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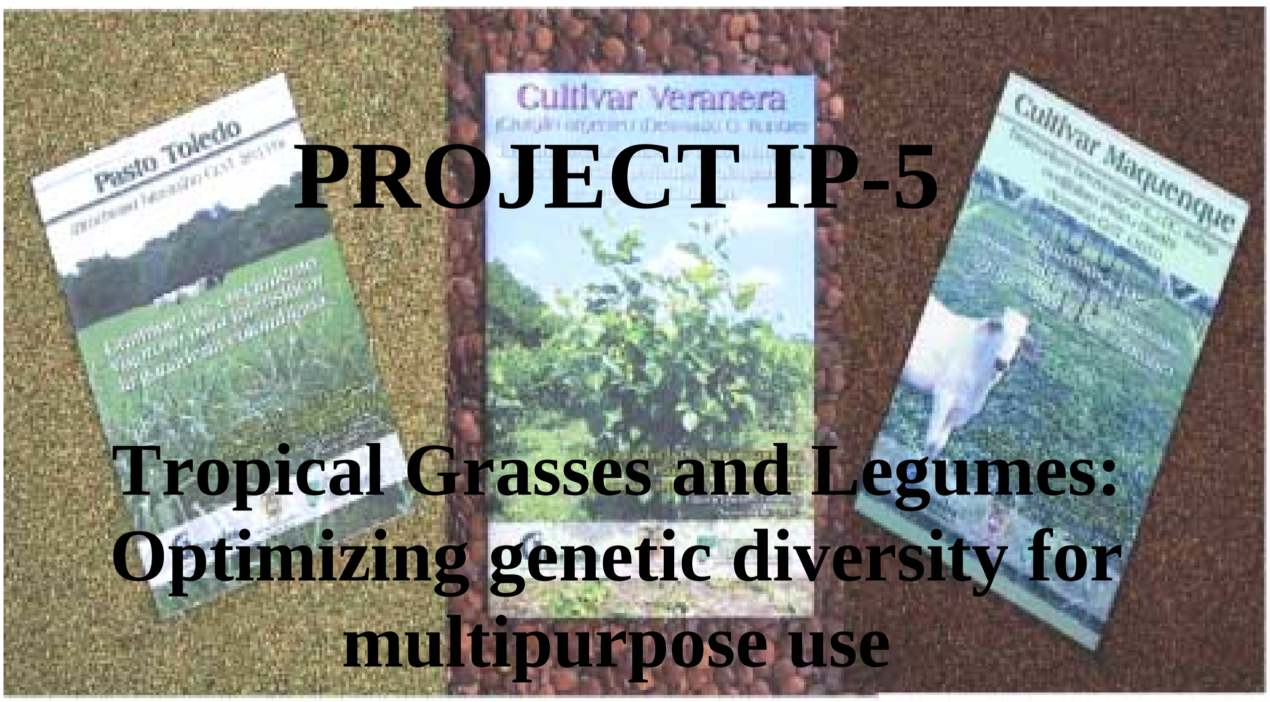

PROJECT IP-5

Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

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International Center for Tropical Agriculture

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Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use
(Project IP5)

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Cali, Colombia

TABLE OF CONTENTS

PROJECT OVERVIEW

IP5: Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

Project Description

Objective: To develop and utilize superior gene pools of grasses and legumes for sustainable agricultural systems in subhumid and humid tropics.

Outputs:
1. Optimized genetic diversity for quality attributes, for host-parasite-symbiont interactions, and for adaptation to edaphic and climatic constraints, for legumes and selected grasses.
2. Selected grasses and a range of herbaceous and shrubby legumes evaluated with partners, and made available to farmers for livestock production and for soil conservation and improvement.

Gains: Defined genetic diversity in selected grass and legume species for key quality attributes, disease and pest resistance, and environmental adaptation. Known utility in production systems of elite grass and legume germplasm. New grasses and legumes will contribute to increased milk supplies to children, cash flow for small dairy farmers, while conserving and enhancing the natural resource base.

Milestones:

2002 Defined patterns of variation in spittlebug bioecology in contrasting ecosystems.
Known animal production potential of *Brachiaria* hybrids with resistance to spittlebug. Forage availability and livestock productivity increased in selected provinces of 6 countries in S.E. Asia.

2003 Methods and tools available to enhance targeting and adoption of multipurpose forage germplasm in smallholder production systems in the hillsides of Central America. Role of Endophytes in Rhizoctonia resistance in *Brachiaria brizantha* defined.

2004 Multipurpose legumes validated for use in priority crop livestock systems. Prototype field management systems designed for enhancing endophytes' role in drought tolerance of *Brachiaria* species.

2005 Brachiaria hybrid with resistance to spittlebug available as a commercial cultivar to farmers in the tropics

Users: Governmental, nongovernmental, and farmer organizations throughout the subhumid and humid tropics who need additional grass and legume genetic resources with enhanced potential to intensify and sustain productivity of agricultural and livestock systems.

Collaborators: National, governmental, and nongovernmental agricultural research and/or development organizations; SROs (Univ. of Hohenheim, Cornell Univ., IGER, OFI, CSIRO, JIRCAS, ETHZ, Univ. of Gottingen).

CGIAR system linkages: Enhancement & Breeding (30%); Livestock Production Systems (15%); Protecting the Environment (5%); Saving Biodiversity (40%); Strengthening NARS (10%). Participates in the Systemwide Livestock Program (ILRI) through the Tropileche Consortium.

CIAT project linkages: Genetic resources conserved in the Genetic Resources Unit will be used to develop superior gene pools, using where necessary molecular techniques (SB-2). Selected grasses and legumes will be evaluated in different production systems of LAC, Asia and Africa using participatory methods (SN-3) to assess their impact in rural livelihoods and in natural resources conservation (PE-2, PE-3).

PROJECT WORK BREAKDOWN STRUCTURE
2002

Project Purpose

To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable agriculture systems in subhumid and humid tropics

OUTPUTS	**Grass and legume genotypes with high forage quality attributes are developed**	**Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed**	**Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed**	**In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research**
ACTIVITIES	• Selection of *Brachiaria* genotypes for high digestibility and other quality attributes • Assesment of quality and animal production potential of selected legumes • Assessment of quality and animal production potential of selected grass species • Assessment of the potential of tannins in legumes and saponins in tropical fruits to reduce methane in ruminants on grass diets • Adjustment of methods for simultaneous evaluation of tropical legumes for feed and soil improvement	• Bioecology of spittlebug species in contrasting environments • IPM components for spittlebug management • *Brachiaria* genotypes resistant to spittlebug and other biotic stress • Identify host mechanisms for spittlebug resistance in *Brachiaria* • Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* • Define interactions between host and pathogen in *Brachiaria* • Elucidate the role of endophytes in tropical forage grasses • Define interactions between host and pathogen in *Arachis* and *Stylosanthes*	• Genotypes of *Brachiaria*, other grasses, and *Arachis* with adaptation to edaphic factors • Identifiy genotypes of grasses and legumes with dry season tolerance • Shrub legumes with adaptation to drought and cool temperatures • Selection of legumes for multipurpose use in different agroecosystems and production systems • Genotypic variation in inhibition of nitrification and nitrous oxide emission in *Brachiaria*	• Develop of partnerships with NARS, NGO's, IARC's, ARIS and the private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use • Evaluation with farmer participation of multipurpose forages in crop and livestock systems • Forage seeds: reproductive biology, quality, multiplication, and delivery of experimental and basic seed • Expert systems for forages biodiversity by linking geographical information with biological data • Facilitate communication through Newsletters, Journals, Workshops and Internet

Revised Project Log-Frame
(2002)

CIAT
Area: **Genetic Resources Research**
Project: **IP-5 Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use** **Project Manager: Carlos E. Lascano**

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base	• New cultivars of grasses and legumes used by farmers. • Raised productivity of livestock and crops while protecting biodiversity and land in savannas, forest margins and hillsides	Statistics and case studies on socio-economic benefits and natural resource conservation in smallholder livestock farms in LAC and Southeast Asia	Policies are put in place by governments to favor sustainable livestock and forage development in marginal areas occupied by small farmers
Purpose To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable agriculture systems in subhumid and humid tropics.	• Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock and crop farmers in savannas, forest margins, and hillsides	• Range of genetic variation in desirable plant traits • Performance of forage components in systems	• Support from traditional and nontraditional donors • Effective collaboration: • CIAT's Projects • ARO's, partners and farmers, NGOs
Outputs 1. Grass and legume genotypes with high forage quality attributes are developed.	• Defined utility of *Cratylia* silage and cowpea hay as a feed resource for dairy cows by 2002. • New *Brachiaria* genotypes with superior forage quality for improved animal performance characterized by 2003.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (PE2), AROs, partners and farmer groups
2. Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed.	• QTL's for resistance to spittlebug (and high aluminum in the soil) in *Brachiaria* are available for marker-assisted selection by 2003. • Role of endophytes in Rhizoctonia resistance in *Brachiaria* clarified by 2003. • Benefits of endophytes (on drought tolerance) demonstrated under field conditions by 2004. • *Brachiaria* genetic recombinants with combined resistance to different species of spittlebug are available by 2004.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (SB1, SB2), AROs, partners and farmer groups
3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• Improved accessions of *Vigna* and *Lablab* with adaptation and known value to farmers in hillsides of Central America are available to partners by 2004. • *Brachiaria* genetic recombinants with resistance to high aluminum in the soil and with drought tolerance are available to partners by 2004.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (SB1, PE2, PE4), AROs, partners, NGOs and farmer groups
4. In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research.	• New grass (1) and legume (2) cultivars released by partners and farmers are available to farmers in Colombia by 2002. • Scaling process of *Cratylia argentea* and improved *Brachiaria* cultivars Central America is in place by 2003. • New market opportunities in Central America for processed forages assessed by 2003. • Effective forage multiplication systems are established in benchmark sites in SE Asia by 2003. • Widespread adoption of forage technologies in SE Asia by 2004. • An information network on forage research and development is established in SE Asia by 2003. • Improved multipurpose grasses and legumes result in increased on-farm milk, meat, and crop production, and reduced labour requirements in benchmark sites in SE Asia by 2004.	Surveys on adoption impact of new grasses and legumes: • Seed sold • Area planted • Production parameters • Environmental/socioeconomic indicators	• Effective collaboration with CIAT Projects (PE2, SN1, SN2, SN3, BP1 and Ecoregional Program), partners, NGOs and farmer groups

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Activities 2002	**Milestones (2002-2003)**		
1.1 Selection of *Brachiaria* genotypes for high digestibility and other quality attributes (CEL, JWM) 1.1.1 Screening of *Brachiaria* hybrids for digestibility 1.1.2 Screening of selected ***Brachiaria*** accessions and hybrids for saponins	• Efficient and reliable protocol for screening *Brachiaria* hybrids for digestibility with NIRS	• List of *Brachiaria* hybrids with known digestibility • Annual Report	• Effective collaboration with the plant breeder and Biotechnology staff
1.2 Assessment of quality and animal production potential of selected legumes (CEL) 1.2.1 Effect of neutralizing tannins in legumes on in vitro protein degradability 1.2.2 Milk production of cows fed legume-based supplements	• Known effects of tannins with different chemical structure on nitrogen degradation by rumen microbes • Known value of cowpea hay and *Cratylia* bag silage supplements for milking cows	• Annual Report	• Availability of milking cows from neighboring farm in CIAT Quilichao • Availability of fruits of *Sapindus saponaria*
1.3 Assessment of quality and animal production potential of selected grass species (CEL, JWM) 1.3.1 Milk yield with new accessions and hybrids of *Brachiaria*	• Known benefits in annual production of the *Brachiaria* hybrid 46-24, resistant to spittlebug (*A. varia*) as compared with commercial cultivars	• Annual Report	• Availability of milking cows from neighboring farm in CIAT-Quilichao
1.4 Assessment of the potential of tannins in legumes and saponins in tropical fruits to reduce methane in ruminants on grass diets (D. Hess, CEL) 1.4.1 In vitro evaluation of the effect of *Sapindus saponaria* on methane release and microbial population 1.4.2 In vitro evaluation of the effect of tannins in legumes on methane production 1.4.3 In vivo evaluation of *Sapindus saponaria* and legumes as supplements on rumen fermentation and N utilization by sheep fed a low quality grass diet	• Known utility of saponin-rich fruits to reduce methane and enhance N utilization by ruminants • Known effect of tannins in legumes on methane reduction and N turnover in vitro	• Annual Report • Thesis • Scientific publications	• Availability of funds from ETH
1.5 Adjustment of methods for simultaneous evaluation of tropical legumes for feed and soil improvement (K. Tscherning, EB, MP, RJT and CEL) 1.5.1 Assessment of the effect of drying plant material on aerobic and anaerobic decomposition of legumes of contrasting legume quality	• Better defined plant chemical components related to degradation of legumes in the rumen and soil	• Annual Report • Report to Donors • Papers for publication	• Effective collaboration between PE2 and IP5
2.1 Study the bioecology of spittlebug species in contrasting environments (DCP) 2.1.1 Biology of spittlebug species 2.1.2 Phenology of spittlebug populations in the field 2.1.3 Determinants of egg diapause 2.1.4 Taxonomy and distribution of spittlebug species	• Life tables for two species established • Correspondence between rainfall and population fluctuations analyzed • Role of drought and plant age on diapause determined • Taxonomic description of 3 new species completed	• Annual Report • Report to Donors • Published papers	• Collaboration maintained with UniAmazonia • Security conditions permit travel to field sites in Colombia

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
2.2 IPM components for spittlebug management (DCP) 2.2.1 Short-term mass rearing of different spittlebug species 2.2.2 Maintenance of the entomopathogen collection 2.2.3 Screening fungal entomopathogens for virulence to spittlebugs 2.2.4 Field evaluation of spittlebug entomopathogens in two contrasting regions	• Entomopathogen collection maintained and improved • Variation in virulence of select isolates to different spittlebug species and life stages quantified • Effect of time and number of entomopathogen applications on spittlebug abundance obtained from two field sites	• Annual Report • Report to Donors • Published papers	• Security conditions permit travel to field sites in Colombia
2.3 *Brachiaria* genotypes resistant to spittlebug and other biotic stresses (JWM, CC) 2.3.1 Development of new hybrid population for spittlebug screening (JWM) 2.3.2 Establishment of *Brachiaria* hybrids in field nurseries in Mexico (JWM) 2.3.3 Identification of *Brachiaria* genotypes resistant to spittlebug 2.3.4 Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (CC)	• New *Brachiaria* hybrids (10,000) established nurseries in México • List of sexual and apomictic *Brachiaria* hybrids resistant to one or more species to spittlebug under greenhouse conditions • List of *Brachiaria* hybrids with resistance to spittlebug under greenhouse and field conditions	• Annual Report • Published papers	• Effective flow of *Brachiaria* genetic recombinants to screen for spittlebug resistance • Effective collaboration from Corpoica in caqueta for spittlebug field screening • Funds are available from Papalotla seed company to carry out to screen for spittlebug

2.4 Identify host mechanisms for spittlebug resistance in *Brachiaria* (CC)
2.4.1 The effect of mixed infestations on resistance expression
2.4.2 Diagnostic survey of a 'new' pest affectin pará grass in Colombia
	• Mechanisms of resistance of *Brachiaria* to five spittlebug species
	• Defined genetic variation among and within spittlebug species as related to resistance levels in selected *Brachiaria* genotypes
		• Annual Reports
		• Published papers:
		  • Mechanisms of resistance to SPB complex
		  • Improved methodology for field screening of genotypes
		  • Breeding for SPB resístanse in *Brachiaria*
			• Funds from Papalotla seed company are available to carry out strategic research

2.5 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* (JWM, JT, CC) 2.5.1 Isolation and characterization of sequences associated with resistance to spittlebug in Brachiaria 2.5.2 Validation of PCR tag of "apo-locus" and initial application in Brachiaria breeding program	• Usefulness of spittlebug QTL's assessed • Reliability of N14 for routin phenotyping determined. • Screened of hybrid populations for identification of QTLs	• Annual Reports • Mapping population	• Effective collaboration between IP5 and the Biotechnology Group
2.6 Define interactions between host and pathogen in *Brachiaria* (SK, JWM) 2.6.1 Screening of *Brachiaria* hybrids for Rhizoctonia foliar blight 2.6.2 Studies on conditions for sclerotial production and germination of *Rhizoctonia solani* 2.6.3 Bacterial wilt of *Brachiaria*	• Reactions of *Brachiaria* hybrids to foliar blight determined • Anthracnose pathogen diversity in *Stylosanthes* analyzed • Bacterial blight of *Brachiaria*, its biology and sources of resistance better defined	• Annual Report • Published papers	• *Brachiaria* hybrids provided on time by the breeder to screen for Rhizoctonia resistance

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
2.7 Elucidate the role of endophytes in tropical forage grasses (SK, JWM, IMR) 2.7.1 Endophyte seed transmission studies in *Brachiaria* 2.7.2 PCR analysis and screening of *Brachiaria* genotypes for endophyte presence 2.7.3 Effect of *Acremonium implicatum* on fungal pathogens 2.7.4 Drought tolerance in endophyte-infected plants under field conditions **(IMR)** 2.7.5 Search for new endophytes in Brachiaria and characterization.	• Alkaloid profile of endophytes determined • Effect of endophytes on diseases better defined • New endophyte isolates identified • Endophyte-specific primer applied for screening *Brachiaria* routinely	• Annual Report • Published papers	• Collaborators in NZ will deliver results on alkaloids • Enough seed will be collected from endophyte-infected *Brachiaria* for seed transmission studies • Effective collaboration with breeder
2.8 Define interactions between host and pathogen in *Arachis* and *Stylosanthes* (SK) 2.8.1 Biodiversity studies on the anthracnose pathogen of *Stylosanthes* 2.8.2 Characterization of transgenic *Stylosanthes* plants with chitinase gene—T-3 generation	• Spectrum of antifungal properties (ie. is the activity broad spectrum) determined • Gene encoding antifungal protein cloned • Characteristics of cloned gene determined	• Annual Report	• Effective collaboration with patent lawyers
3.1 Genotypes of *Brachiaria*, other grasses and *Arachis* with adaptation to edaphic factors (IMR, JWM, MP, AS) 3.1.1 Edaphic adaptation of *Brachiaria* **(IMR)** 3.1.2 Edaphic adaptation of *Arachis pintoi* **(IMR, MP)**	• *Brachiaria* hybrids (apomictic and sexual) with Al resistance identified • *Brachiaria* hybrids with superior performance under field conditions with adaptation to low soil fertility identified	• Annual Report • Publications	• Seed availability of selected grasses and legumes • Willingness of NARS partners to collaborate • Funds are available from Papalotla seed company and restricted core for screening for Al • Continued support from forage agronomist in Costa Rica • Continued collaboration with University of Gottingen, Germany; JIRCAS, Japan; and ETHZ, Switzerland
3.2 Identify genotypes of grasses and legumes with dry season tolerance (IMR, JWM, PJA, AS) 3.2.1 Drought tolerance of Brachiaria (IMR, JWM) 3.2.2 Field studies to determine DM yields and quality of forage sorghum lines and pearl millet in a subhumid environment of Costa Rica (PJA) 3.2.3 Participatory evaluation of multipurpose legumes for enhanced tolerance to drought in Central America (feed, cover, green manure) (AS)	• *Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars identified • Progress toward improved screening method to evaluate drought tolerance in *Brachiaria* • New sorghum and pearl millet forage genotypes identified for subhumid environments of Central America	• Annual Report • Seed of selected accessions of sorghum	• Effective collaboration with ICRISAT
3.3 Shrub legumes with adaptation to drought and cool temperatures (M. Andersson, MP, JT, RSK, LHF) 3.3.1 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea* 3.3.2 Agronomic characterization of a collection of *Rynchosia schomburgkii*	• List of *C. argentea* and *F. macrophylla* accessions with superior agronomic performance and quality identified	• Annual Report • Seed of selected accessions of *C. argentea* and *F. macrophylla* multiplied	• Effective collaboration with U of Hohenheim, Germany, CIAT Projects (SB2, PE4) • Efficient collaboration with PE-2 • *Brachiaria* lines are available to plant in Atenas and Puriscal • Effective collaboration with MAG and ECAG in Costa Rica

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
3.4 Selection of legumes for multipurpose use in different agroecosystems and production systems (MP, PJA, AS) 3.4.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua and Honduras **(MP, LHF, AS, HC, BH, EA)** 3.4.2 Evaluation of a core collection of Cowpea (*Vigna unguiculata*) for multipurpose use in Atenas, C. Rica **(PJA)** 3.4.3 Evaluation of a core collection of *Lablab purpureus* for multipurpose uses (Quilichao and Palmira) **(MP, LHF, BH)** 3.4.4 G*E trial with Mucuna with focus on L-Dopa content **(MP, LHF, BH)** 3.4.5 Effect of cutting height a frequency on DM yield and quality of *Chamaecrista rotundifolia* and *Desmodium velutinum* in Atenas, C. Rica **(PJA)** 3.4.6 Participatory evaluation of improved forages into smallholder dairy systems in Matagalpa, Nicaragua **(AS, MP, LHF, PJA, LAH)**	• Selected accessionsof *Vigna unguiculata* tested in field sites in Colombia Nicaragua and Honduras, with farmer participation • Accessions of *Lablab purpureus* for acid and alkaline soils identified	• Annual Report	• Reception and phytosanitary release of new cowpea accessions • Negotiations with FONDEAGRO for a joint project in Nicaragua are successful
3.5 Genotypic variation in inhibition of nitrification and nitrous oxide emission in *Brachiaria* (IMR, M. Rondón, EB, CEL (CIAT); T. Ishikawa, G.V. Subbarao, K. Okada, O. Ito (JIRCAS) 3.5.1 The biologica phenomenon of nitrification inhibition in *Brachiaria humidicola* 3.5.2 Tropical forage grasses and their influence on inhibition of nitrification and emission of nitrous oxide from acid soil	• Quantified differences in inhibition of nitrification and emission of nitrous oxide among tropical grasses by 2003	• Annual Report	• Effective collaboration with JIRCAS
4.1 Develop of partnerships with NARS, NGO's, IARC's, ARIS and the private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use 4.1.1 On-farm evaluation of new grass and legume options for livestock systems in the Llanos of Colombia (**CEL**) 4.1.2 Evaluation of legumes as covers for plantations in the Llanos of Colombia (**CP, MP, LHF, BH, AS**) 4.1.3 Evaluation of green manures in the Llanos of Colombia (**CP, MP, LHF, BH**) 4.1.4 Utility of Cratylia in small dairy farms of the Llanos (**CP, CEL**) 4.1.5 Ex-ante economic evaluation of *Cratylia argentea* in dual purpose cattle production systems in the Llanos of Colombia **(FH)**	• Initiated validation of *Brachiaria* hybrid cv Mulato in the Llanos • Release of *Brachiaria brizantha, C. argentea* and *Desmodium ovalifolium* accessions for the Llanos • Effect of green manure species in rice based systems defined for the llanos	• Technical bulletin on the use of *D. ovalifolium* CIAT 13651 as cover crop and for use in pastures • Technical bulletin on the use of *B. brizantha* cv. Toledo in the Llanos • Technical bulletin on the use of *C. argentea* cv. Veranera in the llanos	• Effective collaboration on forage evaluation with partners is maintained • Effective collaboration with CIAT Projects, NARS, NGO's and farmer groups • Continued input from Forage Agronomist in Costa Rica • Effective collaboration with U of Hohenheim, Germany • Continued collaboration with oil palm and rubber growers in the Llanos of Colombia • Effective collaboration with FIDAR (NGO)

