303   |
| MO1                     | 1  | 51.7848  | 14.5992   | 3.5471  | 0.0004   |
| MO2                     | 1  | 55.0610  | 14.6356   | 3.7621  | 0.0002   |
| MO3                     | 1  | 68.1134  | 16.1236   | 4.2245  | 0.0001   |
| MO4                     | 1  | 52.0172  | 16.7257   | 3.1100  | 0.0020   |
| MO5                     | 1  | 91.3192  | 16.3468   | 5.5864  | 0.0001   |
| MO6                     | 1  | 118.4365 | 15.9788   | 7.4121  | 0.0001   |
| MO7                     | 1  | 119.8292 | 15.8663   | 7.5524  | 0.0001   |
| MO8                     | 1  | 115.3758 | 16.5178   | 6.9849  | 0.0001   |
| MO9                     | 1  | 127.7584 | 19.0388   | 6.7104  | 0.0001   |
| MO11                    | 1  | -22.3698 | 15.7593   | -1.4195 | 0.1563   |
| MO12                    | 1  | -38.7204 | 16.1256   | -2.4012 | 0.0166   |
| CASTOR                  | 1  | 10.1063  | 11.3919   | 0.8872  | 0.3753   |
| TILENE                  | 1  | 1.7355   | 11.1591   | 0.1555  | 0.8765   |
| BAMBEY                  | 1  | 20.0246  | 11.3710   | 1.7610  | 0.0787   |
| NIORO                   | 1  | 3.9886   | 12.2334   | 0.3260  | 0.7445   |
| MPAL                    | 1  | 3.2007   | 10.7045   | 0.2990  | 0.7650   |
| F-test = 56.947         |    |          |           |         |          |
| AdjR <sup>2</sup> = 69% |    |          |           |         |          |

willing to pay premiums for these characteristics. Also, the breeder would need to consider insect resistance in order to reduce the price discounts due to insect damage. In order to enable producers to sell their cowpea at the best period (May to July), a policy that will lead to metallic drums price subsidy should be put in place.

## References

- Berndt, E.R. 1991. The practice of econometrics. Classics and contemporary. Addison-Wesley  
Journal of Economics: 29: 239–249.
- Brorsen, W., R.G. Grant, and E.M. Rister. 1984. A hedonic price model for rough rice. Bid/acceptance markets. American Journal of Agricultural Economics 66: 156–163.
- Direction des statistiques agricoles, Rapport annuel République du Sénégal. 1998.
- Espinosa, J.A. and B.K. Goodwin. 1991. Hedonic prices estimation for Kansas wheat characteristics. Journal of Agricultural Economics 16: 72–85.
- Rosen, S. 1974. Hedonic prices and implicit markets: product differentiation in pure competition. Journal of Political Economics 82: 34–55.
- Tall, S.G. 1991. Evaluation socio-économique des essais mini-kits. ISRA/DRCSP/Projet CRSP Niébé 1991. 51p.