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
4.1.6 Release of multipurpose forage for the Llanos **(CEL, CP)** 4.1.7 Use of forages for the recuperation and conservation of degraded areas in hillsides of Colombia (**FIDAR, MP, LHF**) 4.1.8 Analysis of intensification of milk production systems in Colombia **(FH)** 4.1.9 Validation/promotion of *Brachiaria* hybrid cv. Mulato and other forage lines by NARS and farmers (**PJA**) 4.1.10 Participatory evaluation of forages for multipurpose use in Haiti (**PJA**) 4.1.11 Enhancing beef productivity, quality, safety, and trade in Central America (**FH**) 4.1.12 Collaboration with ETHZ-Zurich **(IMR, CEL)** 4.1.13 Collaboration with U. Hohenheim-Germany **(MP)**			• • No further deterioration of security situation in Colombia • Funds for SoFT proposal approved by ACIAR • Participation of INTA in proposal development
4.2 Evaluation with farmer participation of multipurpose forages in crop and livestock systems 4.2.1 Development of participatory methods to enhance adoption of forages as feed resources and for INRM **(Rein van der Hoek, V. Hoffmann, MP)** 4.2.2 Evaluation of forages for multipurpose use with farmer participation in Hillsides of Central America **(MP, AS, LAH, LHF, PA)** 4.2.3 On-farm forage seed production in Nicaragua and Honduras **(MP, AS, Sertedeso)** 4.2.4 On-farm evaluation of green manures in hillsides of Nicaragua **(AS, MP)** 4.2.5 Evaluation of *C. argentea* as live barrier to control erosion and as a source of feed in Costa Rica **(WS, PJA, MP)** 4.2.6 Studies on indigenous fodder trees and shrubs in N. Vietnam **(RR)** 4.2.7 Socio-economic evaluation of forage technologies in Philippines and Vietnam **(RR)** 4.2.8 Participatory evaluation of Brachiaria Mulato (**RR**)	• Model for participatory selection of forages refined and training tool written • New forage options adapted by group of farmers in Honduras and Nicaragua • Initiation of small forage seed enterprises in Nicaragua and Honduras • Brachiaria Mulato evaluated by 10 farmers in focus sites in Thailand, Philippines, Vietnam and China.	• Annual Reports • Published papers • Technical bulletin on seed production of *B. brizantha* cv. Toledo and *C. argentea* cv. Veraniega	• Effective collaboration with NARS and farmer groups
4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed (JWM, PJA) 4.3.1 Multiplication and delivery of selected grasses and legumes • Seed Unit of Palmira **(JWM)** • Seed Unit of Atenas **(PJA)**	• Seed of promising forages produced, harvested and delivered to partners	• Annual Reports • Seed inventories	• Fund are available to support the Seed Unit • Climatic condition allow seed production and harvest

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
4.4 Expert systems for forages biodiversity by linking geographical information with biological data 4.4.1 Development of a database and retrieval system for the selection of tropical forages for farming systems in the tropics and subtropics, SoFT **(AF, MP)** 4.4.2 Development of a forage database **(AF, LHF, GR, BH, MP, CEL)** 4.4.3 Incorporating Socio-Economic Data and Expert knowledge in representation of complex Spatial Decision-Making **(R O'Brien, AF, LHF, BH, MP, TO, SC, R Corner)**	• Forage database released and available through Internet and CD • Analysis of intensification of milk production in Colombia completed	• Annual Report	• Funds from ACIAR to develop SoFT are approved
4.5 Facilitate communication through newsletters, journals, workshops and internet (IP5 Staff) 4.5.1 Development of a Forage Web Site **(S. Staiger)** 4.5.2 Updating FAO's Grassland index **(MP, RSK)** 4.5.3 Twenty years of Pasturas Tropicales **(AR)** 4.5.4 CIAT field day on spittlebug biology and management **(DCP)** 4.5.5 Prepare technical bulletin on spittlebug bioecology and monitoring **(DCP)** 4.5.6 Bibliographic database on spittlebugs available on webpage **(DCP)** 4.5.7 Symposium on spittlebug to communicate research results to PRONATTA **(DCP)**	• Publication of a special edition of Pasturas Tropicales on Bioecology of spittlebug in Colombia • Spittlebug species profiles completed and published on the web • Field day conducted; informative bulletin prepared for publication; bibliography online; PRONATTA symposium conducted	• Annual Reports • Forage Web Page • Pasturas Tropicales	• Funds are available to continue publishing Pasturas Tropicales • Support from FAO for updating forage database

Research Highlights

- **Saponin-rich fruits reduce methane and enhance nitrogen utilization by ruminants**

In last year's Annual Report we indicated that fruits of *Sapindus saponaria* have the potential to suppress methane release and reduce ciliate protozoa populations *in vitro*. However, it was not known whether these effects were dependent on quality of the basal diet or if they could be replicated in vivo. Results from this year show that the interactions of forage quality and addition of saponin-rich supplements (i.e. fruits of *S. saponaria*) were mostly non-significant, except for ciliate protozoa counts. Ciliate protozoa were only suppressed by the addition of *S. saponaria* with improved legume- based diets. The fact that the methane suppressing effect of *S. saponaria* was independent of the quality of the basal diet indicates that fruits of *S. saponaria* have the potential to suppress methane release when feeding low quality grass diets as well as when feeding higher quality grass-legume diets. In a respiratory chamber experiment carried out with sheep, the methane suppressing potential of *S. saponaria* was confirmed *in vivo*, thus validating our results obtained with the Rusitec-apparatus. Additionally, we showed that the supplementation with fruits of *S. saponaria* increased the amount of bacterial nitrogen escaping the rumen and shifted the volatile fatty acid production in the rumen towards a lower acetate: propionate ratio, indicating an improvement of the efficiency of rumen fermentation. These effects of *S. saponaria* were independent and additional to the positive effects observed when the low quality grass was supplement with a legume.

- **Significant progress continues to be made in the Brachiaria Improvement Program**

Biotic Constraints: For years it was assumed that resistance to one spittlebug species applied generally to other spittlebug species. We showed in previous reports that this is not the case and that resistance may be species-specific. Based on results obtained in previous years we developed a successful strategy for the simultaneous but independent screening of large sets of genotypes (~1,000) for resistance to three key Colombian spittlebug species (*Aeneolamia varia*, *Aeneolamia reducta*, and *Zulia carbonaria*), which we implemented this year. Three plants of each clone are propagated and these are infested simultaneously, one with each of the three spittlebug species. Hence, independent assessment of resistance to each of the spittlebug species is obtained for each clone. Resistance is assessed on the basis of damage scores and nymphal survival determined for clones with low mean damage scores. Approximately 90% of the genotypes are culled based on this preliminary unreplicated screening; 10% are re-tested with replication to confirm resistance. Again each clone is independently and simultaneously evaluated for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. For the 2001 sexual population, 32 hybrids showed damage scores that did not differ from those recorded on the standard resistant check (cv. Marandu). Several of the selected hybrids showed levels of antibiosis (reduced nymph survival) to *A. varia*, *A. reducta*, and *Z. carbonaria* that were significantly better than cv. Marandu. This is the first time that *Brachiaria* genotypes combining a high level of antibiosis resistance to three different spittlebug species are reported.

Abitiotic Constraints: This year, as part of the restricted core project funded by BMZ/gtz, Germany, we continued our efforts to separate the physiological and genetic components of aluminum (Al) resistance in *Brachiaria*. Previous research had indicated that *B. decumbens* might exclude phytotoxic Al ions from root apices using a mechanism that does not rely on external detoxification by organic acids. We found evidence for the first time that the surface charge density of root apices of aluminum resistant *B. decumbens* acts as a generic mechanism to avoid uptake of phytotoxic aluminum and other cationic toxicants. Our efforts on phenotypic characterization of *B. ruziziensis* x *B. decumbens* population resulted in identification of few hybrids superior to *B. decumbens* in production of fine roots in the presence of toxic level of aluminum in solution. This work also indicated that growing rooted stem cuttings in the Al treatment and selecting genotypes with long and thin roots could be an effective screen to discard non-adapted segregants in the *Brachiaria* breeding program. Using the screening procedure to evaluate Al resistance, two sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) were identified with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. These sexuals are being used in the breeding program to generate superior hybrids that combine several desirable attributes. Field evaluation of most promising *Brachiaria* hybrids and accessions in wet and dry seasons over 3 years in the Llanos of Colombia indicated that the *Brachiaria* hybrid, FM9503-S046-024 is well adapted to acid soil conditions and drought and its superior performance was related to its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.

- **Endophytes present in Brachiaria are transmitted through seed**

Endophytic fungi have one of the most complex associations with their host resulting in physiological responses in terms of growth, nutrient acquisition, drought resistance, and production of secondary metabolites, like alkaloids. The association of fungal endophytes with grasses is well documented for temperate species but not for tropical species. Work carried out in CIAT showed for the first time that endophytes are present in tropical grasses and that fungal and bacterial pathogens are inhibited *in vitro* by isolates of endophytes from *Brachiaria*. In addition, we found endophytes in a *Brachiar*ia accession (CIAT 16320) that is resistant to *Rhizoctonia solani*. In a greenhouse study we found that plants infected with *A. implicatum* had greater leaf expansion than non-infected plants grown under severe drought stress. Fieldwork is in progress to verify this finding. This year we developed and applied PCR-based method, for detection of endophytes in *Brachiaria* seed. Seed samples collected from plants were confirmed to be endophyte-infected or endophyte-free based on the PCR test with leaf DNA and fungal endophyte isolation on culture media. A specific primer pair developed and used in the PCR assay allows precise and rapid detection of endophytes in *Brachiaria* plants and permits differentiation between endophytic and non-endophytic fungi. All DNA from seeds of endophyte-infected plants had amplified products, which was not the case in seed from non-infected plants. This method will be extremely useful for testing *Brachiaria* seed lots for the presence or absence of endophytes.

- **New forage options are rapidly being tested and adopted by small farmers in hillsides of Honduras and Nicaragua**

Improved forages can play an important role for ensuring socio-economic and environmental sustainability of smallholders production systems in the fragile hillsides of Central America. In a project funded by BMZ/gtz, Germany we are currently applying participatory methods to promote the use of improved forages as feed resources and for conservation of natural resources in references sites in Honduras and Nicaragua. This year we developed a methodology to analyze and rank plant criteria that are important to farmers for selecting grasses and legumes for multipurpose use in the dry and wet seasons. For example, for farmers in Honduras, major selection criteria are that the forage species should establish fast and compete with weeds. Based on farmer criteria we clustered groups of grasses and legumes for seed multiplication and for on –farm participatory testing. Among the grasses the preferred cultivars have been *B. brizantha* cv Toledo (CIAT 26110) and *Brachiaria* hybrid cv Mulato given that they establish fast and have drought resistance. Legumes selected by farmers include the herbaceous *Centrosema pubescens* (CIAT 15160-- good cover and high biomass production) and the shrub *Cratylia argentea* (CIAT 18668-- good drought tolerance). In the reference site in Honduras, a total 170 farmers (30 % of the total farmers in the reference site) have sown new forage options (*B. brizantha* cv Toledo and *C. argentea*). In the reference site in Nicaragua there is also evidence of fast adoption of grasses (*B. brizantha* cv Toledo). In addition, a total of 13 ha of *Brachiaria* hybrid cv Mulato were established in smallholders farms in different regions of Nicaragua as part of a diffusion strategy funded by the Papalotla Seed Company. Finally, the growing interest of farmers for *B. brizantha* cv Toledo and *C. argentea* has resulted in farmers getting involved in seed multiplication. The Project is providing technical assistance on the management of seed plots, on harvest and on post-harvest treatment of the seed.

- **Forages are contributing to improved rural livelihoods of small farmers in the Philippines (Mindanao) and in Vietnam (Tuyen Quang Province)**

Philippines: Improved forage species being promoted by the FSP (Forages for Smallholder Project) increased animal production, improved soil conservation and saved farmers time. The reduced labor time enabled households with low labor and land availability to acquire animals, and allowed others to increase their herd size. Planting forages in contour lines increased crop production slightly and allowed the establishment of sustainable production systems through the returns from livestock. These changes resulted in a significantly higher household income from livestock for the adopters of new forages. In addition, the introduction of new forages reduced the time dedicated by women and children to tasks like herding and cutting forages. Time saved, was generally invested in extending crop activities. The participatory approach of FSP changed the attitude of fieldworkers and increased the number of farmers interested in training, workshops and cross project visits. This extended the impact of FSP to farmers' knowledge of soil conservation, crop rotation and intercropping. Farmers also began to use participatory tools to facilitate decision making in their other activities. Finally, the adoption of new forages by smallholders in Philippines was highly dependent upon the livestock dispersal and credit programs. These systems resulted in a large number of opportunistic participants in FSP activities. Planting improved forages for short cycle species, like chickens and goats should be encouraged as it reduced the time needed to attain positive returns from labor investment.

Vietnam: The overall effect of the adoption of new forages promoted by the FSP on welfare of farm households has been very significant. The mean income from mixed ruminant–fish production systems increased from $US6.50 to $US17.00/month/household member within 2 to 4 years of the adoption of new forages. Time saved has also allowed households to increase their income from other, mainly agricultural, activities. Due to the increased income from investments in tree crops, the overall income improved but the contribution of livestock to total income decreased. The adoption of new forage species has also had extremely positive social and gender effects. New forages reduced the time spent by children and women in herding, and cutting and carrying forages for animals. This allowed children more time to study and also had educational and cultural advantages for women. Poorer farmers, who depended more on livestock due to their smaller land area, benefited most from forages. Improved forages allowed them to keep large ruminants that increased their income and also provided a stepping-stone to general intensification of their production systems. The extension of new forages introduced by FSP in Tuyen Quang province did not depend on livestock distribution schemes or credit programs. The many advantages associated with the adoption of improved forages, along with favorable socio-economic conditions, provide support for the development of an autonomous process of adoption of improved grasses and legumes.

- **New grass and legume alternatives were released for the Llanos of Colombia**

CIAT and CORPOICA, with financial support from the Ministry of Agriculture of Colombia, have been evaluating for the last 5 years a number of grass and legume options for the well drained savannas (acid soils with very low fertility) and for the piedmont (acid soils with moderate level of soil fertility) region. Several forage technologies were evaluated: a) improved grasses in terms of high carrying capacity and drought tolerance, b) herbaceous legumes with multiple use such as to recuperate degraded pastures, cover crops in plantations and green manures in crop-livestock systems and c) shrub legumes for cut and carry systems in dairy farms. The strategy followed has been to evaluate the different forage technologies on –station and on- farm. One very important outcome of the collaborative work of CIAT and CORPOICA in the Llanos was the release this year of one grass (*Brachiaria brizantha* cv Toledo for fattening systems in the piedmont), one herbaceous legume (*Desmodium heterocarpon* subsp *ovalifolium* cv Maquenque) for recuperation of degraded pastures in the well drained savannas and as cover crop in plantations), and one shrub legume (*Cratylia argentea* cv Veranera for cut and carry in more intensive dairy systems in the piedmont). Technical Bulletins were produced and distributed among researchers, extension workers and interested farmers. In addition, basic seed was delivered to CORPOICA to establish commercial seed plots.

Output 1: Grass and legume genotypes with high forage attributes are developed

Activity 1.1 Selection of Brachiaria genotypes for high digestibility and other quality attributes

Highlights

- Variability in IVDMD hybrids of Brachiaria seems to be less than anticipated, but with NIRS even small differences in IVDMD among genotypes can be detected.

- Confirmed that a high correlation exists between successive samplings in saponin- like activity in Brachiaria and there is variability among hybrids produced in the breeding program

1.1.1 Screening of *Brachiaria* hybrids for digestibility

Contributors: P. Avila, J. Miles and C. Lascano

Rationale

In the on going *Brachiaria* improvement the main objective has been to breed for spittlebug resistance and for adaptation to acid-low fertility soils. In terms of quality attributes, such as IVDMD and crude protein, our approach has been to maintain the quality of *Brachiaria* bred lines at least as equal to that of *B. decumbens* cv Basilisk, which is the most widely planted cultivar in tropical America.

A justification for this strategy had been that with the current in vitro system in the Forage Quality Laboratory it is not possible to handle the large number of genotypes (over 3,000) generated annually by the breeding program. However, with the Near-Infrared Spectroscopy (NIRS) it is now possible to analyze large number of samples in the Forage Quality Laboratory provided good calibration curves are available.

In 1999 we developed a narrow – based NIRS equation and when applied found that the resulting parameters had high precision as indicated by low SE of the calibration (0.98). In addition, estimates of IVDMD of few samples using NIRS had a high correlation ($r = 0.89$) with IVDMD values obtained with the two-stage Tilley and Terry in vitro procedure. Later in 2000 we tested the NIRS calibration curve with leaves of 176 *Brachiaria* hybrids that form part of a population (tetraploid *B. ruziziensis* x *B. brizantha* cv. Marandu) used to develop molecular markers for digestibility. Results showed a high correlation ($r = 0.84$) between observed and values of IVDMD estimated using NIRS.

Las year we determined the effect of age of plant material on the precision of the NIRS equation to estimate IVDMD in *Brachiaria.* The in vitro digestibility values ranged from 68 to 80 % and from 71 to 83 % in samples of 51 and 71 days of regrowth, respectively, which indicate little effect of maturity on IVDMD. High correlations ($r = 0.73$ to 0.80) were observed between IVDMD values from the laboratory and values predicted with NIRS in the two sets of samples. These results further confirmed that the NIRS equation we had developed to screen Brachiaria hybrids for IVDMD is adequate given the high correlation with IVDMD values measured in the laboratory and relatively low SE of predicted values.

We had also reported a very low correlation between IVDMD values obtained in samplings of the same Brachiaria population in different times. Thus we carried out three successive samplings of the same mapping population used before. Results showed higher correlations between samplings than reported previously, but still low ($r = 0.4$ to 0.5; $P<0.05$). This suggested the need to sample replicated plants from the Brachiaria population used for developing molecular markers for quality and other traits.

Materials and Methods

This year we sampled (leaves) from 50 *Brachiaria* hybrids planted in three reps in the greenhouse. All samples were oven dried (50°C), ground in a 1 mm screen and analyzed for in vitro digestibility using NIRS and the two stage in vitro method. The results on IVDMD were subject to analysis of variance with rep and genotype as sources of variation. We also carried out correlation analysis between IVDMD values obtained with two calibration equations used in NIRS and the IVDMD values obtained in the laboratory.

Results and Discussion

Results indicated that as expected the IVDMD measured in the laboratory had a high correlation (r = 0.79; P< 0. 001) with IVDMD estimated with NIRS (Figure 1). In addition, our results showed a high correlation (r = 0.98; P < 0.0001) between IVDMD values calculated with the two NIRS equations developed in CIAT's Forage Quality Laboratory (Figure 1).

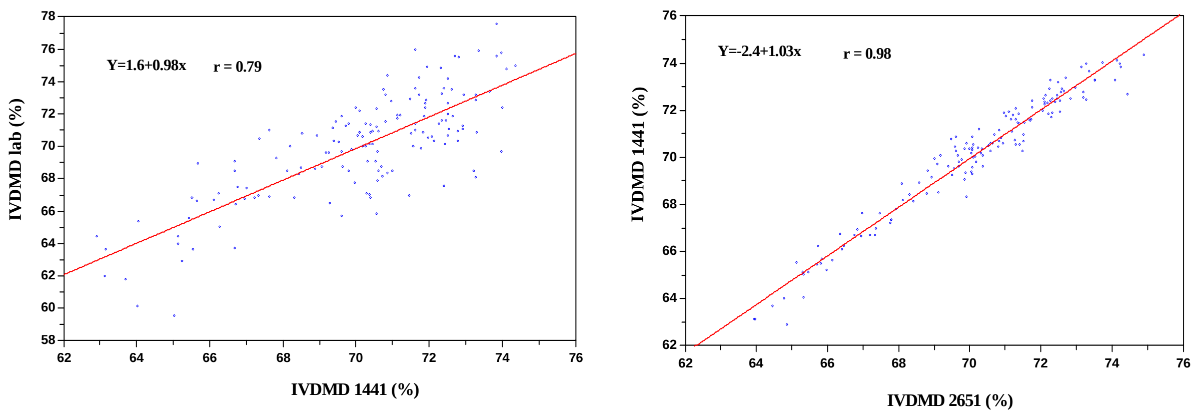

Figure 1. Relationship between IVDMD values obtained in the laboratory and IVDMD values obtained with NIRS (left) and between IVDMD values obtained with NIRS using two calibration equations (right).

The ANOVA performed in the IVDMD data estimated with NIRS showed that reps were not significant (P>0.39), but that differences due to genotype were significant (P <0.05). Values of IVDMD of the 50 Brachiaria genotypes evaluated ranged from 65.6 to 73.3 %, which is considerable less than was expected from previous results. It was also interesting to observe that the differences in IVDMD between two parents (*B. brizantha* cv Marandu -70.4% and *B. ruziziensis* - 69.0%) used in the breeding program were not significant. These results indicate that differences in IVDMD among hybrids of Brachiaria may be less than anticipated, but that with NIRS even small differences in IVDMD among Brachiaria genotypes could be detected.

Selection for improved forage quality is justified if genetic variance for quality traits is greater than the variance resulting from the interaction of genotype with environment (G x E). Previous work at CIAT with accessions of *B. brizantha* and *B. decumbens* had shown that the variance in IVDMD caused by genotype was four times greater than the variance from G x E.

We conclude that there is justification to screen *Brachiaria* hybrids for IVDMD using NIRS even though the variability digestibility seems to be less than expected. One of the advantage of Brachiaria hybrid cv Mulato is its high yield and crude protein (CP) content relative to commercial cultivars. Thus we also believe that hybrids should be screened for CP. Screening for IVDMD and for CP in the *Brachiaria* improvement program will be implemented next year with populations (approximately 1000) that go to the spittlebug screen. With this strategy it will be possible to establish correlations between quality traits and resistance to spittlebug.

1.1.2 Screening of selected *Brachiaria* accessions and hybrids for saponins

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

Based on the hypothesis that saponins are responsible for photosensitization in *Brachiaria decumbens* last year we determined the presence or absence of these compounds in accessions and hybrids of *Brachiaria*. Results (See AR 2000 and 2001) indicated differences in saponin activity among the few *Brachiaria* accessions included in the assay. Saponin activity was very high in the commercial cultivar *B. decumbens* CIAT 606, which in fact was as high as that recorded in the positive control. In contrast saponin activity was low in *B. humidicola* and in *B. brizantha* cv Marandu, which is the source of spittlebug resistance in the Brachiaria breeding program. Results from last year also showed that saponin activity in new *Brachiaria* hybrids was more variable than what was found in 2000. Out of 10 new hybrids included in the test, 7 had very low saponin activity and similar to what was recorded in one of the parents (*B. brizantha* CIAT 6789) used in the cross.

This year we were again interested in determining saponin- like activity in *Brachiaria* hybrids chosen at random from a *B. brizantha* cv Marandu (apomictic) x *B. ruziziensis* (sexual) population used for developing molecular markers for spittlebug, apomixes and digestibility.

Materials and Methods

A large (>230 sibs), bi-parental (tetraploidized, sexual *B. ruziziensis*-x-*B. brizantha* CIAT 6294 [cv. Marandu]) F_1 hybrid population (full-sib family) was produced and propagated for determinations of saponin content. Unintentional maternal selfs were identified by isozyme analysis, and eliminated.

The laboratory procedure used to estimate saponin activity in the test forage samples is based on the hemolisis of red blood cells obtained from rabbits, with a solution extracted (80% aqueous methanol) from fat free samples (0.1 g) of test forages. A dilution of 1:20 was used to determine absorbance in a spectrophotometer in the Forage Quality Laboratory. Leaves of wheat and of the tree *Entorolubium ciclocarpum* (Orejero) were used as negative (low levels of saponins) and positive (high levels of saponins) controls in the assay, respectively.

Results and Discussion

A high and significant correlation ($r = 0.96$; $P<0.001$) was found between Absorbance value recorded in 2000 and in 2001 and between Absorbance values recorded in 2001 and 2002 ($r = 0.98$; $P<0.0001$) (Figure 2). However, results shown in Table 1 indicate that the absorbance values recorded in the same accessions and hybrids of *Brachiaria* harvested in 2001 and 2002 were similar in magnitude in some groups but not in others.

It would seem from the results from 3 consecutive years, that two of the parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6780) used in the *Brachiaria* breeding program have very contrasting levels of saponins and this is reflected in the variability of saponin activity measured in the hybrids included in the test. The high concentration of saponins in *B. decumbens* is consistent with the observations of photosensitization in cattle and sheep fed with this grass.

Table 1. Saponin like activity in *Brachiaria* accessions and hybrids recorded in samples harvested in two consecutive years.

Classification	Forage Sample	Absorbance (2001)	Absorbance (2002)	Significance
Very High	*E. ciclocarpum* (Control)	762	548	*
	B. decumbens CIAT 606	695	485	**
	Hybrids:			
	1084-3	631	548	*
	1084-10	808	658	**
High	*B. ruziziensis* CIAT 26164	630	528	**
	Hybrids:			
	1092-5	624	537	NS
	1092-15	520	469	*
	1094-15	602	501	*
Intermediate	Hybrids			
	1092-14	451	404	**
	1092-2	491	395	**
	1092-3	589	405	**
	1092-11	462	342	*
	1094-19	478	371	**
Low	Hybrids			
	1092-1	251	200	*
	1092-4	195	183	NS
	1092-13	196	170	*
	1083-7	149	142	NS
Very Low	Paja de trigo (Control)	28	32	NS
	B. humidicola 16871	49	32	**
	B. brizantha 6780	56	40	**
	Hybrids			
	1078-31	28	35	NS
	1079-18	30	35	NS
	1081-25	31	29	NS
	1093- 31	31	86	*
	1095- 4	29	38	*
	1097- 6	33	39	NS

** $P < 0.01$ * $P < 0.05$

We conclude from these results that there is more than justified to screen *Brachiaria* hybrids for saponins. However, as indicated last year we need to adapt a laboratory screening procedure that is fast and that allows us to quantify the concentration of saponins present in large populations of Brachiaria. We have not yet been able to standardize a laboratory procedure or to establish collaboration with an advanced laboratory investigate the chemical nature of saponins. Thus next year we will calibrate NIRS to measure saponin-like activity in Brachiaria

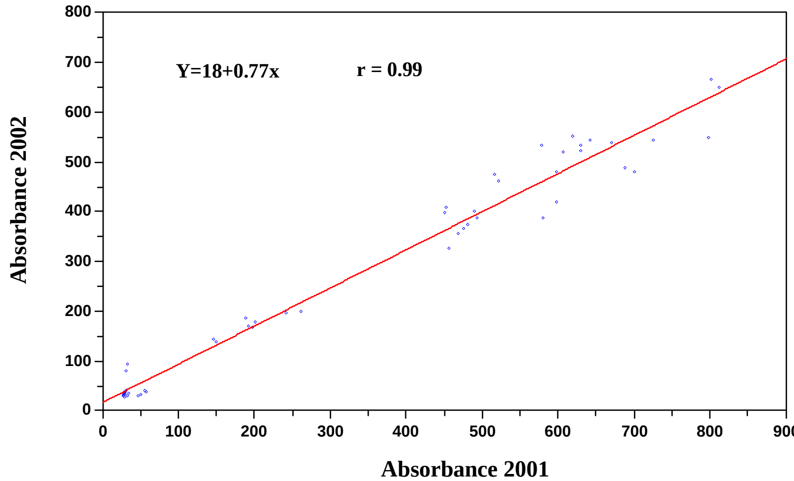

Figure 2. Relationship between Absorbance values in two consecutive years related to saponin like activity in different Brachiaria genotypes.

Activity 1.2 Assessment of quality and animal production potential of selected legumes

Highlights

- Determined that the proteolytic enzyme (Ficin) is useful to study relative effects of condensed tannins on degradation of protein in tropical legumes

- Documented that milk yield of cows grazing pastures in the dry season can be increased from10 to 20% by feeding Cratylia silage and Cowpea hay packed in bags

1.2.1 Effect of neutralizing tannins in legumes on in vitro protein degradability

Contributors: N. Narvaez, C. Lascano (CIAT)

Rationale

It is well documented through in vivo trials that condensed tannins influence the degradability of the protein fraction of tropical legumes in the rumen and that as result there is less ammonia production and a greater proportion of the feed protein by passing the reticulo- rumen. However, in vivo experiments are expensive and time consuming, which limits their use to screen legume species in terms of protein degradability. Researchers have used the well-known Tilley and Terry two stage in vitro method to estimate nitrogen digestibility of legumes. Results have shown that provided that corrections factors are applied for the presence of microbial N the method is sensitive to the presence of tannins in legumes.

However, one drawback of the two stage in vitro method is the need to have a fistulated animal as a source of rumen liquor, which is not always possible in some forage quality laboratories. Thus we were interested in adapting a protocol published in the US (Poos-Floyd et al. 1985, J of Dairy Science. 68: 829-839) that could allow us to use a proteolytic enzyme to screen legumes on the basis of protein degradability.

Material and Methods

Leaves of shrub legumes species with tannins (*L. leucocephala, C. fairchildiana* and *Bauhinia sp*) and without tannins (*G. sepium, and E. fusca,*) were harvested in the Cauca Valley, Colombia. Forage samples were treated with PEG (6 mg of PEG/100 mg of DM) and with water (control). The forage samples (600 mg of DM) were weighed into tubes with a buffer solution (15 ml), which were then placed in an incubation bath (39^0C) for two hours to get the samples soaked. This pre-soaking treatment in the buffer solution was followed by the addition of 10 ml of ficin (proteolytic enzyme extracted from the latex of Ficus glabrata- Sigma Chemical No F- 3266 of 0.5 Units/mg) obtained by dissolving 4.3 g of the enzyme in one liter of buffer solution. After one hour of incubation in the water bath, the tubes were removed and the contents filtered in a Whatman No 41 paper filter. The supernatant and the solid residue in each tube were then analyzed for protein (N x 6.25).

Results and Discussion

The degradability of protein in leaves of species without tannins was higher than the degradability of species with tannins, regardless of the addition of PEG (Table 2). There were also differences in protein degradability between species with no tannins, being higher in *G. sepium* than in *E. fusca,* regardless of the addition of PEG. In legumes with tannins the addition of PEG to the media resulted in large increments (from 2 to 6 fold) in protein degraded by the proteolytic enzyme used in the assay.

We also performed a correlation analysis between protein degraded by the enzyme and tannin content in legumes and results showed as expected a high negative relationship ($r = -0.83$; $P < 0.001$).

In this study we also evaluated the effect of leaf maturity on protein degradation (data not shown) and found that protein degradability by the enzyme was lower in mature leaves, regardless of species and addition of PEG to reduce the activity of tannins.

Table 2. Protein of legumes (immature leaf tissue) with and without tannins that was degraded by a proteolytic enzyme (Ficin) under incubation in a water bath

Treatment	Legumes with no Tannins		Legume with Tannins		
	G. sepium	*E. fusca*	*L. leucocephala*	*C. fairchildiana*	*Bauhinia* sp
	(Protein degraded, %)		(Protein degraded, %)		
-PEG	58 a	45 b	15 e	13 e	2 f
+ PEG	56 a	46 b	29 d	37 c	27 d

a, b, c, d, e and f means are different (P<0.05)

The range of values on degradation observed with the Ficin assay in this study are considerable lower than values observed in other in vitro and in vivo studies where a similar rage of legumes with and without PEG were evaluated. Thus from these results it seems that the use of a proteolytic enzyme such as Ficin can be useful only to determine the relative effects of tannins on protein degradability of tropical legumes.

1.2.2 Milk production of cows fed legume-based supplements

Contributors: P. Avila, C. Lascano, M. Peters and L. H. Franco (CIAT)

Rationale

In CIAT's Forage Project we are developing forage alternatives to feed livestock in the dry season. One of such alternatives is the shrub legume *Cratylia argentea* (Cratylia) that has proven to be very drought tolerant in many tropical regions where it has been tested. Farmers in dry hillsides of CA have been using Cratylia mainly in cut and carry systems. However, some progressive farmers have ensiled surplus Cratylia forage in the wet season using conventional silage technology. The use Cratylia as a silage in the dry season has resulted in the replacement of purchased feed supplements, which in turn has had a favorable impact profits to the farmer.

The forage Project has also been evaluating different annual legumes such as cowpea (from IITA) and LabLab (from CSIRO) as green manures in crop –livestock systems. In addition, we have been interested in looking at the feed value in the dry season of cowpea and LabLab hay harvested at pre-flowering and after grain harvest.

There is abundant literature on production and utilization of silage and hay, but in most cases the technology available is not useful to small farmers given that it relies on machinery (i.e. tractors and mechanical forage harvesters) out of the reach of these farmers. Thus we have been investigating alternative technologies such as the " bag silage" and hay packed in bags that could be more appropriate for smallholders livestock systems.

In this section we report the results of feeding Cratylia silage and Cowpea hay to milking cows grazing a *Brachiaria decumbens* pasture in the dry season.

Materials and Methods

Cratylia Bag Silage: Cratylia forage with 4 months regrowth was harvested by hand in a fodder bank in Quilichao and passed through an electrical forage chopper. The resulting forage was placed in plastic bags (0.52 m x 089 m) and molasses (10 kilos/100 kilos of DM) was then added to improve the fermentation. The forage in the bags (17 kilos/bag) was well mixed with molasses before compacting and storing in a dark place at room temperature. The little bag silage that was made is shown in Photo 1 together with the silage that was fed to cows after three months storage (Photo 1).

Photo 1. Cratylia silage packed in bags

Cowpea Hay: Whole plants of cowpea in the pre-flowering stage (8 weeks after planting) were harvested by hand and passed through an electrical forage chopper. The chopped forage was then sun-dried for 3 days and then placed in polypropylene bags (0.57 m x 0.90 m). Bags with approximately 8 to 9 kilos of sun dried cowpea hay were stored at room temperature for 30 days before being fed to dairy cows. The bags and cowpea hay fed is shown in Photo 2.

Photo 2. Hay of cowpea packed in bags

Dual- purpose cows in early lactation were assigned to one of the following treatments arranged in a 3 x 3 LSD: T1: *B. decumbens*, T2: *B. decumbens* + Cratylia silage and T3: *B. decumbens* + Cowpea hay. The Cratylia silage and cowpea hay were supplemented daily (0.8 % DM of BW) to cows grazing a *B. decumbens* pasture in the dry season (Photo 3). Each period had duration of 14 days of which 7 were for adjustment and 7 days for measurements.

Photo 3. Cows grazing *a Brachiaria decumbens* pasture in the rainy season (Quilichao Research Station)

Results and Discussion

The quality of the silage and hay fed are shown in Table. It is evident that Cowpea hay was of very high quality as indicated by high digestibility (89%), high crude protein (22%) and low fiber (22% NDF). On the other hand, the Cratylia silage had high CP (18%) but lower digestibility (57%) given its higher fiber content (44% NDF) when compared to the cowpea hay.

Table 3. Chemical composition and digestibility of Cowpea hay and Cratylia bag silages fed to milking cows as supplements.

Parameter	Cowpea Hay	Cratylia Silage
DM (%)	94.3	37.5
Crude Protein (%)	22.0	18.0
Neutral Detergent Fiber (%)	19.1	44.1
In vitro DM digestibility (%)	88.6	56.5

Results shown in Table 4 indicate that intake of cowpea hay was higher than the intake of Cratylia silage, but that this difference did not translate into a significantly higher milk yield. The supplementation of silage and hay resulted in 11 to 18% more milk yield when compared with production of cows receiving no supplement.

Table 4. Milk production of cows grazing a *Brachiaria decumbens* pasture in the dry season and supplemented with Cratylia silage and cowpea hay.

Treatment	Supplement offered (kg of DM / kg of BW /d)	Supplement Consumed (kg of DM/ kg of BW/ d)	Milk yield (l/cow/d)
Brachiaria			5.6 b
Brachiaria + Cratylia bag silage	50.2	37.3	6.2 a
Brachiaria + Cowpea hay	57.3	52.3	6.6 a

a, b means different (P<0.05).

These results confirm previous findings that had indicated that through the use of Cratylia silage produced and stored with conventional methods (stored in Bunker type-silo and compacted with a tractor) farmers could replace concentrates to supplement cows in the dry season and consequently increased their profits.

The use of plastic bags to make silage is very appropriate for smallholders that have few milking cows. This simple technology could also be attractive to small farmers who do not own livestock but who could benefit from selling “little bag silages” to livestock owners who need extra feed for the dry season. We need to explore the market potential of little bag silages in smallholder systems in hillsides of Honduras and Nicaragua so that we can then start evaluating the feasibility and economics of this technology with farmers.

We have been evaluating cowpea accessions from IITA mainly as green manures in annual cropping systems in the llanos and hillsides of Central America. However, double purpose cowpeas developed by IITA could be of interest to small livestock farmers in Central America as high quality hay for the dry season. The results of our experiment with dairy cows clearly show that supplementing cows in the dry season with small quantities of hay resulted in one more liter of milk per day. Thus we also need to assess with farmer participation the feasibility and economics of hay production with high quality annual legumes.

Activity 1.3 Assessment of quality and animal production potential of selected grass species

Highlight

- Milk production with a new *Brachiaria* hybrid that is resistant to spittlebug (*A. varia*) was as high as with the *Brachiaria* hybrid cv Mulato and higher than with commercial accessions (*B. brizantha* cv Toledo and *B. decumbens* cv Basilisk)

1.3.1 Milk yield with new accessions and hybrids of Brachiaria

Contributors: P. Avila, C. Lascano and J.W. Miles (CIAT)

Rationale

In previous experiments we had shown that milk yield with the commercial *Brachiaria* Hybrid Mulato was greater than with *B. brizantha* cv Toledo and *B. decumbens* cv Basilisk. It was also interesting to observe that MUN values were two times greater in cv Mulato as compared to the other two *Brachiaria* cultivars, suggesting a higher concentration of CP in the forage on offer.

This year we completed two short-term grazing experiments to compare milk yield in a new Brachiaria hybrid (FM 9503-5046-024) resistant to spittlebug (*A. varia*) with milk yield recorded in released cultivars (Mulato and Toledo).

Materials and Methods

The two grazing trials were carried out in a rainy period (February to May, 2002) using 2 cows/ha. In each experiment a total of 6 cows (3 Holstein and 3 Zebu Crossbreds in mid lactation) arranged in a 3 x 3 Latin Square were used to measure milk yield in pastures that were mowed 3-4 weeks prior to grazing. Each period was of 14 days of which 7 were for adjustment to the treatment and 7 for measurement of milk yield milk composition parameters and pasture attributes.

In the first experiment we had the following treatments: T1: *B. brizantha* cv Toledo (control), T2: *Brachiaria* hybrid cv Toledo and T3: *Brachiaria* hybrid 4624.

Treatments in the second experiment were: T1: *B. brizantha* cv Toledo, T2: *B. decumbens* cv Basilisk and T3: *Brachiaria* hybrid 4624.

Results and Discussion

Our results did not show a significant interaction of cow group and pasture for milk yield, so mean values across cow types are presented in Table 5.

In the first experiment milk yield was higher in cows grazing *Brachiaria* hybrid cv Mulato and in the new *Brachiaria* hybrid FM 9503-5046-024 as compared to what was recorded in the recently released *B. brizantha* cv Toledo. In the second experiment we also observed that milk yield was higher in the new *Brachiaria* hybrid FM 9503-5046-024 as compared to *B. decumbens* cv Basilisk and *B. brizantha* cv Toledo.

As observed in previous experiments, MUN (Milk Urea Nitrogen) was greater in cows grazing Mulato and the new hybrid FM 9503-5046-024 as compares with *B. brizantha* cv Toledo. This lower MUN values have been consistently associated with lower CP in the leaf tissue of Toledo, particularly when pastures are not stocked heavily.

Our results from this year confirm that the *Brachiaria* hybrid cv Mulato has a higher quality than commercial cultivars. However, it is also of interest to see the *Brachiaria* hybrid FM 9503-5046-024 with resistance to spittlebug (*A. varia*) seems to have a similar quality to the commercial cv Mulato as reflected by milk yields. This new hybrid is currently under regional evaluation and particular attention is being placed on its seed production potential.

Table 5. Milk yield of cows grazing contrasting Brachiaria pastures (Quilichao Research Station).

Treatments	Milk yield (l/cow/d)	Fat (%)	MUN (mg/dL)
Experiment 1			
B. brizantha cv Toledo	6.0 b	4.2	6.2 b
Brachiaria hybrid cv Mulato	6.7 a	4.2	10.4 a
Brachiaria hybrid FM 9503-5046-024	7.1 a	4.3	12.1 a
Experiment 2			
B. brizantha cv Toledo	5.1 b	4.6	5.9 b
B. decumbens cv Basilisk	5.5 b	4.3	7.2 a
Brachiaria hybrid 4624	6.1 a	4.2	8.2 a

a,b Means different ($P<0.05$)

Activity 1.4 Assessment of the potential of tannins in legumes and saponin in tropical fruits to reduce methane in ruminants on grass diets

Highlights

- The fruit *S. saponaria* suppressed in vitro methane release by over 10% with grass-alone diets as well as from grass-legume diets. However, ciliate protozoa were only reduced by *S. saponaria* in the grass-legume diets.

- The supplementation with *S. saponaria* significantly reduced daily methane release from sheep by over 10%.

- The supplementation with *S. saponaria* increased bacterial protein flow to the duodenum by 50% in sheep fed a nitrogen deficient grass diet and by 26% in sheep fed a grass-legume diet.

- The addition of legumes with high levels of tannins reduced methane release per unit of organic matter fermented.

1.4.1 In vitro evaluation of the effect of *Sapindus saponaria* on methane release and microbial populations

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Rationale

Previous studies (See AR IP-5 2002) had shown that the supplementation with fruits of the tropical tree *Sapindus saponaria* reduced methane release from tropical diets. However, it was not clear if this methane suppressing effect was dependent on the quality of the basal diet or not. Thus an in vitro experiment was set up to test the hypothesis that the effect of *Sapindus saponaria* on methane release was independent of the quality of the diet.

Materials and Methods

A Rumen-Simulation Technique (Rusitec) experiment was performed to compare the effects of the dietary inclusion (1/3 of the basal diet DM) of the following three tropical forage legumes of contrasting quality:

1) *Calliandra calothyrsus* (low quality, high tannin content),
2) *Cratylia argentea* (medium quality, moderate tannin content) and
3) *Arachis pintoi* (high quality, negligible tannin content), to a basal diet of *Brachiaria dictyoneura*, which was also tested without legume.

All diets were evaluated with and without the addition of *Sapindus saponaria* fruits (8% of the daily DM supply).

Results and Discussion

The mean rumen fluid pH across all diets was 7.01 and the effects of legumes and *S. saponaria* on this variable were minor. *C. argentea* and *A. pintoi* increased ($P<0.05$) rumen fluid ammonia from 0.3 mmol (control) to 1.8 and 2.0 mmol, respectively, but *C. calothyrsus* had no effect ($P>0.05$) on ammonia levels and *S. saponaria* tended ($P<0.10$) to reduce rumen ammonia by 17% on average.

The legumes *A. pintoi* and *C. argentea* increased bacteria counts while *A. pintoi* increased protozoa counts ($P<0.05$). Both legumes enhanced organic matter and fiber (cellulose) degradation ($P<0.05$) (Figur 3, left). *C. calothyrsus* had no effect on microbial counts ($P>0.05$) and reduced organic matter and fiber degradation ($P<0.05$). Methane release was increased by *C. argentea* and *A. pintoi* by almost 3 to 4-fold relative to the grass-alone diet, whereas *C. calothyrsus* reduced methane release by 50% ($P<0.05$). *S. saponaria* reduced methane release by 11% on average ($P<0.05$) (Figure 3, right).

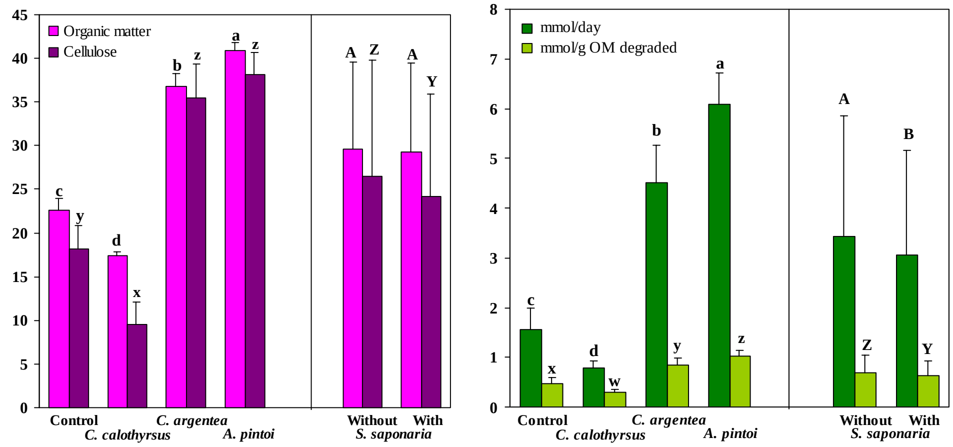

Figure 3. Apparent organic matter and cellulose degradability (%) (on the left) and methane release (on the right) of tropical diets containing no legumes (control) or 1/3 of *C. calothyrsus*, *C. argentea* or *A. pintoi*. All diets were evaluated with and without the addition of *S. saponaria* fruits (8% of daily DM supply).

Adding *S. saponaria* to the diets tended to decrease ($P<0.10$) methane release relative to organic matter degraded. Interactions between addition of legumes and *S. saponaria* were mostly insignificant, except in protozoa counts (suppressed by *S. saponaria* only in the diet containing *A. pintoi*).

In general, our results indicate that the inclusion of legumes in the diets clearly elevated N supply in all cases and this resulted in highly significant effects of legumes in all variables related to rumen fluid N turnover. Apparent N degradation rates from the grass-legume diets, compared to the grass-alone diet, were reduced by about 87% with *C. calothyrsus* but showed a 2-fold increase with *A. pintoi* and a 1.5-fold increase with *C. argentea*.

These results indicate that methane release can be reduced with *S. saponaria* when fed in combination with pure low quality grass diets or with low quality grass diets supplemented with legumes. In addition, our results suggest that the tannins present in *C. calothyrsus* could be associated with methane reduction and that methane production per unit of OM digested was reduced when high quality legumes were used as supplements of low quality grass diets. The higher organic matter and fiber degradation of low quality grass basal diets supplemented with *C. argentea* and *A. pintoi* would probably results in higher animal productivity. Therefore, the increased methane produced with these legumes will be counterbalanced by a correspondingly higher animal productivity.

1.4.2 In vitro evaluation of the effect of tannins in legumes on methane production

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Rationale

Results from the experiment reported in the previous section showed clearly that the replacement of 1/3 of low quality *Brachiaria dictyoneura* in the diet by *Calliandra calothyrsus* significantly reduced methane release per unit of organic matter digested. However, it is not known which properties of *C. calothyrsus* (*e.g*. high tannin content, low digestibility) are responsible for this effect. Thus an experiment was set to test the hypothesis that feeding legumes high in tannins could significantly reduce methane production.

Materials and Methods

A Rumen-Simulation experiment using the RUSITEC was performed to evaluate the effects of a partial (1/3) or total (1/1) replacement of *Brachiaria dictyoneura* by *Calliandra calothyrsus*. *Brachiaria dictyoneura* was also tested without the legume. All diets were evaluated with and without the addition of PEG, a tannin-binding agent that is commonly used to inactivate tannins and to evaluate tannin related effects.

Results and Discussion

These results indicate that tannins could be responsible to some extent for the suppression of methanogenesis caused by *C. calothyrsus*. The addition of PEG (i.e. inactivation of tannins) drastically increased methane release from diets containing *C. calothyrsus* and remained without any effect in the grass-alone diet (Figure 4).

It is well documented that high tannins in tropical legumes have a negative effect on digestibility and on intake by ruminants. In addition, tannins are known to bind feed protein in the rumen and make it unavailable in the small intestine. Low fiber digestibility of *C. calothyrsus* could also contribute to low methane production.

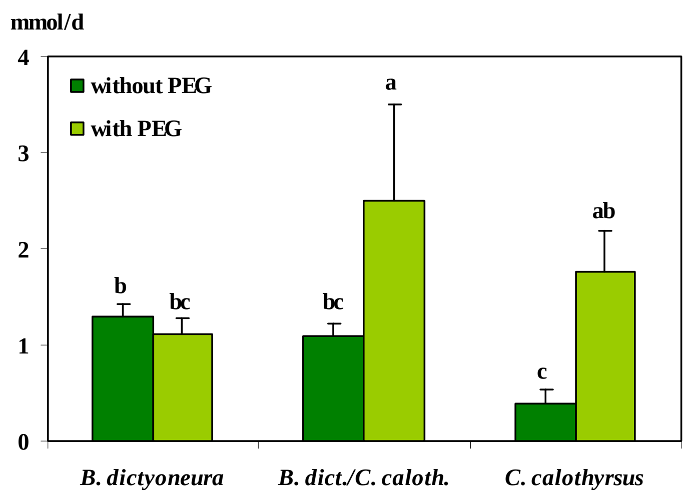

Figure 4. In vitro methane release from grass-alone (*B. dictyoneura*), grass-legume (*B. dictyoneura/C. calothyrsus*) and pure legume (*C. calothyrsus*) diets, expressed as daily release per fermenter supplied with 15 g/d of forage DM. All diets were evaluated with and without the addition of PEG (30 mg/g of forage DM).

One could speculate that by diluting tannins through the combination of legumes with and without tannins it would be possible to reach a tannin threshold that has limited effect on digestion and on intake but can still significantly reduce methane production. However, to better define feeding systems with legumes that have tannins we first need to understand the underlying mechanism on how tannins and possibly fiber in tropical legumes reduce methane.

1.4.3 In vivo evaluation of *Sapindus saponaria* and legumes as supplements on rumen fermentation and N utilization by sheep fed a low quality grass diet

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Experiment 1 - CIAT

Intake, digestion and duodenal nitrogen flow in sheep fed a low quality grass diet supplemented with fruits from *Sapindus saponaria* and legumes

Rationale

It is known that complete and partial defaunation increase the flow of microbial and dietary protein from the rumen to the duodenum and consequently can improve nitrogen utilization in ruminants. We have demonstrated through in vitro studies that the incorporation of the saponin-rich fruit *Sapindus saponaria* into forage-based diets reduces rumen protozoa population. However, little information is available on how *S. saponaria* affects nitrogen flow to the duodenum and whether these effects are dependent on the quality of the basal diet. Therefore an experiment was carried out to study the influence of a supplementation with fruits from *S. saponaria* on intake, digestion and duodenal N-flow in sheep fed tropical forage diets of contrasting quality.

Materials and Methods

Six African-type sheep were fitted with ruminal and duodenal cannulae and allotted to one of four treatments in an unbalanced simple crossover design with a 2×2 factorial arrangement of treatments: 2 basal diets $\times$ 2 supplementations (with and without *S. saponaria*).

The forage required for the trial was harvested, dried and stored at CIAT's research station in Quilichao (Cauca valley, Colombia) and fruits of *S. saponaria* were collected in the northern cost region of Colombia and in the Cauca Valley.

The traditional low–quality diet consisted of a grass hay (*Brachiaria dictyoneura* cv. Llanero, 3.7% crude protein, 72.8% neutral detergent fiber, 41.1% acid detergent fiber) and the improved diet consisted of 75% (on a DM basis) of the same hay complemented with 25% sun -dried *Cratylia argentea* leaves (18.6% CP, 60.2% NDF, 36.5% ADF).

Animals housed in individual metabolism crates were offered daily 80 g DM/kg $BW^{0.75}$ of forage in two meals (8:00 and 14:00). Both diets were fed either without supplementation or with fruits of *S. saponaria* as a supplement (8 g/kg $BW^{0.75}$ per day) dosed directly into the rumen. The fruits of *S. saponaria* were ground using a 3 mm–screen and administered via rumen cannula. Animals were offered a mineral mix *ad libitum* and water 4 times a day. Sheep used in the trial were allowed to graze for one week between experimental periods, which had a duration of 19 days, of which 7 were for adjustment and 13 for measurements. Forage refused and feces were collected daily and on day 17 and 18 of the measurement period, samples of duodenal digesta were taken every 6 hours and on the last day rumen samples were collected every 3 hours to determine purines and bacterial nitrogen flowing to the duodenum. Cr-EDTA and indigestible acid detergent fiber were used as flow markers for the liquid and the solid phase, respectively.

Results and Discussion

Results for intake and digestibility are presented in Table 6 and as expected, voluntary dry matter intake was higher (+20%, $P<0.001$) with the grass-legume diet than with the grass-alone diet. Basal forage intake was no affected by the administration of *S. saponaria* and consequently total DM intake of animals receiving *S. saponaria* was increased. Interactions of basal diet and *S. saponaria* on intake were non significant ($P>0.05$), but significant interactions in digestibility were observed. The supplementation of *S. saponaria* reduced DM and NDF digestibility in the grass-alone diet, but no in the grass-legume diet.

Table 6. Intake and digestibility of dry matter, neutral detergent fiber and acid detergent fiber of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria*.

Variables	Grass only		Grass-Legume		SE	Significance		
	Control	Sap.	Control	Sap.		Diet	Sap.	Interact
Total intake	g/d							
Dry matter	692	774	834	918	46.9	0.009	0.097	0.989
DM/kg $BW^{0.75}$	42.3	50.0	53.5	57.8	2.57	0.003	0.035	0.534
Organic matter	633	719	769	858	42.0	0.006	0.056	0.981
NDF	515	545	575	626	36.8	0.075	0.282	0.790
ADF	282	310	341	362	21.3	0.022	0.258	0.881
Digestibility	%							
Dry matter	51.4a	47.6b	47.6b	49.4ab	1.12	0.385	0.388	0.036
Organic matter	57.5	58.5	58.5	54.7	3.38	0.682	0.679	0.498
NDF	58.0a	51.0b	51.8b	53.7ab	1.98	0.392	0.219	0.051
ADF	49.6a	42.4b	42.3b	40.2b	1.38	0.005	0.005	0.099

The main objective of carrying out this feeding trial was to test the hypothesis that the supplementation with *S. saponaria* would result in a suppression of rumen ciliate protozoa and consequently in an increased bacterial and total N flow to the duodenum. Results presented in Table 7 show that rumen ciliate protozoa were not suppressed by *S. saponaria*. Instead, counts of the group of Entodinium and of total ciliate protozoa were even increased by *S. saponaria* ($P<0.01$).

Table 7. Ciliate protozoa counts in rumen fluid of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria.*

Ciliate protozoa counts	Grass only		Grass-Legume		SE	Significance		
(10^4/ml)	Control	Sap.	Control	Sap.		Diet	Sap.	Interact.
Holotrichs	1.6	2.0	1.6	1.3	0.33	0.264	0.906	0.361
Entodinium	8.5	17.1	9.1	14.3	1.58	0.494	0.001	0.308
Total	10.4	19.2	10.7	15.6	1.80	0.421	0.002	0.290

Results on N intake, digestion and utilization are presented in Table 8. Total N intake was 128% higher ($P<0.001$) with *C. argentea* than with the grass-alone diet and the supplementation with *S. saponaria* had no effect on total N intake ($P>0.10$). The higher N intake with *C. argentea* was the result of the higher DM intake and of the higher N content in the forage offered. A higher amount of total N (+55%, $P<0.001$) and bacterial N (+30%, $P<0.05$) reached the duodenum when *C. argentea* was fed.

The supplementation with *S. saponaria* tended to increase total N flow (+18%, P<0.10) and significantly increase bacterial N flow (+36%, P<0.01) to the duodenum. This increase was considerably higher with the grass-alone diet (+50%) than with the grass-legume diet (+27%). Apparent N absorption was increased when *C. argentea* was fed (+48%, P<0.05) but was not affected by the supplementation with *S. saponaria* (P>0.05). However, it is worth mentioning that the apparent N absorption was numerically increased by over 40% with the grass-alone diet when *S. saponaria* was administered, which was not the case with the grass-legume diet, but due to the high variability this difference failed to be significant.

Table 8. Nitrogen intake, digestion and utilization and volatile fatty acid concentrations in rumen fluid of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria*.

Nitrogen	Grass only		Grass-Legume		SE	Significance		
	Control	Sap.	Control	Sap.		Diet	Sap.	Interact.
N intake (g/d)	5.54	5.74	11.86	13.91	0.800	0.001	0.179	0.29
N intake (g/kg $BW^{0.75}$/d)	0.18	0.21	0.40	0.46	0.030	0.001	0.167	0.65
Duodenal flow (g/d)	6.64	9.14	11.85	12.74	0.958	0.001	0.098	0.44
Bacterial	3.10	4.65	4.43	5.64	0.430	0.018	0.007	0.72
Duodenal flow (% of intake)	126.2	155.7	109.9	100.6	13.71	0.021	0.468	0.20
Apparent absorption (g/d)	3.67	5.41	6.68	6.74	0.925	0.035	0.344	0.40
N digestibility (%)	43.4	36.9	46.2	53.2	7.69	0.230	0.974	0.42
Ruminal escape (g/d)	3.53	4.48	7.40	7.07	0.591	0.001	0.605	0.32
Rumen ammonia N (mg/l)	30.3	17.8	89.3	79.3	8.263	0.001	0.192	0.89
Volatile fatty acids (mmol/l)	103.7	121.2	116.6	125.1	5.95	0.18	0.0047	0.48
Acetate	81.5	91.5	90.4	92.8	4.58	0.27	0.20	0.44
Propionate	14.8	19.6	18.2	22.0	1.15	0.024	0.002	0.66
n-Butyrate	7.1	9.9	7.3	9.8	0.61	0.98	0.001	0.83
iso-Butyrate	0.41	0.27	0.67	0.50	0.048	0.001	0.006	0.73
Acetate/Propionate	5.63	4.77	4.98	4.24	0.173	0.005	0.001	0.75

Nitrogen apparent digestibility was similar with all diets (P>0.05), whereas ruminal escape N and rumen ammonia N were increased when *C. argentea* was fed but remained unaffected by the supplementation with *S. saponaria* (P>0.05). The interactions between quality of the diet offered and *S. saponaria* on nitrogen intake, digestibility and duodenal N-flow were non-significant. This indicates that the potential of *S. saponaria* to increase bacterial N flow is not dependent on quality of the basal diet.

The supplementation with *C. argentea* and *S. saponaria* affected concentration of individual volatile fatty acids. Both supplements had no effect on acetate concentration but increased the concentration of propionate. While the legume did not affect n-butyrate, it was clearly increased by *S. saponaria*. This higher n-butyrate concentration could be related to the relatively high sugar concentration of *S. saponaria* (21% of DM). The legume also increased the concentration of iso-butyrate, which is one of the end products of degradation of true protein in the rumen.

This finding is an indication that ruminal protein degradation was enhanced when the legume was fed and is in agreement with the higher rumen ammonia levels observed with diets containing *C. argentea*. In contrast, supplementation with *S. saponaria* reduced the iso-butyrate concentration, suggesting that this feed ingredient suppressed protein degradation. Finally, *C. argentea* and *S. saponaria* independently shifted the volatile fatty acid production towards lower acetate to propionate proportion.

Results from this study confirm previous studies that indicated that the inclusion of *Cratylia argentea* into diets based on low-quality hay increased total dry matter and nitrogen intake and improved nitrogen absorption. They also show that supplementation with the saponin-containing fruits of *Sapindus saponaria* may increase bacterial nitrogen flow to the duodenum independently of the quality of the basal diet. It is interesting to note that supplementation with *S. saponaria* may increase bacterial N flow to the duodenum even though rumen protozoa counts are not suppressed.

Finally, our results indicate that supplementations with *C. argentea* and *S. saponaria* improve the efficiency of rumen fermentation by shifting the volatile fatty acid production towards lower acetate to propionate ratio. The effects of the two supplements were independent and additive, thus the highest nutritional efficiency was obtained with the combination of *C. argentea* and *S. saponaria*.

Experiment 2 –ETH-Zurich

Methane release and energy and nitrogen utilization of sheep fed a low quality grass diet supplemented with fruits from *Sapindus saponaria* and legumes

Rationale

Previous in vitro-experiments showed that the inclusion of fruits of *Sapindus saponaria* into tropical forage-based diets could suppress methane released by over 10%. However, no information was available on its effect on methane release in vivo. Thus a respiratory chamber experiment was carried out to study the influence of a supplementation with fruits of *S. saponaria* on energy and nitrogen utilization and methane release of sheep fed tropical diets.

Materials and Methods

Three basal diets with contrasting forage quality were used. The traditional diet consisted of a low quality hay (*Brachiaria dictyoneura* cv. Llanero), and in the other two diets 1/3% and 2/3% of the grass sun-dried leaves of the shrub legume *Cratylia argentea* replaced hay. All three diets were fed either with a concentrate containing 25% *S. saponaria* or with a control concentrate without *S. saponaria*. Animals were offered 60 g/kg $BW^{0.75}$ of forage and 21 g/kg $BW^{0.75}$ of concentrate per day. Six growing Swiss White Hill sheep (initial body weight 30.1±2.8 kg) were allotted to one of the six treatments in a complete Latin-Square design with 3×2 factorial arrangement of treatments (3 basal diets $\times$ 2 concentrates) and 6 experimental periods of 21 days each.

The first 12 days of each experimental period were used for adaptation, days 13 to 20 for measurement of forage intake and total collection of feces and urine (Photo 4, right) and day 21 for blood and rumen liquid sampling. Respiratory measurements were carried out (Photo 4, left) during two 22.5 h-periods on days 19 and 20.

The forage required for the trial was harvested and dried at CIAT's Research Station in Quilichao (Cauca Valley, Colombia) and fruits of *S. saponaria* were collected in the rural area near Cali (Cauca valley, Colombia). Forage and fruits were then shipped to Switzerland and stored at the ETH-Zurich. The two concentrates were formulated to contain similar amounts of protein, fiber and energy. Approximately 180 kg of each concentrate were mixed and pelleted at the Institute of Animal Sciences at the ETH-Zurich.

 

Photo 4. Sheep during measurements in respiratory chamber (on the left) and during collection period in metabolic crates (on the right).

Results and Discussion

Up to now, only data from four of the six measurement periods have been analyzed. However, some effects are already evident. Supplementing legume significantly increased:

1. Total daily dry matter, energy and nitrogen intake
2. N apparent digestibility and retention,
3. Rumen ammonia and blood urea levels, and
4. Flows of dietary and microbial nitrogen to the duodenum.

On the other hand, legume supplementation reduced apparent digestibility of OM, neutral detergent fiber and acid detergent fiber but did not affect energy retention and methane emissions. Supplementation with *S. saponaria* had no effect on dry matter and nutrient intake, but reduced the apparent digestibility of organic matter, nitrogen, fiber and energy. Additionally, *S. saponaria* shifted N excretion from urine to feces (more N in feces and less in urine), tended to reduce total N losses and to increase the flow of microbial nitrogen to the duodenum. *S. saponaria* had no significant effect on rumen ammonia but reduce the concentration of urea in blood.

Finally, the supplementation with *S. saponaria* clearly suppressed rumen ciliate protozoa by 70% and daily methane release per animal by over 10%. No interactions between the legume and the *S. saponaria* supplementation were observed.

The results from this study are in line with those from our in vitro experiments that indicated that *S. saponaria* has a high potential to suppress rumen ciliate protozoa and methane emission of ruminants fed tropical diets of contrasting quality.

Activity 1.5 Adjustment of methods for simultaneous evaluation of tropical legumes for feed and soil improvement

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Highlight

- Found that IADF (Indigestible acid detergent fiber) fraction was highly correlated with extent and rate of decomposition of legumes under aerobic (soil) and anaerobic (rumen) conditions

1.5.1 Assessment of the effect of drying plant material on aerobic and anaerobic decomposition of legumes of contrasting legume quality

Rationale

It is recognized that legume species are useful to enhance existing feed resources and to contribute to soil fertility in mixed livestock-cropping systems through their use in associated grass-legume pastures, as green manure or as mulch.

In mixed crop-livestock production systems legume quality is a key factor to obtain maximum benefits in terms of rate and extent of N release in the rumen or soil. Consequently, Animal Nutritionists and Soil Scientists have been interested in defining plant quality parameters that are correlated with release of nutrients from topical legumes. However, research in quality of legumes as it relates to ruminants or soil has been carried out in an independent manner and consequently there has been very little information sharing on methodological aspects.

Decomposition of plant material in the soil is mainly mediated by microbial population and to lesser effects by soil macrofauna. Plant decomposition is often determined using the litterbag technique whereby plant material is placed in or on the soil in series of nylon litterbag. Decomposition is then determined by sampling the bags over time (usually several weeks or months) and the results (DM disappearance and N release) are then related to plant chemical entities. This method is resource -and time- consuming, but provides valuable data for comparing plant species in terms of their relative decomposition and nutrient release patterns.

Ruminants also decompose plant material through microbes that degrade plant protein and cell wall constituents to ammonia, amino acids and volatile fatty acids, which are then used by microbes for protein synthesis. To measure the extent and rate of nutrient release from plant material used as a feed resource, samples are incubated with rumen microbes using in vitro systems or alternatively using in situ techniques, which follow the same principle of the soil nylon litterbag method.

It is recognized that soil and rumen processes involved in plant degradation have fundamental differences namely an anaerobic aqueous environment in the rumen, higher number of microbes and much faster degradation rates in the rumen compared with soil. Despite these differences, the extent and rate of nutrient release from plants in the two processes is greatly affected by compositional factors of the plant (i.e. N level, lignin, condensed tannins).

Thus we are interested in testing the hypothesis that that similar plant chemical entities control decomposition and the release on nutrients in the rumen and soil and that in vitro values on rates and extents of digestion by rumen microbes can be used to predict decomposition values of legume plant material in the soil.

To test these hypotheses we setup a research program, which involves three phases:

a) Laboratory studies to determine rates and extent of aerobic and anaerobic degradation of plant material from legumes with contrasting quality subject to different drying treatments and using different methods.
b) Laboratory studies to determine relationships between plant chemical entities and aerobic and anaerobic decomposition and release of nutrients in a range of legumes of contrasting quality.
c) Field studies using selected legumes as green manures and indicator crops to validate predictions of equations of nutrient release and in vitro anaerobic and aerobic results.

We hope that through this research we can produce the following outputs:

a) Know applicability of in vitro methods used to assess feed value of forages to define potential decomposition and release of nutrients from legumes when used as feed resource or to improve soil fertility.

b) Known chemical entities in plant material that control the extent and rates of decomposition of tropical legumes in the rumen and soil.

c) Guidelines for quick and reliable assessment of the value of tropical legumes as feed resources and to improve soils

In this report we summarize results from the first series of laboratory studies and greenhouse trials not shown in the 2001AR. The second phase of the work was started in August, 2002 and results will be reported next year.

Materials and Methods

In the first phase the following woody tropical legumes were utilized: a) *I. constricta* (low tannin content), b) *C. argentea* (medium tannin content) and, c) *Calliandra* spp (high tannin content). Plant material from the three legumes growing in a hillside site (Pescador, Cauca) was harvested after 6 weeks of regrowth and selected plant material (leaf + fine stem) where subject to the following drying treatments prior to aerobic and anaerobic incubation: fresh, frozen, freeze-dried, oven-dried (60^{o}C) and air-dried.

Results on gas production, DM decomposition and mineralisation over time were fitted to appropriate regression models to estimate rates. Correlation analysis was then performed between dependent and independent variables.

Results and Discussion

Results presented last year showed that drying treatments had a significant effect on the chemical composition (quality) of the three legumes included in the test (see Annual Report 2001). For all legume species, oven drying resulted in more fiber and lignin than freeze-drying or air-drying possibly as a consequence of artifacts formed by heat damage (Maillard reaction). However, this effect did not lead to a consistent reduction of DM degradation (litterbag and in-vitro digestibility experiments) or N mineralisation (leaching tube experiment) of the legumes under test as we had expected based on results in the literature.

Of the legumes included in our study, *I. constricta* (no tannins) was the one with the highest quality given its lower fiber and lignin concentration and higher N level when compared to the other two species. In the case of *C. argentea* (low levels of condensed tannins), the main factor affecting its quality would seem to be the high and lignified cell wall fraction. On the other hand, degradation of *Calliandra* spp (high levels of fiber and condensed tannins) could be more related to its high tannin content than to the high lignin content.

The rate of DM disappearance of *I. constricta* under aerobic conditions was 4 times greater than *C. argentea* and 7 times greater than *Calliandra* spp. However, under anaerobic conditions the rate of gas production of *I. constricta* when averaged across drying treatments was only 1.4 times greater than with *C. argentea* and 3.5 times greater than with *Calliandra* sp. (see table in annual report 2001).

As anticipated, net mineralisation rate (leaching tube experiment) was significantly higher for *I.constricta* than for *C. argentea*. The lowest mineralisation rate was observed with *Calliandra* spp, which also showed a mineralisation pattern with an initial net immobilization rate. Given that the pattern of mineralisation was so different for *Calliandra* spp. relative to other species, it was not possible to adjust the *Calliandra* spp mineralisation pattern to the same exponential model used for *I. constricta* and *C. argentea*.

Results shown in Table 9 indicate that cell wall content (NDF) was poorly correlated to DM loss in the anaerobic rumen in vitro system and in the aerobic soil litterbag or leaching tube system, but that negative and significant correlations were observed with ADF (cellulose + lignin) and with lignin content. The highest correlations were obtained after adjusting the lignin fraction with condensed tannins under aerobic and anaerobic conditions and with indigestible fiber (INDF).

Table 9. Relationship between different plant chemical components, gas production and dry matter (DM) loss (rate and extent) in an anaerobic in vitro gas production system and an aerobic soil litterbag and leaching tube system.

	Anaerobic Conditions- Rumen Microorganisms Gas Production r		Aerobic Conditions- Soil Microorganisms DM loss r		Aerobic Conditions- Soil Microorganisms Mineralisation r
Plant chemical entities	Extent	Rate	Extent	Rate	Extent
NDF	-0.13 *	-0.21 *	-0.28 *	-0.27 *	-0.35 (P<0.1570)
ADF	-0.65 *	-0.65 *	-0.68 *	-0.6 *	-0.73 (P<0.0005)
Lignin	-0.74 *	-0.66 *	-0.79 *	-0.70 *	-0.72 (P<0.0007)
Condensed Tannins (CT)	-0.80 *	-0.56 *	-0.76 *	-0.58 *	-0.74*
Lignin + CT	-0.95 *	-0.92 *	-0.91 *	-0.68 *	-0.84*
IADF	-0.91 *	-0.87 *	-0.92 *	-0.75 *	-0.90*

* = P<0.0001

Our results showed high positive correlations for the majority of rates and extents of degradation measured under aerobic and anaerobic conditions (Table 10). The highest positive correlation between anaerobic and aerobic conditions was found between extent of gas production under anaerobic conditions and extent of dry matter loss under aerobic conditions. Also a high positive correlation was found between extent of gas production under anaerobic conditions and extent of mineralisation under aerobic conditions.

Results confirm that decomposition and mineralisation of legume plant material in the soil using the litterbag and leaching tube technique is highly correlated with gas production using *in vitro* anaerobic methods, indicating potentials for time and cost savings in evaluation of forages. While with the litterbag it takes 20 weeks to determine the extent and rate of decomposition of plant material in the soil with the in vitro anaerobic system it only takes 72 h to determine the extent of degradations of DM from plant material.

Our results also suggest that differences in plant quality attributes could be more important than sample preparation in determining the extent and rate of decomposition of plant material in the soil and rumen. In 2001, we concluded that the aerobic and anaerobic degradation of the legumes used is a function of indigestible fractions of the cell wall such as lignin alone or corrected for the presence of condensed tannins. These findings were confirmed in 2002, showing the high correlations between IADF with aerobic and anaerobic degradation.

Table 10. Relationships between rates and extents of anaerobic (gas production with rumen microbes) and aerobic (DM disappearance in litterbags, mineralisation in leaching tubes) degradation of legumes.

System and Variable	Aerobic Conditions- Soil Microorganisms DM Loss **r**		Aerobic Conditions- Soil Microorganisms Mineralisation **r**
	Extent	Rate	Extent
Anaerobic-Conditions Rumen Microorganisms			
Gas Production-Extent of degradation	0.92*	-	0.87*
Anaerobic Conditions- Rumen Microorganisms			
Gas production -. Rate of degradation	-	0.49*	-

* = P<0.0001

OUTPUT 2: Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed

Activity 2.1 Study the bioecology of spittlebug species in contrasting environments

Highlights

- Information on the comparative biology of nine spittlebug species summarized to establish trends and patterns of variation

- Life table analysis of three spittlebug species carried out for the first time to describe population parameters

- Verbal and quantitative models developed to explain the phenology of spittlebug populations in seasonal environments and predict the arrival of the first generation outbreak

- Evidence obtained to explain two key aspects of spittlebug phenology: acceleration of diapause termination through exposure of eggs to drought conditions and post-diapause quiescence with drought tolerance

- A new report of spittlebugs attacking sugar cane in Colombia was investigated and documented

2.1.1 Biology of spittlebug species

Contributors: Jairo Rodriguez, Oscar Yela, Ulises Castro, Daniel Peck

Rationale

A major barrier for advancing the management of spittlebugs in grasses is the lack of biological information on specific pest species. This has led to a weak understanding of the patterns of variation in this insect group and has contributed to an overgeneralization of this complex pest problem. Describing the patterns of variation in biology across this diverse group will depend on more detailed studies of currently unknown species. Tailoring IPM strategies to the particular species and habitats of concern will also depend on this information. To establish this foundation, results from the studies of Colombia's grass-feeding spittlebugs are summarized and some trends highlighted. In addition, life table analyses were carried out for the first time in spittlebugs. *Zulia carbonaria* was chosen as the model species to establish methodologies to further quantify relevant demographic parameters, overcome technical difficulties in the comparative study of reproductive biology, and compare results to previous life cycle studies. Although not presented here, we have gone on to obtain life table information for *M. andigena* and *P. simulans* as well.

Materials and Methods

Comparative biology. The biology of nine grass-feeding spittlebug species from Colombia were studied from 1996 to 2002 using comparative methodologies previously established at CIAT. To differentiate among the life stages, morphological measures were made of eggs, nymphs and adults. To quantify the life cycle, duration of life stages were followed under controlled conditions. And to begin to describe the reproductive behavior, oviposition site preferences were determined. To have access to all life stages, small colonies were established in the greenhouse with eggs collected from field-caught adults. To differentiate among the developmental stages, these were characterized morphologically. With the aid of a stereoscope and ocular micrometer, certain aspects of the external morphology were measured for four developmental stages of the eggs, five nymphal instars, both sexes of late instar V (Vb) and both adult sexes.

To quantify the duration of life stages, eggs, nymphs and adults were observed under controlled conditions. For the adults, tenerals (<12 hours old) from the colony were confined in cohorts of four individuals under acetate sleeve cages over pots of the host plant; mortality was assessed daily. For the nymphs, recently emerged first instars (<12 hours old) were placed in individual pots of host plant established with abundant surface roots that nymphs require as feeding sites. Transformation from one instar to the next was determined by direct observation of the nymph itself or the molted exuvia . The mean longevity of each life stage was calculated from observations of 40 individuals. For the eggs, duration of the developmental stages was determined under controlled conditions (27°C, 100% RH, total darkness). Recently laid eggs (<24 hours old) were maintained on moist filter paper in petri dishes and observed daily. The mean duration of each of the four generalized developmental stages was calculated from observations on 100 eggs.

To study oviposition sites as part of the description of reproductive biology, field conditions were replicated in the screenhouse. The soil surface was specially prepared with soil oviposition substrate and 2 g leaf litter was dispersed on top. Each pot was infested with two females and two males from the colony and 10 days later eggs were recovered from three oviposition substrates: soil, leaf litter and the plant surface.

Life table analysis. Life tables were established for spittlebugs for the first time, using *Zulia carbonaria* as the model species. This research was carried out in 2002 under laboratory conditions and in the screenhouse (mean min/min temperature 19.5/29.5°C, and minimum RH of 56.3%). Adult female *Z. carbonaria* were collected in the field and confined in groups of six individuals in each of 10 large petri dishes lined on the bottom with humid filter paper that served as oviposition substrate. Stems of *Brachiaria ruziziensis* held in a microvial filled with water served as a food source. After 24 hours all eggs were removed and put in groups of seven in sectioned dishes. Eggs were kept under controlled conditions (27°C, 100% RH, complete darkness) and observed daily to record survivorship and mortality in each of the four developmental stages. A life table was calculated from these data based on an initial cohort of 100 eggs. Upon emergence, each nymph (<12 hours old) was transferred to the screenhouse and placed in pots of *B. ruziziensis* specially prepared with abundant surface roots for feeding sites and spittle mass establishment. These pots were covered with a lid that had a hole through which the plant stems emerged. Nymphs were observed daily to determine advancement to the next instar through direct observation or detection of the exuvia.

Once adults emerged, data were collected for the female fertility table. Females were maintained under acetate sleeve cages over pots of *B. ruziziensis*. Oviposition substrate (2 cm thick) was provided in an inverted lid fitted in the top of the pot with an opening through which the plant stems emerged. Each pot was infested with one female and two males, the latter replaced when dead to provide continual presence of the opposite sex. Every three days females were transferred to new pots. Eggs were recovered from the soil substrate and the plant stem. Calculations were based on an initial cohort of 30 females.

The fecundity of *Z. carbonaria* females was determined based on the number of eggs laid by the initial cohort of 30 individuals. The fertility table was calculated from the longevity of the females, rate of oviposition and sex ratio (based on previous biological studies). Once the fertility table was constructed the following demographic parameters were calculated. Generation time, T, was the mean period between birth of the parents and birth of the progeny, based not only on duration of the immature and adult stages but on the preoviposition period and duration of the oviposition period. Net replacement rate, Ro, was represented as the mean number of female progeny a female leaves in a generation, a function of survival and fecundity. The innate capacity for increase, r_m, is the potential increase in a population under optimal conditions given an initial cohort.

Results

Comparative biology. Eggs of all species passed through four developmental stages (S1, S2, S3, S4) distinguished by external characteristics and usually accompanied by an increase in size from one stage to the next (Table 11). Eggs were spindle-shaped and varied from white to creamy yellow to brownish-yellow in color. Generally, in S1 eggs were recently laid with no particular externally visible signs of development. In S2 a spot of red pigment was usually visible and the operculum had darkened to reveal a gray streak below the chorion. In S3 the chorion opened to expose the black operculum and the red spot was usually no longer visible. In S4 two pairs of red spots were visible, the posterior representing the Batelli glands of the abdomen and the anterior representing the eyes of the developing nymph. Each progressive stage was usually accompanied by a statistically significant increase in both length and width. Total egg development time varied from 14.1-18.0 among species, and up to a maximum of 310 days (*A. reducta*) for quiescent or diapausing eggs.

Table 11. Differentiation of egg developmental stages.

Species	Length				Width			
	S1	S2	S3	S4	S1	S2	S3	S4
Aeneolamia lepidior	a	b	c	c	a	b	c	d
Aeneolamia reducta	a	a	b	c	a	b	c	d
Aeneolamia varia	a	a	b	c	a	b	c	d
Mahanarva andigena	a	b	c	d	a	b	c	d
Mahanarva trifissa	a	ab	b	c	a	a	b	c
Prosapia simulans	a	b	c	d	a	b	c	d
Zulia carbonaria	a	a	b	c	a	b	c	d
Zulia pubescens	a	a	b	c	a	b	c	d
Zulia sp. nov.	a	ab	b	c	a	b	c	d

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Five instars were confirmed for the nymphs of all species, accompanied by an increase in head capsule width, body, stylet and wing pad length; head capsule width was the most diagnostic because of little overlap among instars (Table 12, Table 13).

Table 12. Differentiation of nymphal instars: head capsule width and stylet length

Species	Head capsule width					Stylet length				
	I	II	III	IV	V	I	II	III	IV	V
Aeneolamia lepidior	-	-	-	-	-	-	-	-	-	-
Aeneolamia reducta	a	b	c	d	e	a	b	c	d	e
Aeneolamia varia	-	-	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	c	d	e	a	b	c	d	e
Mahanarva trifissa	a	b	c	d	e	a	b	c	d	e
Prosapia simulans	a	b	c	d	e	a	b	c	d	e
Zulia carbonaria	a	b	c	d	e	a	b	c	d	e
Zulia pubescens	a	b	c	d	e	a	b	c	d	e
Zulia sp. nov.	a	b	c	d	e	a	b	c	d	e

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Table 13. Differentiation of nymphal instars: anterior wing pad and body length.

Species	Anterior wing pad length			Body length				
	III	IV	V	I	II	III	IV	V
Aeneolamia lepidior	-	-	-	-	-	-	-	-
Aeneolamia reducta	a	b	c	a	b	c	d	e
Aeneolamia varia	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	c	a	b	c	d	e
Mahanarva trifissa	a	b	c	a	b	c	d	e
Prosapia simulans	a	b	c	a	b	c	d	e
Zulia carbonaria	a	b	c	a	b	c	d	e
Zulia pubescens	a	b	c	a	b	c	d	e
Zulia sp. nov.	a	b	c	a	b	c	d	e

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Stylet length decreased from the fifth instar to the adult in most species. There was sexual dimorphism expressed as the smaller size of male adults and late fifth instars in most species, and a trend toward brighter coloration in male adults in some species (Table 14).

Table 14. Differentiation of adult sexes.

Species	Head capsule width		Stylet length		Forewing length		Body length: Without wings		Body length: With wings		Body width	
	M	F	M	F	M	F	M	F	M	F	M	F
Aeneolamia lepidior	a	b	a	a	a	b	a	b	a	b	a	b
Aeneolamia reducta	a	b	a	b	a	b	a	b	a	b	a	b
Aeneolamia varia	-	-	-	-	-	-	-	-	-	-	-	-
Mahanarva andigena	a	b	a	b	a	b	a	b	a	b	a	b
Mahanarva trifissa	a	b	a	b	-	-	-	-	-	-	a	b
Prosapia simulans	a	b	a	b	a	a	a	b	a	b	a	b
Zulia carbonaria	a	b	a	a	a	b	a	b	a	b	a	b
Zulia pubescens	a	b	a	b	b	b	a	b	a	a	a	a
Zulia sp. nov.	a	b	a	a	a	b	a	b	a	b	a	b

For each species and parameter, different letters indicate statistically significant differences in the mean (a, b, c, d assigned smallest to largest).

Life cycle varied by 30 days (45.3-75.5) (Table 15). For eggs, nymphs and adults, the range of variation in duration was 14.1-18.0, 26.1-48.4 and 6.2-21.4 days, respectively. Seven of the nine species oviposited primarily in the soil, while *Prosapia simulans* preferred the stem surface and *Zulia pubescens* both stem and soil. Litter was the least preferred substrate receiving 0.0-8.2% of eggs with the exception of 22.7% in the case of *Mahanarva trifissa*.

Life table analysis. Under the conditions of the egg to adult study, mortality was 46.6%, that is 53 of the original cohort of 118 individuals died in the immature stages. The life table parameters for *Z. carbonaria* are summarized in Table 16. During the egg stage, dx (proportion of the original cohort that dies during each life stage) was highest (9.3%) in S1. Nymphs had the highest dx (25.4%) in instar I. The total mortality exhibited during the immature stages was 46.6%, divided in 10.1% and 36.3% for the egg and nymphal stages, respectively. The k-values for immature *Z. carbonaria* under the conditions of this study were 0.27 represented by 0.05 for the eggs and 0.23 for the nymphs (Table 16, Figure 5).

Table 15. Duration of life stages and oviposition sites of nine spittlebug species in Colombia

Species	Duration (days)				Oviposition sites [1]		
	Egg	Nymph	Adult	Total[2]	Soil	Litter	Surface of plant stem
Aeneolamia lepidior	14.1	35.4	6.2	52.6	79.7	5.7	14.6
Aeneolamia reducta	15.8	261	6.8	45.3	90.4	8.2	1.4
Aeneolamia varia	17.2	30.8	7.2	51.6	97.6	1.7	0.7
Mahanarva andigena	16.4	48.4	21.4	75.5	67.6	0.0	32.4
Mahanarva trifissa	17.0	44.2	6.8	64.6	74.0	22.7	3.3
Prosapia simulans	18.0	45.6	17.8	72.5	17.4	0.0	82.6
Zulia carbonaria	17.4	42.4	19.6	69.6	99.4	0.6	0.0
Zulia pubescens	14.3	38.0	18.4	61.5	40.4	0.4	59.2
Zulia sp. nov.	14.6	42.7	14.2	64.4	10.0	0.0	0.0

[1] Percent of eggs recovered in each substrate.
[2] Equivalent of egg + nymph + ½ adult

Table 16. Life table parameters for immature stages of *Z. carbonaria* on *B. ruziziensis*.

Stage	*ax*	*lx*	*dx*	*qx*	*kx*
Egg S1	118	1.000	0.093	0.093	0.042
Egg S2	107	0.907	0.000	0.000	0.000
Egg S3	107	0.907	0.008	0.009	0.004
Egg S4	106	0.898	0.000	0.000	0.000
Instar I	106	0.898	0.254	0.283	0.144
Instar II	76	0.644	0.025	0.039	0.017
Instar III	73	0.619	0.042	0.068	0.031
Instar IV	68	0.576	0.000	0.000	0.000
Instar V	68	0.576	0.042	0.074	0.033
Adult	63	0.534			
∑			0.466		0.271

ax =number of individuals at start of each life stage
lx =age-specific survivorship
dx =proportion of individuals dying in each life stage
qx =rate of mortality
kx = age-specific mortality ("killing power")

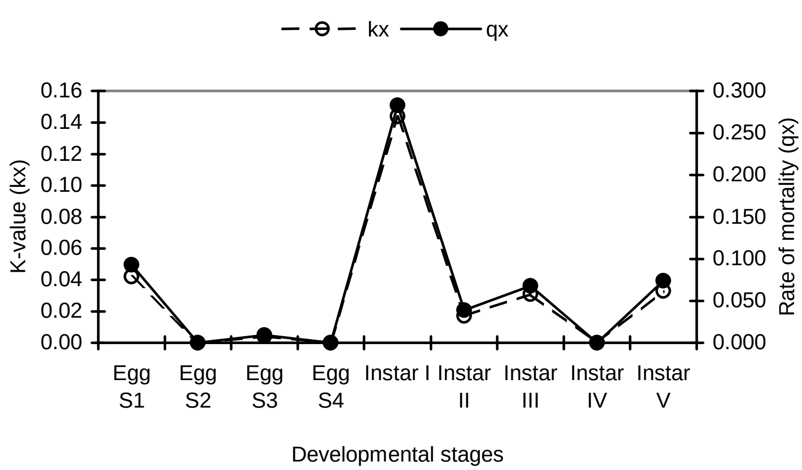


Figure 5. Rate of mortality and killing power for the immature stages of *Z. carbonaria* in laboratory conditions.

Mean (± S.E.) longevity of the females under the conditions of this study was 34.2±11.6 days (Figure 6). Mean lifetime fecundity was 125.8±82.9 eggs per female, with a fertility of 97.9% (Table 17). During the study oviposition rate peaked near the half life of the females where 16.6% of eggs were recovered (Figure 7). The demographic parameters generation time (T), net replacement rate (Ro), and intrinsic rate of growth (r_m) summarize the behavior of *Z. carbonaria* on *B. ruziziensis* (Table 18). The generation time was calculated as 78.2 days, net replacement rate (Ro) as 125.8 times; the intrinsic rate of natural increase showed that *Z. carbonaria* produces 0.06 females/day throughout the generation time. Finally, finite rate of multiplication was 1.06 females/day.

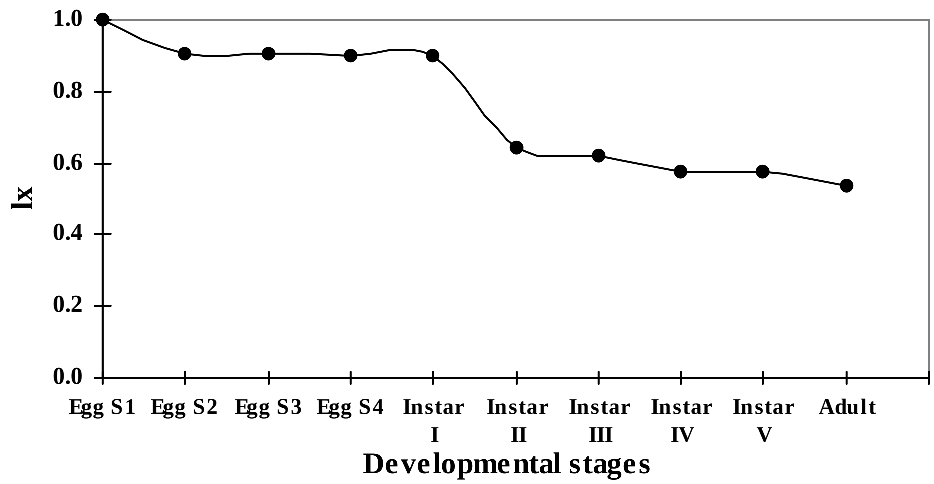


Figure 6. Survivorship curve of *Z. carbonaria* on *B. ruziziensis* under laboratory conditions

Table 17. Fecundity of *Z. carbonaria* females in under screenhouse conditions on *B. ruziziensis.*

Day	Total eggs laid	Mean ± S.E.	*n* (females)
3	1	0.03±0.18 a	30
6	88	3.03±8.22 b	29
9	264	9.43±16.00 d	28
12	392	15.08±13.60 e	26
15	626	25.04±13.74 f	25
18	401	16.04±13.34 e	25
21	405	16.88±11.05 e	24
24	431	17.96±17.43 e	24
27	434	17.67±11.31 e	24
30	330	13.75±9.37 e	24
33	231	10.04±7.25 d	23
36	134	6.38±5.53 d	21
39	27	3.86±4.81 c	7
42	16	4.00±2.16 c	4
45	3	3.00±0.00 b	1

Means followed by different letters are significantly different (P<0.05).

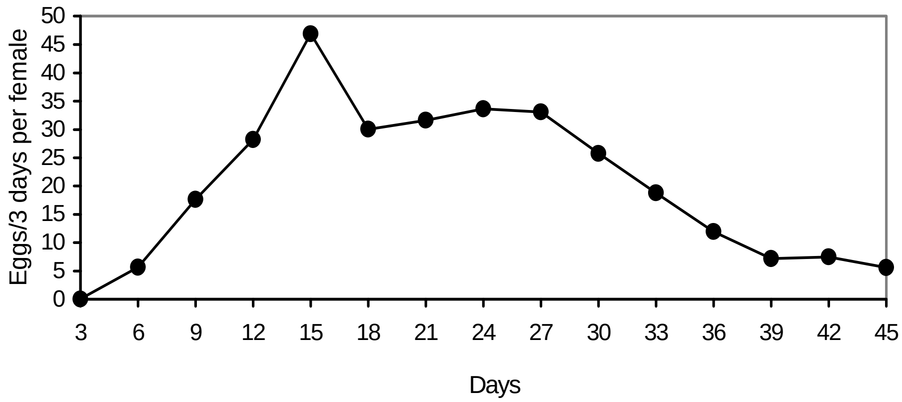


Figure 7. Oviposition rate of *Z. carbonaria* on *B. ruziziensis* under laboratory conditions

Table 18. Demographic parameters for females of *Z. carbonaria* on *B. ruziziensis*

Parameter	Value
Time to development (days)	59.83
Rate of survival (%)	53.40
Sex ratio (M:F)	1:1
r_m, Innate cpacity for increase (individuals/day)	0.063
Ro, Net replacement rate (progeny/individual)	125.77
T, Generation time (days)	78.20
λ, Finite rate of multiplication (females/day)	1.06

Discussion

The biological variation exhibited by Colombia's grass-feeding spittlebug complex is relevant to effective pest management. This new information both strengthens certain trends and broadens the known variation in this diverse and damaging pest complex of Neotropical forage grass and sugar cane.

The survival of *Z. carbonaria* from egg to adult was 21.3% less than that determined for *Prosapia simulans* under the same conditions (CIAT 2001). The highest mortality rate was suffered by the nymphal stage, 26.2% higher than the egg stage. The mortality measured for the immature stages of *Z. carbonaria* (46.4%) was higher than that measured in *P. simulans* where mortality in eggs and nymphs was 2.1 and 19.1%, respectively. The overall k-value for *Z. carbonaria* (0.27) was 0.14 times higher than *P. simulans*. The highest k-value for *Z. carbonaria* was for the nymphal stage while in *P. simulans* the greatest value was for the egg stage.

The longevity of females under these conditions exceeded by 13.8 days the longevity reported in previous biological studies (CIAT 2000). This difference is partially due to the provision of fresh material every 3 days in the case of the present study. Daily fecundity of females (3.7 eggs/day) is lower than that observed in *A. varia* (10.6 eggs/day, unpublished data). Fertility rates were similar to *M. andigena* (94.3%) and *P. simulans* (95.1%). The generation time (78.2 days) is considerably higher that that estimated from field population studies (63.2 days) partially because the latter did not include an estimation of the age of first reproduction and sequence of oviposition as included in the fertility table.

2.1.2 Phenology of spittlebug populations in the field

Contributors: Ulises Castro, Daniel Peck, Anuar Morales, Jairo Rodriguez, Oscar Yela (CIAT), Antonio Pérez (UniSucre), Guillermo León (C.I. La Libertad)

Rationale

In ecoregions with a defined rainy season, management of spittlebug populations may depend on suppression of the first population outbreak before the adults lay eggs and contribute to future generations or before they colonize previously uninfested areas of the farm (CIAT 2001). Predicting when and where on the farm the first focal outbreaks occur will help target control tactics and establish control strategies. Given the correspondence between the rainy season and the occurrence of spittlebug nymphs and adults, it should be possible to predict the arrival of the first generation based on the nature and timing of the first rains together with information on the determinants of diapause termination.

Predicting the first generation of insects in regions with a marked seasonality will depend on a detailed understanding of the behavior of the eggs in combination with population dynamics surveys and meteorological data. It is known that the abrupt outbreak of nymphs that occurs at the beginning of the rainy season is not related to the eclosion pattern of eggs under continually humid conditions in the laboratory. It is postulated that eggs terminating diapause are able to enter a state of post-diapause quiescence in which they are still tolerant to drought conditions (Output 2.1.3). These eggs are poised to continue developing and eclose once adequate conditions of moisture return. The nature of the return of the wet season rains, therefore, could determine the synchrony and magnitude of the first generation.

In Colombia, the spittlebug complex varies across different ecoregions, therefore any predictive models need to be tailored according to the diapause syndrome of the major species and the agroecological characteristics each region. In this output we summarize the status of a verbal and quantitative model to predict the time of arrival of the first spittlebug generation.

Materials and Methods

This research was carried out in three contrasting ecoregions of Colombia: the Cauca River Valley (Cauca), Caribbean Coast (Sucre) and Orinoquian Piedmont (Meta). The Cauca Valley features a bimodal precipitation pattern where the rainiest months are March-May and September-November with a mean of 1800 mm/yr; the predominant spittlebug species is *Zulia carbonaria*. The Orinoquian Piedmont is unimodal for precipitation where the rainiest months are March-November and mean annual precipitation is 2730 mm/yr; the predominant spittlebug species is *Aeneolamia varia*. The Caribbean Coast is unimodal for precipitation where the rainiest months are April-October, and mean annual precipitation is 1039 mm/yr; the predominant spittlebug species is *Aeneolamia reducta*. Specific site characteristics are described elsewhere (CIAT 2001).

Three plots of 0.5 ha were selected in each survey site in pastures of *Brachiaria* spp. (Cauc, Meta) and *Bothriochloa pertusa* (Sucre). Each plot was divided into four subplots to facilitate subsampling of the different spittlebug life stages. Surveys were initiated two months before the rainy season and were carried out twice weekly. Once the rainy season was underway surveys were increased to twice weekly until the end of the first population peak of nymphs and adults.

Nymph surveys comprised counts in two quadrats (0.25 m^2) per subplot, or 8 per plot, in which the occupants (nymphs, teneral adults) of all spittle masses were collected and identified to instar in the laboratory. This provided a measure of the absolute abundance of the different life stages and documentation of the progression of the spittlebug generation through the nymphal instars to teneral adults. Adult surveys comprised 50 sweeps with an insect per subplot, or 4 series of 50 per plot, between 09h00 and 11h00; spittlebug adults were collected and identified in the laboratory to sex and species. This provided a measure of the relative abundance of adults.

In this analysis repetitions were the years in which these population surveys were carried out in the three sites: 3 years for Cauca and Meta and 4 years for Sucre. For each region and year the population fluctuation data for nymphs and adults were analyzed in terms of cumulative insect-days (or cumulative area under the population curve) to control for variation in number and frequency of surveys. From these data we calculated the date of 50% cumulative insect-days as a measure of when that life stage reached peak abundance. This analysis allowed a comparison of the life cycle of each species and the precipitation pattern that originated the first generation. It was considered that the precipitation event that prompted the eclosion of eggs was that which occurred during the diapause egg stage S2; to identify this event we back-calculated from the nymph and adult population peak according to known information on duration of the life stages (Figure 8).

To predict the timing of the first generation, repetitions were the different survey dates and the respective percent cumulative insect-days. This relationship was best described as linear and therefore a simple linear regression model was applied to estimate time of arrival of the first generation in each region. Models were adjusted so that time 0 was considered as the date of precipitation that gave origin to the population.

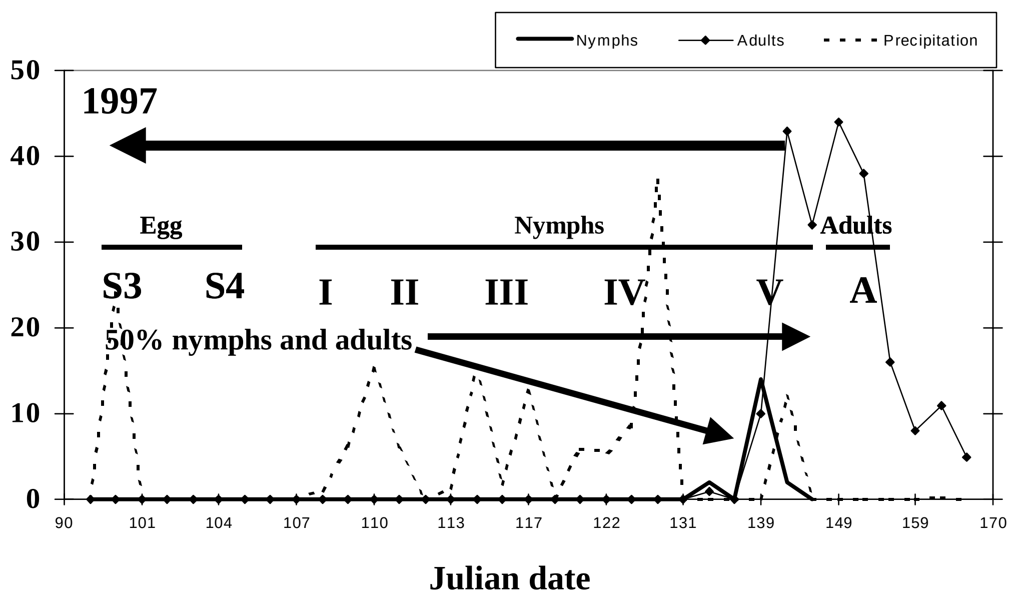


Figure 8. Precipitation and life cycle of *A. reducta*, Sucre, 1997.

Results

Using the dates of 50% cumulative insect-days to back-predict the precipitation events that promoted the population appeared adequate for all years in the two unimodal sites (Meta, Sucre). These precipitation events were predicted as being sufficient for promoting the synchronous eclosion of post-diapause quiescent eggs and thereby the first generation of nymphs. For example, in Sucre 1997, the 12.5 and 24.0 mm of rain that fell during a period of 4 days was probably responsible for promoting the first generation of nymphs and adults 28.7±6.7 and 39.3±11.2 later (Table 19, Figure 8). In Meta 1997, 34.0 and 108.9 mm of rain falling in a period of 4 days promoted nymph and adult peaks 32.3±7.3 and 39.3±11.2 days later. In Cauca the situation was more complicated because it was difficult to identify the precipitation events responsible for initial outbreaks. For that reason analysis of this site is considered more preliminary and it was necessary to analyze population data at the plot rather than farm level. It was estimated that in this region 35.8 and 75.0 mm of rain falling in a period of 4 days was sufficient to promote nymph and adult peaks 44.9±5.9 and 61.1±9.7 days later (Table 19).

Table 19. Date of 50% cumulative insect-days and precipitation events that originated first generation spittlebug populations in two regions.

Region	Year	Stage	50% cumulative insect-days (Julian date)	Precipitation: Julian date[1]	Precipitation: Amount (mm/day)	Difference
Sucre	1997	Nymph	138.3	100	24.0	38.3
		Adult	148.8			48.8
	1998	Nymph	134.6	106	15.3	28.6
		Adult	154.0			48.0
	2000	Nymph	145.5	121	12.5	24.5
		Adult	146.2			25.2
	2001	Nymph	148.4	125	13.7	23.4
		Adult	160.1			35.1
	Mean	Nymph				28.7±6.7
	± (S.E.)	Adult				39.3±11.2
Meta	1998	Nymph	56.5	32	75.1	24.5
		Adult	72.7			40.7
	2000	Nymph	117.1	84	108.9	33.1
		Adult	121.5			37.5
	2001	Nymph	107.2	68	34.0	39.2
		Adult	115.0			47.0
	Mean	Nymph				32.3±7.3
	± (S.E.)	Adult				41.7±4.83
Cauca	1999 P1	Nymph	47	10	75.0	37
		Adult	70			60
	1999 P2	Nymph	60			50
		Adult	71			60
	1999 P3	Nymph	51			41
		Adult	57			47
	2000 P1	Nymph	382*	335	35.8	47
		Adult	413*			78
	2000 P2	Nymph	389*			54
		Adult	403*			68
	2000 P3	Nymph	375*			40
		Adult	390			55
	2001 P3	Nymph	391*	346	68.0	45
		Adult	406*			60
	Mean	Nymph				44.9±5.9
	± (S.E.)	Adult				61.1±9.7

[1]Back-calculated according to the known life cycle
*Includes 365 days of previous year

The simple linear regression model to estimate the arrival of nymphs and adults in each region is summarized in Table 20 and Figure 9. There was a statistically significant positive correlation between survey day and cumulative insect-days for all three regions ($P<0.01$). For *A. reducta* in Sucre, the correlation (Pearson correlation coefficient, r) was 0.86 for nymphs (n=40) and 0.77 for adults (n=50).

For *A. varia* in Meta the correlation was 0.86 for nymphs (*n*=32) and 0.84 for adults (*n*=30). For *Z. carbonaria* in Cauca, the correlation was 0.90 for nymphs (*n*=57) and 0.80 for adults (*n*=72).

Table 20. Model to predict date of arrival of first generation nymphs and adults given known causative precipitation date. T = days to appearance, X = percent of the population expected.

Region	Life stage	Formula	Correlation (r)	50% cumulative insect -days (Julian date)
Sucre	Nymph	T=10.2+35.2(X)	0.86	27.8
	Adult	T=18.9+39.7(X)	0.77	38.9
Meta	Nymph	T=9.7+44.8(X)	0.86	32.1
	Adult	T=19.9+41.4(X)	0.84	40.6
Cauca	Nymph	T=16.7+56.6(X)	0.90	45.0
	Adult	T=25.2+58.2(X)	0.80	54.3

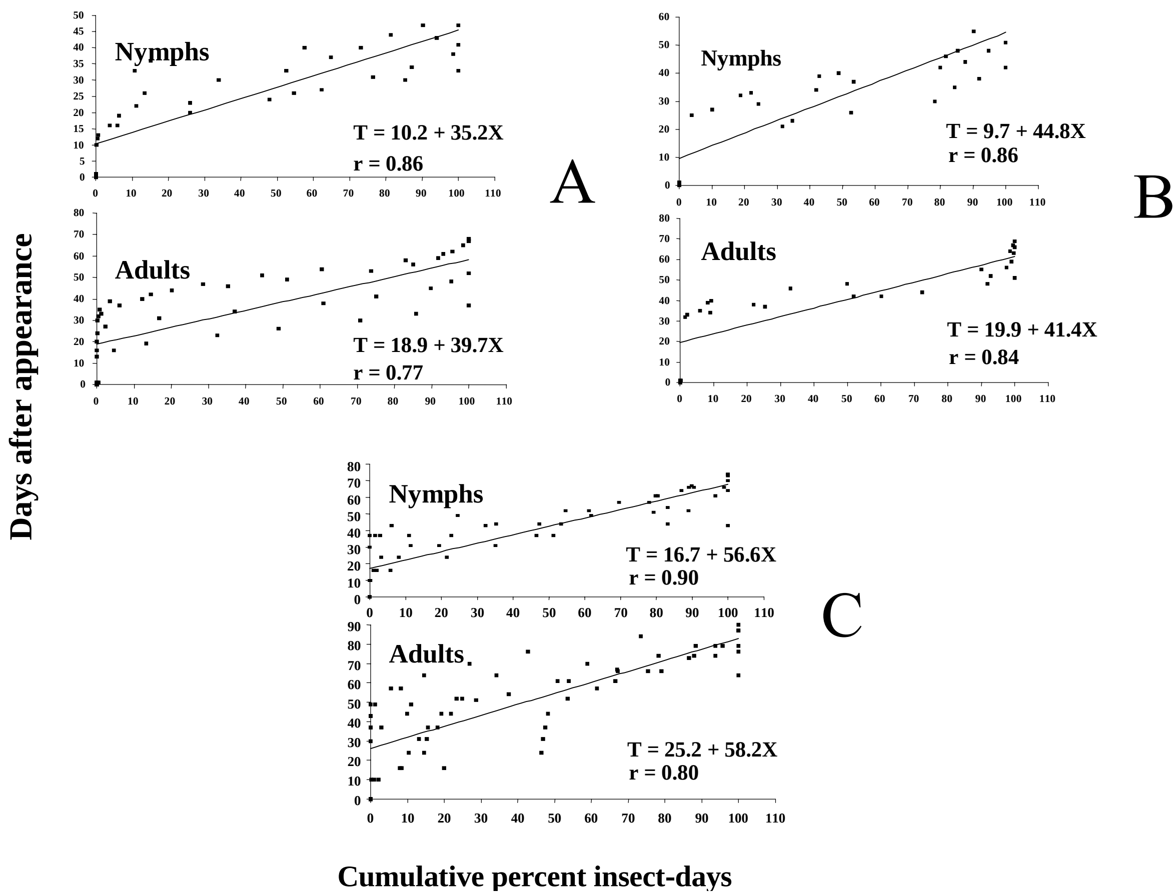


Figure 9. Prediction of the first generation of spittlebugs in Sucre, *A. reducta* (A); Meta, *A. varia* (B) and Cauca, *Z. carbonaria* (C).

Discussion

Information obtained previously (CIAT 2000, Output 2.1.3) indicates that the marked seasonality in Sucre and Meta causes population synchrony in early season spittlebug populations given the number of post-diapause quiescent eggs ready to continue their development upon arrival of the first rain. The difference between the date of 50% cumulative insect-days of appearance of the nymphs and adults and the date of the first major rainfall event can explain the arrival of the first generation population peak based on the duration of the egg life stages S3 and S4 and the five instars and at least a third of the adult longevity for all three species in all three regions. The information obtained suggests that *A. reducta* responds to 12.5-24.0 mm and *A. varia* to 36.4-112.0 mm of rain. It has been previously determined for *A. varia* that 25 mm was sufficient to promote the first population outbreak (King 1975). Given the bimodal seasonality in Cauca, it will be necessary to broaden the studies to have more certainty in defining the day in which the amount of precipitation was sufficient to promote population outbreak in the case of *Z. carbonaria*.

The quantitative model for predicting the appearance of the three species in the different regions is important because this tool could reduce the need for population surveys or complicated scouting. If it is possible to reduce the abundance and diffusion of the first generation adults the impact from subsequent generations will also be reduced as will economic losses in pasture production, especially in highly seasonal areas such as the Caribbean Coast and Orinoquian Piedmont of Colombia. This prediction will have its greatest impact in combination with control tactics directed at the nymphs before they lead to the highly mobile and injurious adult stage of the first generation.

With all the information collected and analyzed during the development of this study and parallel studies (Output 2.1.3), the following is a verbal model to describe spittlebug phenology. This model should be considered a general theory to explain the population synchronization of spittlebugs in seasonal agroecosystems.

In the Neotropics, grass-feeding spittlebugs synchronize their life cycle with the season most appropriate for their development and reproduction. After an extended dry season the first rains of the wet season stimulate the regrowth of new plant tissue which offers abundant sites that are adequate for nymphal development. Once the insect has established in the field under favorable humid conditions the first generation of females lay eggs which are immediately developing (not diapausing) to take advantage of the favorable food sources and habitat by producing a second generation. With the progression of the wet season the incidence of diapausing eggs increases to up to100% in the last generation in anticipation of the imminent dry season.

It is the immature stage (nymph) of the later generations that is responsible for perceiving the environmental signals or “token stimuli” that indicate that the dry season. The response to this challenge is egg diapause. Once the nymphs have transformed to the adult stage, they are conditioned to lay a higher proportion of diapausing eggs so that the next generation may survive the dry season and return during the next rainy season.

In the temperate zones, photoperiod and temperature are the major regulators of diapause induction. In tropical regions there is less variation in photoperiod and temperature and therefore other factors may be more important in diapause regulation. Both phenology of the plant (greater age, poorer nutritional quality) and soil humidity (drought stress) could be perceived by nymphs and lead to adults that lay eggs with a higher incidence of diapause. Other factors such as temperature should also be explored for their role as token stimuli.

The insect survives the long dry period as eggs temporarily delayed in developmental stage S2, tolerant to drought. Diapause and quiescence are natural phenomena where development is suspended, thereby offering a defense against the adverse environment. The difference is that eggs in diapause cannot respond directly to the return of wet conditions, while quiescent eggs are able to continue development in direct response to favorable conditions.

In the case of *A. varia*, the majority of eggs in the dry season are in diapause. As the dry season progresses, more and more eggs terminate diapause. The same conditions of drought accelerates this process as shown after 15 and 30 days of complete dryness (Output 2.1.3). When diapause termination occurs under unfavorable dry conditions, the eggs of *A. varia* enter post-diapause quiescence. In this phase, the eggs remain tolerant to drought stress but they are poised to continue their development and eclose in direct response to higher levels of soil humidity. Therefore with the progression of the dry season the proportion of diapause eggs decreases while the proportion of post-diapause quiescent eggs increases.

Few days after the rains the nymph population appears in the field, often in a highly abrupt and synchronous fashion. The life stage responsible for this synchrony is the quiescent eggs. The model to predict the arrival of the appearance of the first generation based on rainfall patterns is summarized in Table 20. A large and highly synchronous outbreak would therefore be the result of a long dry season (diapause terminated in the majority of the eggs) ended by an abrupt arrival of rains that soak the pasture completely (stimulating the continued development of quiescent eggs en masse). In contrast, it is predicted that a small and asynchronous initial outbreak would be the results of a shorter dry season with interrupted rains going into the wet season.

2.1.3 Determinants of egg diapause

Contributors: Ulises Castro, Oscar Yela, Daniel Peck

Rationale:

The influence of moisture on the induction or termination of diapause has been little studied . The function of moisture as a regulator in the termination of diapause has been documented in insects of the Tropics where temperature and moisture appear to replace photoperiod as the primary regulator of diapause. Field studies on certain species of cercopids have shown a relationship between the arrival of the rainy season and the appearance of the insect's first population peak in the field. It has been suggested that this population synchrony is achieved because in a stage of post-diapause quiescence eggs are capable of responding to the new humid conditions to terminate their development and eclose, thereby contributing to the first generation of nymphs. At least in the Brazilian species *Deois flavopicta*, diapausing eggs enter a physiological period of post-diapause quiescence if conditions are not favorable upon diapause termination. Once such quiescent eggs are subjected to moist conditions, however, development continues and eggs eclose.

To understand and predict the synchrony and timing of the arrival of the first generation outbreak, it is necessary to elucidate the environmental factors that could serve as post-oviposition signals in the termination of egg diapause. For the widespread Colombian species *Aeneolamia varia*, it is predicted that a period of drought, like that experienced by the eggs in the dry season, could serve as a signal to accelerate the termination of diapause and that eggs then enter a state of post-diapause quiescence. This physiological stage would permit the synchronized eclosion of field populations in response to the return of the wet season rains. In this study we sought to determine the influence of drought as a post-oviposition signal in the regulation and termination of diapause in eggs of *A. varia*.

Materials and Methods

The diapause eggs used in this experiment were obtained from a colony of *A. varia* maintained at CIAT that originated 1-2 generations previously from field-caught adults in Villavicencio, Meta. Earlier studies showed that *A. varia* from this region are capable of diapause, increasing the proportion of diapausing eggs as the dry season approaches.

The experiment was performed in two trials. Eggs in the first trial came from females reared in June 2001 while eggs in the second trial came from females reared between December 2001 and January 2002. For each trial, a completely random design was used with three repetitions of the following three treatments: control (eggs continually moist), 15 d drought (eggs exposed to 15 days of dryness and then remoistened), and 30 d drought (eggs exposed to 30 days of dryness and then remoistened). The experimental units were large petri dishes (15 cm diameter, 2 cm height) lined with filter paper, sealed with parafilm and kept under controlled conditions (27ºC, 100% RH, total darkness). Eggs were evaluated twice weekly with the aid of a stereoscope to record the number of eclosed and damaged/dead eggs. Differences in treatments for mortality and days to eclosion were tested with an ANOVA. Trials were analyzed separately due to differences in egg origin and eclosion patterns.

Results

In both trials the eclosion curves showed an initial peak for the treatments under drought stress (Figure 10). This initial peak was followed by a period of no eclosion corresponding to the period of drought stress (15 and 30 days). Eggs did not resume development and eclose until after the return of moist conditions. In Trial 2, three defined eclosion peaks were observed in experimental eggs, corresponding in chronological order to the control, 15 d and 30 d treatments. In Trial 1, eggs submitted to drought eclosed in a similar pattern of one major peak in contrast to the control where eclosion occurred sporadically without evidence of synchronized peaks.

No differences in the mortality of diapausing eggs were detected between the two drought treatments (Table 21). These treatments had a higher mortality than the control, varying from 22.9 to 71.8%. The mortality in the control was 5.1 and 3.9% for a trials 1 and 2, respectively.

Table 21. Mortality of diapausing *A. varia* eggs under drought stress.

Treatment	Mortality (%)		Mean days to eclosion	
	Trial 1	Trial 2	Trial 1	Trial 2
0 days drought (control)	5.1 b	3.7 b	75.3 a	16.4 c
15 days drought	22.9 a	54.5 a	54.0 b	32.0 b
30 days drought	69.7 a	71.8 a	51.0 c	50.0 a

For each trial, means followed by the same letter were not statistically different ($P<0.05$).

For the variable of time to eclosion, in Trial 2 the mean for diapausing eggs held under moist conditions was 16.1 days (Table 21). After terminating diapause the eggs continued with their post-diapause development without a period of quiescence given the conditions of adequate humidity. In the drought treatments, the period of eclosion was delayed by approximately the duration of the drought period in comparison to the control.

Compared to Trial 2, Trial 1 eggs were under longer diapause. The eclosion patterns showed a long and relatively constant pattern of eclosion with a mean of 75.3 days for the control (Figure 10, Table 21). In addition, the drought-stressed eggs eclosed earlier than the control, the 30 d earlier than 15 d. Compared to the control, the eclosion curve showed a peak of eclosion a few days after return of moisture, which did not appear in the control eggs.

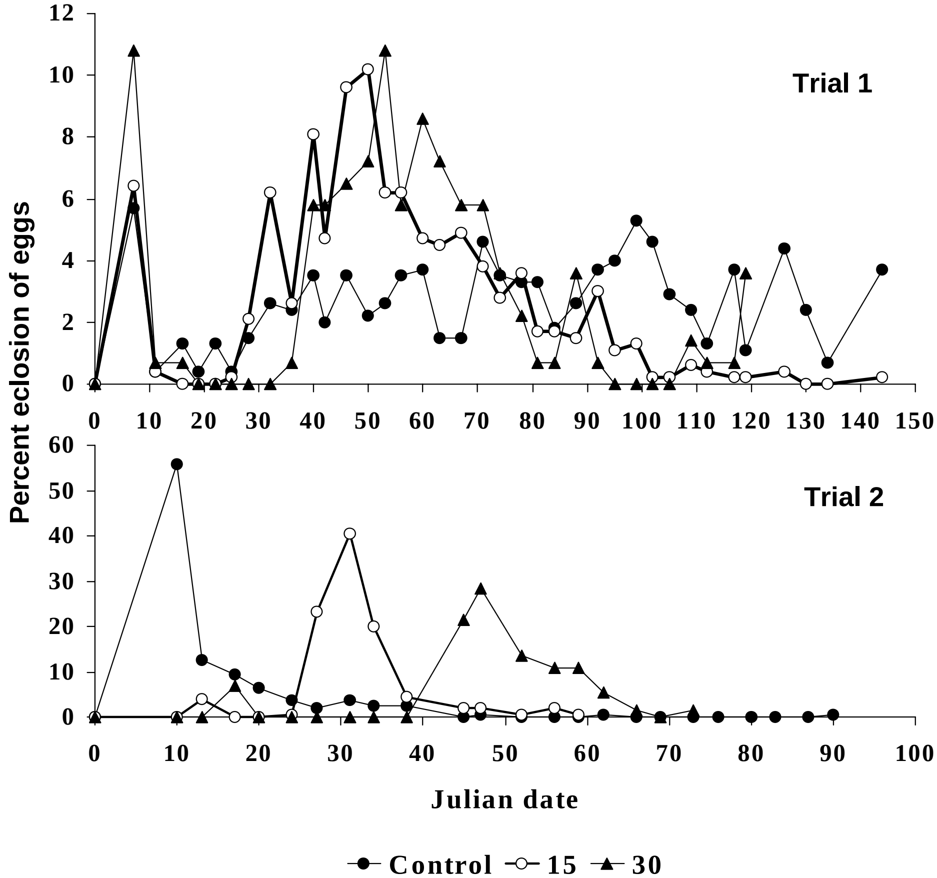


Figure 10. Eclosion pattern of diapausing *A. varia* eggs under drought stress.

Discussion

The initial peak observed in the eclosion curves is probably attributed to eggs already in post-diapause development that were able to continue development and eclose despite the dry conditions. After this brief period, no other eclosion was observed during the period of drought stress. The higher mortality in drought-stressed versus control eggs is attributed to the severity of the drought conditions and to some eggs being in early post-diapause development; in this phase the eggs are sensitive to the lack of moisture because water is required for continued development.

In terms of time to eclosion, Trial 2 showed an early and synchronized pattern of egg eclosion due to the period of drought. According to the control, during the period of drought the majority of eggs would have terminated diapause, completed post-diapause development and eclosed. Under drought conditions, however, a proportion of eggs survived the drought-stress and delayed development until the return of moist conditions. Eggs that terminated diapause while under drought stress entered a drought-resistant post diapause quiescence, delaying continued development until the return of moist conditions.

The same evidence was obtained from Trial 1. In eggs subjected to drought stress, development was delayed until return of humid conditions at which point development proceeded once again. In addition, this Trial offered evidence for accelerated diapause termination. Besides confirming the idea of post-diapause quiescence with drought-tolerance, these results indicate that a period of drought accelerates the termination of diapause. The eclosion peak caused by the return of moist conditions is the result of a large proportion of eggs that had terminated diapause and entered post-diapause quiescence during the drought period. This proportion was higher in the 30 d drought eggs indicating that a longer period of drought accelerated diapause termination even more. In the Trial 1 eggs that had a longer diapause period, eggs actually eclosed earlier when subjected to drought than when kept under continually moist conditions.

The differences in time to eclosion between the eggs in Trial 1 and 2 could be attributed to the origin of the adults that contributed these eggs. Trial 2 eggs came from generations posterior to the adults that provided the eggs for Trial 1. Field studies have shown that the duration of diapause varies among generations and increases in the generations prior to the arrival of the dry season. Although early generations have an decreased incidence of diapause compared to later generations, the duration of this diapause is longer.

2.1.4 Taxonomy and distribution of spittlebug species

Contributors: Daniel Peck, Jairo Rodríguez

Rationale

For several reasons our taxonomic foundation for the neotropical grass-feeding spittlebugs is weak. First, a high degree of intra- and inter-specific variation in color patterns complicates species identification. Second, the male genitalia of relatively few species have been described so this key character is not available for species determinations. Third, there are few taxonomists working in the Cercopidae and there are very few regional taxonomic keys. As a result, getting correct species determinations is difficult for researchers working in this pest system. In this output we summarize new information on the taxonomic identity of certain economically important spittlebug species.

Results and Discussion

Identity of *Mahanarva trifissa*. A summary of the identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador (CIAT 2000) left unclear the status of two species, *Mahanarva* sp. from the Amazonian piedmont of Colombia and Ecuador, and *Zulia* sp. from the south Pacific Coast of southern Colombia and northern Ecuador. After a visit to the British Museum of Natural History (London, UK) and discussions with the Homoptera specialist M. Webb, additional information was obtained on the identity of these two species. The undescribed species of *Mahanarva* reported from Caquetá, and previously thought to be a new species, is identified as *Mahanarva trifissa* (Jacobi). This species was originally described as *Tomaspis trifissa* by Jacobi in 1908 based on a single specimen collected in Peru. After studying the type specimen loaned to the British Museum, we determined that the form of the male genitalia, profile of the rostrum and color pattern fell within the range of variation exhibited by specimens of *Mahanarva* sp. from Caquetá and different regions of Ecuador. However, the color pattern expressed by the Peru type is uncommon, exhibited by only one individual of the several dozen observed from Colombia and Ecuador. The locality of the type specimen conformed to the lowlands tropics of the interior (Amazonian Piedmont). This species will be transferred to the genus *Mahanarva* as part of a taxonomic review pending publication by the British Museum.

In a pending CIAT publication on the biology and habits of this species (CIAT 1999) we will describe the color pattern variation.

Identity of *Zulia* sp. The status of *Zulia* sp. nov. is still unclear. Whether it is a new species distinct from *Zulia vilior* (Costa Rica, Panama) will depend on more studies of the degree of variation in populations from those two countries and those in Colombia and Ecuador.

Identity of new sugar cane pest. In collaboration with Cenicaña, we investigated a recent report of spittlebugs reaching injurious levels on panela sugar cane in Risaralda (September 2002). This species has been initially identified as *Mahanarva bipars* (Walker). As far as we know this species has not yet been reported from Colombia, has not yet been cited as feeding on sugar cane or other graminoids, and has not yet been studied biologically. Similar to *Mahanarva andigena* in Nariño, spittle masses are aerial (at the base formed by leaf axils). This insect is being managed locally by removing old leaves from the stem and using insecticides (chorpyrifos). An initial summary of this report is being prepared for publication including a review of known host plants and distribution, taxonomic status, identification, color pattern variation and pest status.

Identity of Venezuelan spittlebug complex. We received specimens from Venezuela for identification. Specimens from Barinas, Venezuela were identified as *Aeneolamia varia* and *Aeneolamia reducta*. The pest complex seems to be similar to the Colombian Llanos (mostly *A. varia,* some *A. reducta* and apparently some *Zulia pubescens*). Spittlebugs are increasing problems in this region of Venezuela and current outbreaks are more severe than anything we have seen over the last five years in our Colombian sites.

Identity of Guatemalan spittlebug complex. A long series of specimens from 28 sites in Santa Lucía Cotzumalguapa, Guatemala was received from collaborators of Cengicaña. The two species present in forage grasses and sugar cane were confirmed as *Prosapia simulans* and *Aeneolamia postica*. *Prosapia simulans* comprised 3.4% of the examined specimens, detected in 10/28 sites. In sites where this species was collected it comprised 3.3-16.7% of the adults present with the exception of one site where they comprised 100%. Various color patterns were observed; males tended to have 3 lateral yellow bands dorsally (one on the posterior edge of the pronotum, two across the wings) while females tended to be darker due to the reduction in the bands. With little exception this species is longer and wider that *A. postica*. *Aeneolamia postica* comprised 96.6% of the examined specimens, detected in 27/28 sites where it comprised 83.3-100% of the adults present. Like *P. simulans* it has two dorsal lateral bands but it differs in having a third anterior band in the form of a “V” along the border of the scutellum; in only a few cases were these bands reduced. *Aeneolamia postica* presented three general color morphs, red comprising 2.5% of specimens, orange 1.6% and yellow 95.8%.

Activity 2.2 IPM components for spittlebug management

Highlights

- The population phenology of *Prosapia simulans* in the Cauca Valley was documented
- Select isolates of fungal entomopathogens were evaluated on nymphs and adults of *P. simulans*, confirming significant variation in pathogenicity across spittlebug species
- Field studies in Caquetá failed to detect an effect of fungal entomopathogens on spittlebug populations but highlighted strategies to guide future advances

2.2.1 Short-term mass rearing of different spittlebug species

Contributors: Anuar Morales, Oscar Yela, Ulises Castro, Jairo Rodríguez, Daniel Peck

Six spittlebug species (*Aeneolamia reducta*, *A. varia*, *Mahanarva andigena*, *Prosapia simulans*, *Zulia carbonaria*, *Z. pubescens*) were reared in the screenhouse at CIAT to support research (Table 22). Mass-rearing colonies were established according to an improved box design, previously tested and described (CIAT 1999) while small-scale colonies were maintained with the traditional pot methods. Colonies were used to support biological studies of *M. andigena*, *Z. carbonaria* and *Z. pubescens*; diapause studies on *A. varia*; and evaluations of fungal entomopathogens for *A. reducta*, *P. simulans*, *Z. carbonaria* and *Z. pubescens*.

Table 22. Summary of spittlebug colony maintenance activities.

Species	Months maintained at CIAT (2001-2002)												Rearing scale		Purpose of studies	
	N	D	J	F	M	A	M	J	J	A	S	O	Large[1]	Small[2]	Biology	Entomopathogens
A. reducta	X	X	X										X			X
A. varia	X	X	X	X	X	X	X	X	X	X	X	X		X	X	
M. andigena	X	X	X	X	X	X	X	X	X	X	X	X		X	X	
P. simulans	X	X	X	X	X								X	X	X	X
Z. carbonaria		X	X	X			X	X	X	X	X		X	X	X	X
Z. pubescens			X	X	X								X			X

[1]Improved mass-rearing design
[2]Traditional small scale pot design

2.2.2 Maintenance of the entomopathogen collection

Contributors: Anuar Morales, Rosalba Tobón, Daniel Peck

The fungal entomopathogen collection was established in 2000 as a resource for studies on biological control of major pest complexes. Maintaining and strengthening this collection is vital for advancing non-toxic alternatives to insecticides and other effective tactics as components of integrated pest management. The three main activities related to this ceparium are (1) isolation, entry and storage of new isolates, (2) viability tests, propagation and reactivation, and (3) multiplication for laboratory and field studies on virulence and pathogenicity.

In 2002, 26 new accessions were added to the collection for a total of 184. Of this total, 82 were originally isolated from spittlebugs (Homoptera: Cercopidae), 29 from white grubs (Coleoptera: Scarabaeidae), 63 from whitefly (Homoptera: Aleyrodidae) and burrower bug *Cyrtomenus bergi* (Hemiptera: Cydnidae) and 10 from other hosts.

In 2002 this germplasm bank was a vital resource for studies on (1) the characterization of select isolates for field trials to manage grass-feeding spittlebugs (Outputs 2.2.3, 2.2.4) and (2) evaluation and biological characterization of isolates against life stages of the whitefly *Aleurotrachellus socialis*.

2.2.3 Screening fungal entomopathogens for virulence to spittlebugs

Contributors: Anuar Morales, Rosalba Tobón, Oscar Yela, Daniel Peck

Rationale

Four isolates were selected from CIAT's fungal entomopathogen collection for experimental field trials designed to test application techniques. These isolates are the three *Metarhizium* and one *Paecilomyces* strains screened from 49 isolates as the most virulent to adults of *Aeneolamia varia* (CIAT 2000). CIAT 054 and CIAT 055 are *Metarhizium* sp., CIAT 007C *Metarhizium anisopliae* and CIAT 009 *Paecilomyces farinosis*. Before deploying in the field, these isolates must be characterized for their biological and virulence activity on different species and life stages of spittlebugs. Variation in virulence among adults of four species (*A. reducta, A. varia, Z. carbonaria, Z. pubescens*) was described elsewhere (CIAT 2001) as were the results of studies to determine the LC_{50} and LC_{90} on nymphs of *A. varia*. Here we summarize the results obtained for adults and nymphs of *P. simulans*.

Materials and Methods

Evaluation methods for adults and nymphs were based on previously established protocols (CIAT 2000). For adults, evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (15 cm diameter) covered by acetate cylinders (40 cm tall, 15 cm diameter). These plants were infested with 10 adult tenerals (<24 hours old) obtained from colonies maintained at CIAT. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 conidia/ml) with an airbrush and compressor (10 PSI). All four isolates were evaluated. Ten repetitions (pots) were evaluated for each isolate and a control (water with tween at 0.05%). After spraying, plants and insects were maintained in a growth chamber (27±2°C, RH 80±10%).

Virulence was evaluated 5 days later when all insects were scored as alive, dead, and dead with evidence of mycosis. Dead insects with no visible signs of fungus attack were stored in petri dishes with moist filter paper for 3-4 days to ascertain whether they were infected with fungus. Differences were evaluated with an ANOVA and Tukey multiple range test.

For nymphs, evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 weeks after planting with *B. ruziziensis*, surface roots were sufficiently established for nymph development and egg infestation. Eggs of *P. simulans* about to hatch were prepared for treatments and infestation by placing 10 on each of 10 small pieces of filter paper in a petri dish that corresponded to one treatment. Nine different concentrations of conidial suspensions ($1x10^4$, $5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $5x10^6$, $1x10^7$, $5x10^8$, $1x10^9$ conidia/ml) were prepared for the three *Metarhizium* isolates (CIAT 007C, CIAT 054, CIAT 055) with a control (water and tween at 0.05%). Applications were made on the substrate before infestation and on the eggs in petri dishes before infestation. An airbrush and compressor (10 PSI) were used at a volume of 1 ml for substrate and <1 ml for direct egg application.

Plants were maintained in the greenhouse until evaluation of mortality 30-32 days after infestation. During this period, plants were fertilized twice (just before and 15 days after infestation) with urea at 2g/l. There were ten repetitions per treatment. Mortality data were analyzed with Probit (SAS).

Results

Results are shown for *P. simulans* alongside results previously obtained from identical studies in other species (CIAT 2001). The selected isolates of *Metarhizium* did not give good control of *Prosapia* or *Zulia*, compared to *Aeneolamia* (Figure 11). The most virulent isolate against *P. simulans* was CIAT 054 with 35.6% mortality.

The mortality recorded at the different concentrations demonstrated virulent activity of each isolate on nymphs of *P. simulans*. As expected, mortality increased with concentration (Figure 12). Probit analysis showed low X^2 values but X^2 probabilities a little high (Table 23). The lowest LC_{90} was obtained for CIAT 054 ($2.7x10^8$ conidias/ml) followed by CIAT 007C (3.4×10^8 con/ml) and CIAT 055 (6.4×10^8 con/ml).

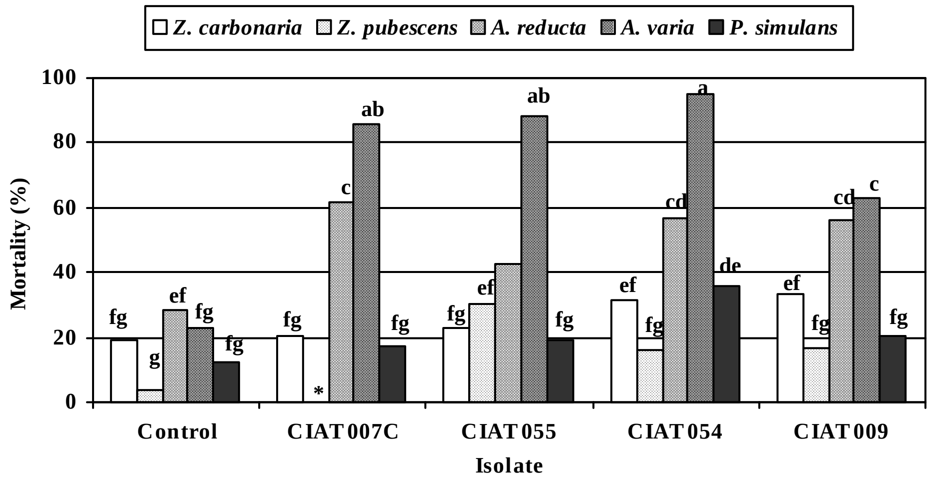


Figure 11. Mortality (absolute percent) of four isolates of fungal entomopathogens on five spittlebug species. Means followed by different letters are significantly different at P<0.05. * CIAT 007C was not evaluated on *Z. pubescens*.

Table 23. Probit analysis of mortality caused by three isolates of fungal entomopathogens on nymphs of *P. simulans*.

Isolate	*n*	LC_{50} (95% CI)	LC_{90} (95% CI)	X^2	Prob X^2	b±S.E.
CIAT 054	765	$1.01x10^7$ ($5.7x10^5$-$2.7x10^7$)	$2.7x10^8$ ($7.4x10^7$-$1.3x10^{10}$)	10.9	0.092	0.39±0.05
CIAT 055	637	$1.58x10^5$ ($5.9x10^5$-$3.5x10^7$)	$6.4x10^8$ ($1.4x10^8$-$1.6x10^{14}$)	7.06	0.219	0.34±0.06
CIAT 007-C	593	$1.04x10^5$ ($4.4x10^3$-$1.1x10^6$)	$3.4x10^8$ ($6.1x10^7$-$3.4x10^9$)	3.8	0.429	0±0.17

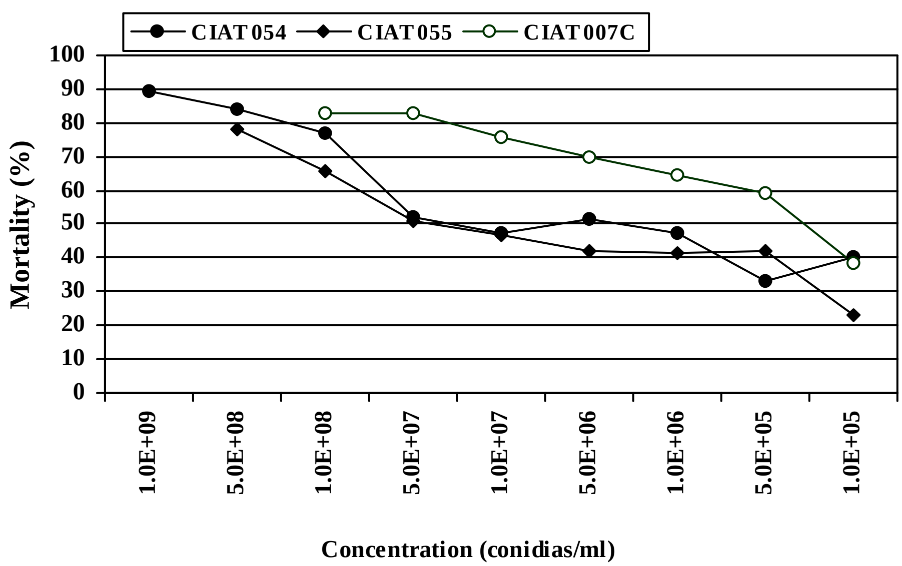


Figure 12. Mortality in *P. simulans* caused by of three fungal entomopathogen isolates at different concentrations.

Discussion

It is important to determine the LC_{90} to establish an appropriate concentration for field applications. This reduces the risk of applying material that is too low in concentration to have any effect in the field or too high that material and production time is wasted.

These results confirm that virulence of fungal entomopathogen strains varies among spittlebug species. Deploying these pathogens as agents of biological control therefore depends on an understanding of the species complex in the area where control is desired, selecting isolates specific to spittlebug species, and reassessing the broad effectiveness of commercial products. On the other hand, results indicate that the diverse collection of isolates in CIAT's ceparium probably has strains highly virulent to species other than *A. varia*, which up to this point has been used as the model species for developing evaluation methodologies. The most efficient screening process might therefore be evaluating a diversity of isolates to the particular spittlebug species of interest, rather than using preselection (with a model species such *A. varia*) with subsequent confirmation of high control on other species.

2.2.4 Field evaluation of spittlebug entomopathogens in two contrasting regions

Contributors: Anuar Morales, Jairo Rodríguez, Ulises Castro, Oscar Yela, and Daniel Peck (CIAT); Daniel Corradine, Germán Chacón, Fabio Obregón, and Orlando Narváez (Universidad de la Amazonía)

Rationale

In general, previous attempts to evaluate the efficiency of fungal entomopathogens as biological control agents of spittlebugs in pastures have focused on laboratory assays. The few that have gone to the field have demonstrated highly variable and low levels of control due to a variety of factors including poor evaluation and application techniques. Aspects such as the number of applications and the timing of applications in relation to phenology of the life stages have received no special attention.

To seriously evaluate the potential of fungal entomopathogens as an alternative for managing pasture spittlebugs, we are combining a detailed knowledge of the biology and phenology of spittlebugs with a series of studies to collect, screen, characterize, and formulate select isolates for deployment in field trials. In previous work (CIAT 1999, 2000, 2001) we have established and strengthened a collection of fungal entomopathogens at CIAT that currently houses 84 isolates of 10 fungal genera originating from almost every region in the country where the pest has been detected. In addition, an improved design for a mass rearing colony of *A. varia* was developed to provide adults of this species and others for evaluations. Finally, a reliable and rapid method to evaluate fungal entomopathogens against spittlebug nymphs and adults has been established. With these protocols we have information on the lethal concentration of the two most promising isolates and variation in their efficiency on other spittlebug species.

The objective of this phase of the project is to establish an application methodology for fungal bioinsecticides. In this report, we summarize the field trials established in two contrasting ecoregions of Colombia, the Amazonian Piedmont and the Cauca River Valley to evaluate the effect of timing and frequency of applications.

Materials and Methods

The Amazonian Piedmont ecoregion (Caquetá) is continuously humid, corresponding to presence of spittlebug nymphs and adults throughout the year with little population synchrony. In this site the number of applications required to achieve an effect was evaluated. The Cauca River Valley ecoregion (Valle del Cauca) is a highly seasonal site with bimodal precipitation and here spittlebug nymphs and adults are present only during the rainy months but with high population synchrony. In this site the timing of the applications in relation to the insect's life cycle was evaluated. The premise is that the diverging environmental conditions of these two ecoregions will require different strategies and control tactics for management of spittlebugs in pastures.

Caquetá. Studies in Caquetá were carried out in collaboration with four undergraduate thesis students from the Universidad de la Amazonía. Five plots of 1200 m^2 were selected and marked at the C.I. Macagual of Corpoica. Each plot was in a separate pasture of predominately *Brachiaria decumbens* forage grass with a history of spittlebug attack. Each plot was subdivided into subplots (100 m^2) for application of treatments. Adults were evaluated weekly with 20 sweeps of an insect net per subplot while nymphs were counted in two 0.0625-m^2 quadrats. In the laboratory nymphs were scored to instar and adults to sex and species. Population surveys began 23 March 2001 and ended 10 May 2002. Applications began 22 September 2001 and were made every 2 weeks until 8 February 2002. Treatments were in a completely randomized block design with five repetitions and are summarized in Table 24.

Table 24. Experimental treatments applied in C.I. Macagual, Caquetá to test the effect of application frequency on spittlebug abundance.

Treatment code	Treatment	Frequency[1]
1a	Cepa CIAT 054	2
1b	Cepa CIAT 054	1
1c	Cepa CIAT 054	0.5
2a	Cepa CIAT 007-C	2
2b	Cepa CIAT 007-C	1
2c	Cepa CIAT 007-C	0.5
3a	Chemical control	2
3b	Chemical control	1
3c	Chemical control	0.5
4	Absolute control	0

[1]Frequency is the number of applications per month

Applications were made with a traditional backpack sprayer fitted with a curtain nozzle. The volume of application was set at 2 liters per subplot. The two isolates were CIAT 054 (*Metarhizium* sp.) and CIAT 007C (*Metarhizium anisopliae*), selected from among 49 isolates as the most virulent to adult *A. varia*. Concentration of the suspension was 8.95×10^7 and 3.61×10^8 conidias/ml for the isolates CIAT 054 y CIAT 007C, respectively, equivalent to the LC_{90} for *A. varia* determined in greenhouse studies (CIAT 2001). The chemical control was Malathion applied at 1.5 liters/ha. Efficiency of the isolates was calculated as the cumulative area under the population curve for adults and nymphs for each treatment over the months of the study. The slope of the line was calculated before and after the start of the treatments. Differences among treatments in the cumulative area under the curve and the slope of the line were analyzed with ANOVA and a multiple range test.

Valle del Cauca. Studies in the Cauca Valley were carried out at Hacienda Piedechinche, El Cerrito and established as in Caquetá except plots were 1600 m^2 to accommodate 16 subplots of 100 m^2. Nymph and adult surveys were identical to Caquetá and were carried out weekly from 25 January 2001 to 26 July 2002. Due to security problems and a change in management practices that led to a sharp drop in spittlebug populations, it was decided to carry out this study in Valle del Cauca instead of Cauca where originally anticipated. Unfortunately, we had no previous understanding of the phenology of the pest in the new site. While *Z. carbonaria* predominated in Cauca, *Prosapia simulans* was most abundant in the new site, with some presence of *Z. carbonaria* and *Z. pubescens*. *Prosapia* *simulans* had been reported as a newly detected pest species in South America, Colombia and the Cauca Valley (CIAT 2000). The original application regime was scheduled as three treatments (CIAT 054, Malathion, control) applied 1, 2, 3, 4, 5 or 6 weeks after the start of the first generation outbreak to compare when in the life cycle of the insect a bioinsecticide would be most effective. Because the insect population was low and early life stages were more difficult to detect than anticipated, applications were never made. Nevertheless, population data are reported in this summary since the phenology of *P. simulans* has not yet been described in Colombia or in a seasonally bimodal ecoregion.

Results

Caquetá. A total of 2,603 nymphs and 22,786 adults were examined in population surveys. According to adult specimens *A. varia* was most abundant with 98.5% of the population, followed by *Z. pubescens* with 1.7% and *M. trifissa* with 0.27%. The months of lowest nymph and adult abundance were April and March, respectively, corresponding to the months of highest precipitation (Figure 13). The greatest incidence of nymphs and adults was in September. From March 2001 to May 2002 a mean of 52.3 nymphs/1.5 m^2 and 441.8 adults/240 sweeps was documented.

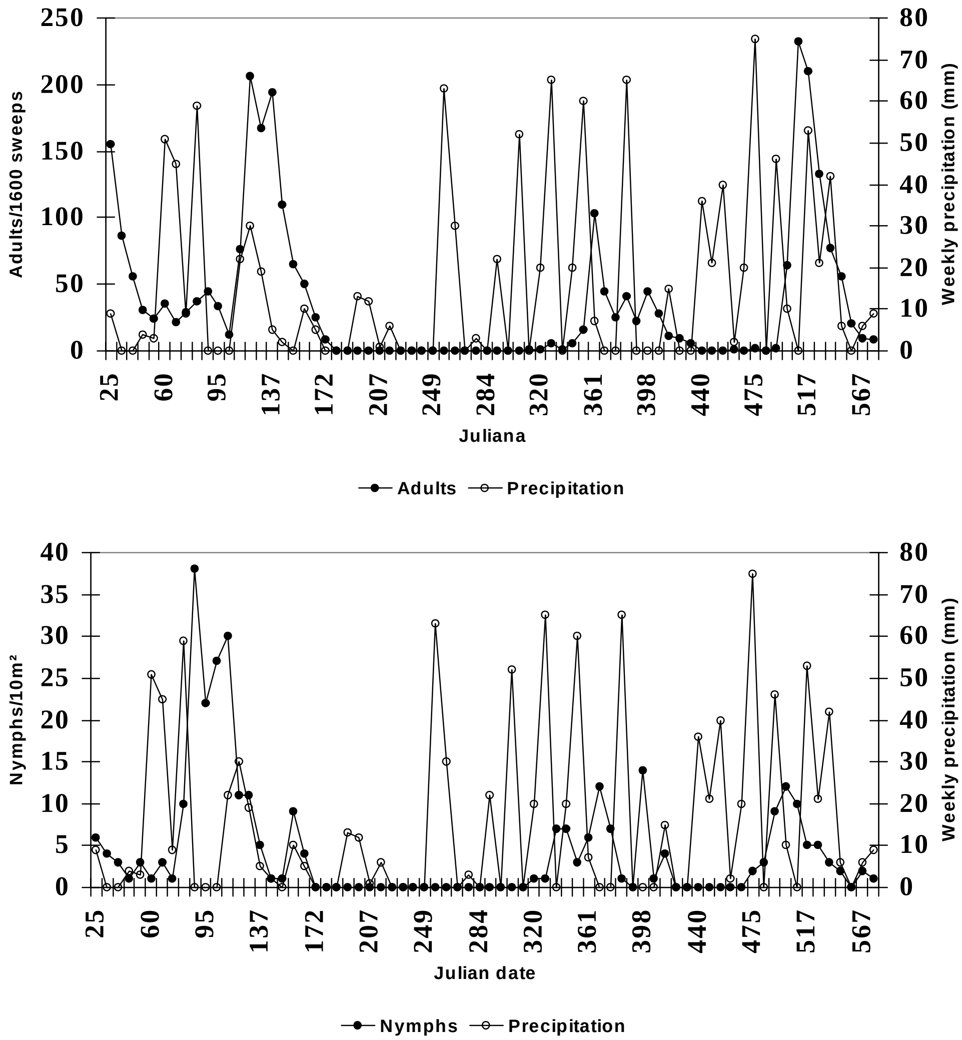


Figure 13. Precipitation and population fluctuation of nymph and adult spittlebugs in C.I. Macagual, Caquetá, 2001-2002.

There was no effect of treatment detected for the final cumulative area under the population curve for nymphs or adults (Figures 14, 15). Separating the analyses into isolate and spittlebug species, the greatest effect was on *A. varia*, although not significantly higher. The insecticide control and CIAT 054 had the lowest cumulative area. For CIAT 054 there were some differences detected; 1 or 2 applications a month showed a greater effect than 0.5 applications per month (Figure 16). Analysis of variance detected differences in the slope of the cumulative population curve measured before and after the treatments.

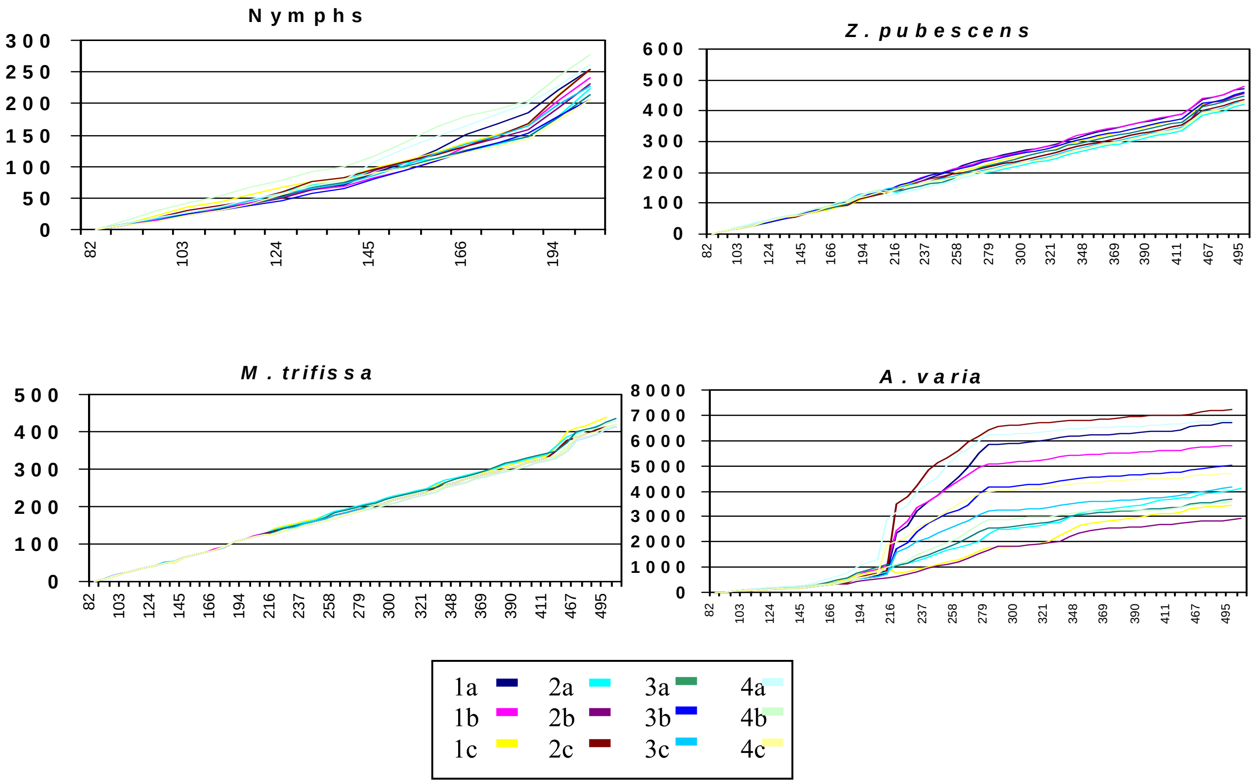


Figure 14. Cumulative area under the population curve (X axis) versus Julian date (Y axis) for adult spittlebug species under different treatment application in C.I. Macagual, Caquetá.

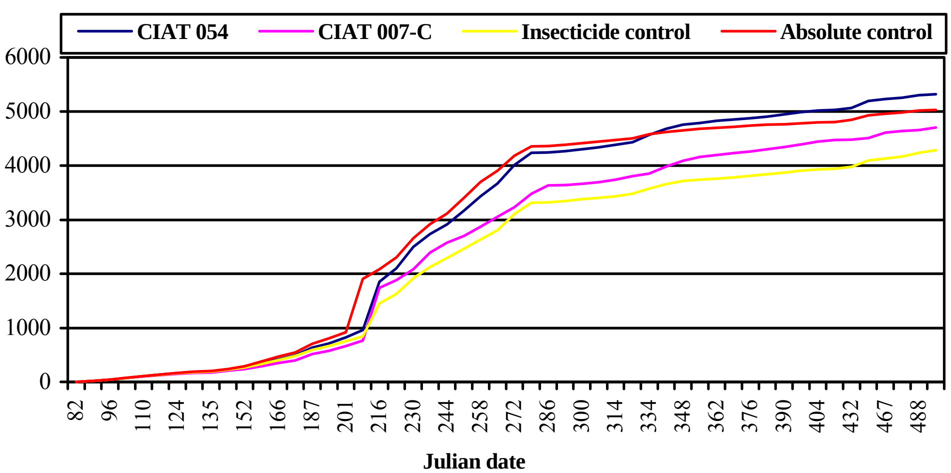


Figure 15. Cumulative area under the population curve (X axis) for adult spittlebugs in each of the treatments applied in C.I. Macagual, Caquetá.

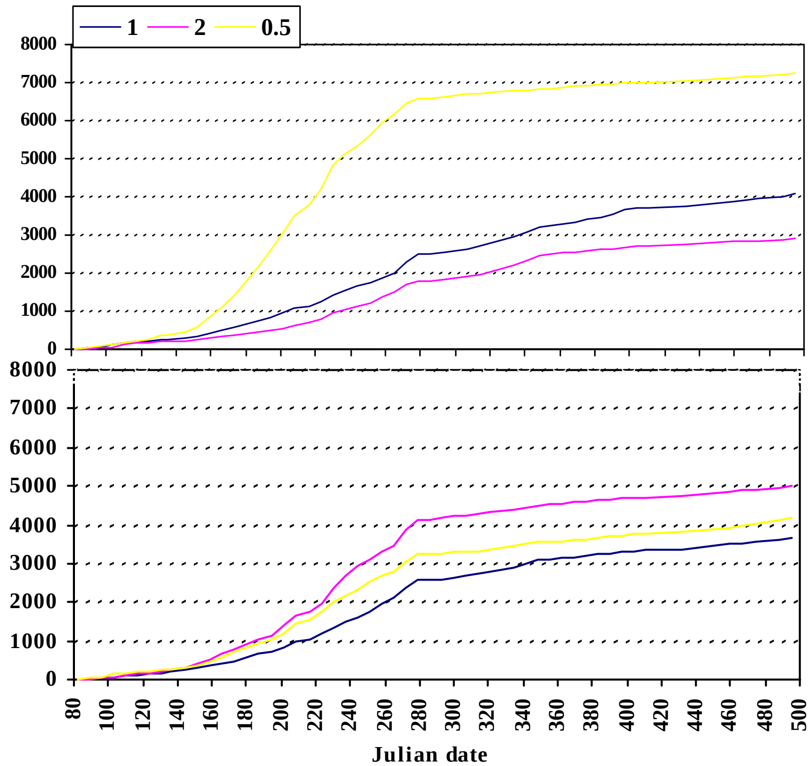


Figure 16. Cumulative area under the population curve (X axis) for adults of *A. varia* according to application frequency. Top: CIAT 054. Bottom: Insecticide control.

For example, CIAT 007C applied once per month had a slope of 1.64 before application of the treatment and a slope of 0.58 afterwards, suggesting that the treatment reduced the velocity of population increase (Table 25). Under this same analysis no effect of treatments was observed on the nymph population. Analysis of the cumulative area under the curve showed a similar growth for all instars in all treatments including the insecticidal and absolute controls. This result could be explained by ineffective deployment of the applications because the products may not have penetrated the spittle mass itself, or penetrated the sward to their feeding sites near the soil surface and litter layer

Table 25. Slope of the cumulative area under the population curve for adults of *A. varia* under different treatments applied in C. I. Macagual, Caquetá.

Treatment	Slope[1]		Treatment	Slope[1]	
2c-Before	1.64	a	1c-After	0.78	abcde
1b-Before	1.62	ab	4 -After	0.69	abcde
1a-Before	1.56	ab	3b-After	0.76	abcde
4 -Before	1.54	abc	1b-After	0.63	bcde
3b-Before	1.34	abcde	2a-After	0.60	cde
1c-Before	1.12	abcde	2b-After	0.59	cde
3c-Before	0.92	abcde	2c-After	0.58	cde
2a-Before	0.90	abcde	3a-After	0.55	de
2b-Before	0.88	abcde	1a-After	0.52	e
3a-Before	0.78	abcde	3c-After	0.50	E

[1]Values followed by the same letter are not significantly different (P<0.01)

Valle del Cauca. A total of 309 nymphs and 2,687 adults were examined in population surveys and all belonged to *P. simulans*, even though *Z. carbonaria* and *Z. pubescens* were detected in these sites previous to the start of surveys. Population analysis of the nymphal life stages at the farm level (all plots summed) and plot level showed two distinct population peaks in 2001 and one for the first semester of 2002 (Figures 17, 18, 19). Analysis at the plot level showed that population peaks occurred in the same period across the three plots, March and April of 2001 and May 2002 (Figure 18). Variation in abundance at the farm level was established by comparing the number of nymphs collected in the surveys. Plot 2 had 46.0% of the total and Plot 5 4.2%, representing the plots of highest and lowest incidence, respectively. At the farm level, over the entire survey period the mean abundance was 3.3 nymphs/2 m². Population analysis of *P. simulans* adults at the farm level showed distinct population peaks in February and May 2001 and January and May 2002 (Figure 17). The same peaks appeared in each of the separate plots and corresponded to nymph peaks indicating that they originated from local nymph populations rather than through immigration (Figures 18, 19). Like nymphs, Plots 2 and 5 had the highest and lowest incidence of adults with 35.3 and 4.0% of total adults captures, respectively. At the farm level, over the entire survey period the mean relative abundance was 27.8 adults/320 sweeps.

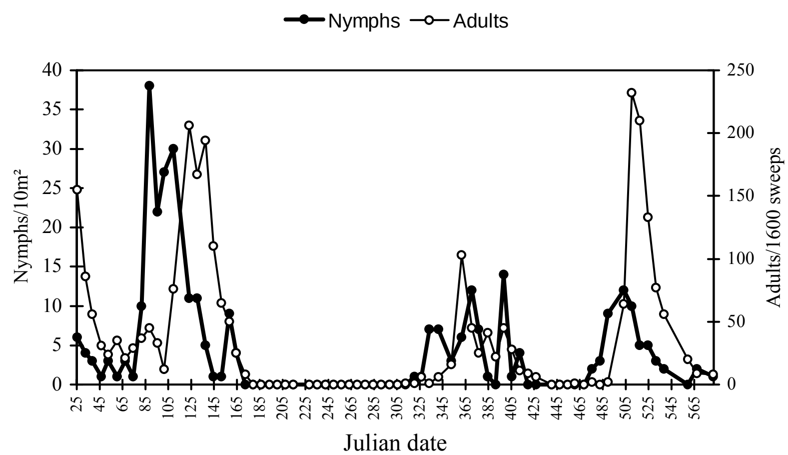


Figure 17. Population fluctuation curve for nymphs and adults of *P. simulans* in Piedechinche, Valle del Cauca, 2001- 2002.

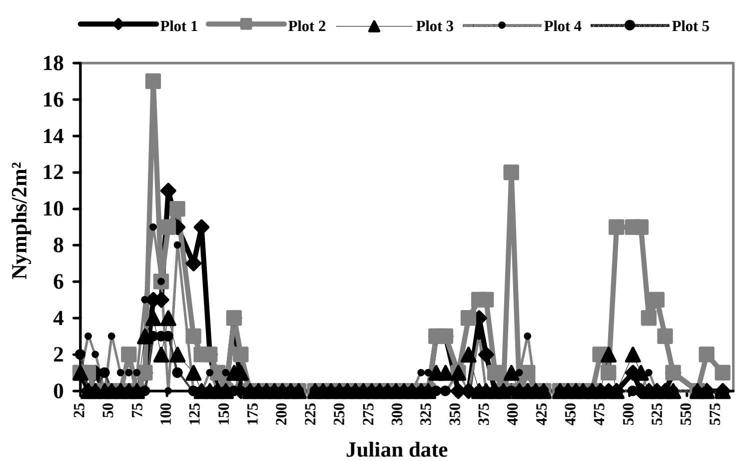


Figure 18. Population fluctuation curve of *P. simulans* nymphs in five plot of *B. decumbens*, Piedechinche, Valle del Cauca, 2001- 2002.

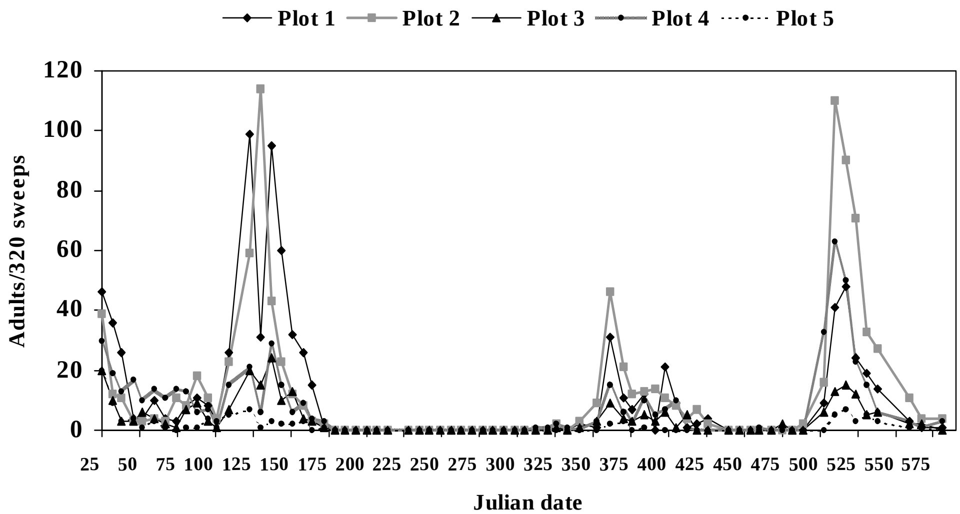


Figure 19. Population fluctuation curve of *P. simulans* adults in five plot of *B. decumbens*, Piedechinche, Valle del Cauca, 2001- 2002.

In the first year of evaluation, nymphs and adults were not detected in the field from July to October, which corresponded to the conditions of drought that preceded and occurred during that period (Figure 20). In the same fashion population peaks of nymphs and adults corresponded to periods of rain that preceded and occurring during that period.

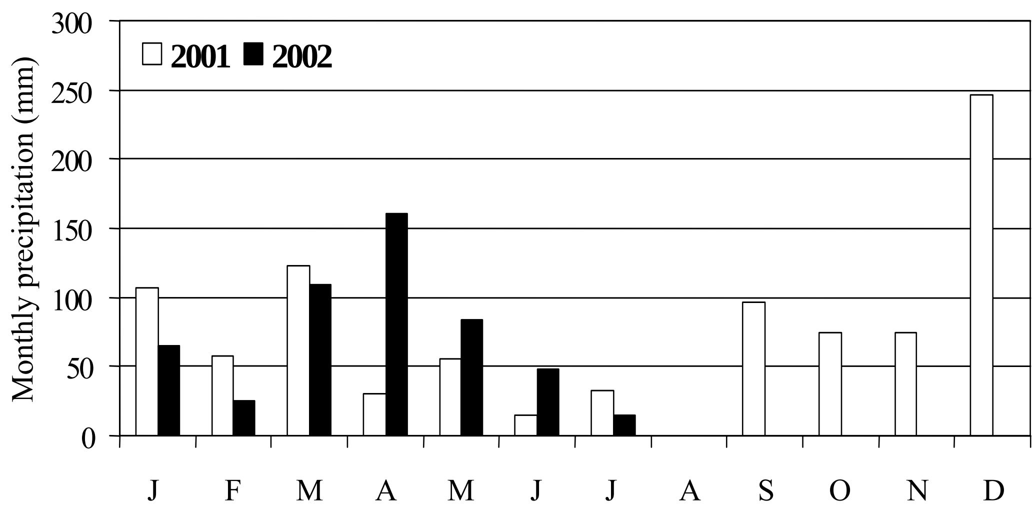


Figure 20. Monthly precipitation (mm) in Piedechinche, Valle del Cauca, 2001- 2002.

Discussion

Although the information obtained from field applications does not permit solid conclusions, certain aspects should be highlighted because they will help to orient future studies on fungal entomopathogens. In ecosystems characterized by continual humidity and continual presence of spittlebugs throughout the year, applications should be directed towards diminishing the velocity of population growth with frequent and regular applications over a brief period of time. This inundative use of biocontrol should initially maintain the population and then slowly cause population decline as the fungus establishes and propagates in the environment. Biological control will not cause a drastic reduction of populations but a slow and longer lasting decline; and it will not contaminate the environment. We believe that integrating elements such as controlled grazing with an improved formulation and delivery system of the product directed towards the nymphs should produce better results.

These results confirm the idea that to establish a spittlebug control program it is indispensable to have a detailed understanding of the habits and behavior of the particular species at that particular site. For example, in Valle del Cauca, we did not know that detection of the early instars of *P. simulans* would be so difficult (compared to other species) because these stages were well-hidden in the soil surface, often a few centimeters below in cracks and fissures. That behavior is relevant to making decisions about when and how to control the first generation of spittlebugs.

Activity 2.3 *Brachiaria* genotypes resistant to spittlebug and other biotic stresses

Highlights

- Approximately 800 clones pre-selected from the tetraploid sexual population were assessed for reaction to three species of spittlebug.

- Thirty-two of approximately 800 sexual clones were selected for crossing block and two of approximately 800 sexual clones showed exceptionally high level of antibiotic resistance to three species of spittlebug (*Aeneolamia varia, A. reducta,* and *Zulia carbonaria*). This is the first time that hybrids combining antibiosis to three different species of spittlebug are reported.

- None of a set of approximately 100 sexual x apomictic experimental hybrids was resistant to any species of spittlebug

- Developed biochemical (isoenzimes) and DNA-based (RAPD) techniques to identify nymphs of different spittlebug species.

- Made progress in the understanding of mechanisms of resistance to *A. reducta* and initiated studies on how multiple spittlebug infestations may affect resistance levels in selected Brachiaria genotypes.

- Continued field screening for resistance to three major spittlebug species in Caquetá.

2.3.1 Development of new hybrid population for spittlebug screening

Contributors: J.W. Miles, A. Betancourt, F. Feijoo (CIAT)

Rationale

As a heterogeneous tetraploid sexual population is improved by intra-population recurrent selection on spittlebug resistance, Al tolerance, Rhizoctonia resistance, and IVDMD, selected clones are crossed with elite apomictic pollen parents to generate large hybrid populations from which elite apomictic clones are sought for development to cultivar status. Comparison of the hybrid families generated using a diversity of Ap pollen parents -- elite CIAT accessions of *B. brizantha* -- will permit identification of those pollen parents that produce superior hybrids when crossed with the sexual population. One or a small number of these apomicts can be used as testers for future improvment of the sexual population based on testcross performance (Miles 1995; 1997).

Materials and Methods

Forty-one tetraploid sexual clones were selected from SX99 cycle of the tetraploid sexual population on general agronomic performance and subsequent assessment of reaction to a single species of spittlebug (*A. varia*). Vegetative propagules of these clones were space planted (approximately 5 x 5 m) in seed plots of 15 *B. brizantha* accessions previously established during second semester 2000, at CIAT-Popayán. Open-pollinated seed was harvested during 2000 and 2001. A small, more or less balanced set of 14 Sx X 14 AP hybrid progenies was retained for observations in Colombia. Approximately 1,700 seedlings were transplanted to the field in May 2002, at CIAT-Quilichao. The remainder of the hybrid seed produced was dispatched to Mexico early in 2002. Seedlings were produced in Mexico and approximately 4000 were established in the field in July 2002.

Results and Discussion

The 15 pollen parents (Ap) of this set of families were chosen from *B. brizantha* accessions with high dry-season yields in the regional trials conducted in Colombia during 1996-1999. Preliminary observations on these families at CIAT-Quilichao permit identification of four (of the 14) Ap parents that produce generally superior hybrid progenies. These will be further assessed to decide upon one (or perhaps two) apomictic tester clones for future population improvement by selection on testcross performance.

This space-planted nursery was cut in July 2002, and we are currently assessing regrowth. During October, we will attempt to assess seed set, harvest open-pollinated seed (for progeny tests in 2003), and further cull the pre-selections that pass on to the next stage of assessment of spittlebug reaction, Rhizoctonia reaction, Al tolerance, and IVDMD. A small set of promising apomictic hybrids should be advancing to wider-scale testing in 2004, using seed harvested from next year's progeny trial.

2.3.2 Establishment of *Brachiaria* hybrids in field nurseries in Mexico

Contributors: Ing. Edgar Guzmán (Semillas Papalotla), J.W. Miles (CIAT)

Rationale

An integral activity of CIAT's collaborative agreement with Semillas Papalotla, new hybrids are to be evaluated in the field in Mexico under conditions of drought stress on the Pacific coastal plain of Oaxaca.

Materials and Methods

Hybrid seed was delivered to Mexico in April. Seeds were germinated and seedlings established for transplanting to the field. In early July, 3,876 seedlings were transplanted to the field as unreplicated, spaced plants.

Results and Discussion

Owing to lack of rainfall following transplant, seedlings are being watered twice a week. Survival is in excess of 90% as of 29 September 2002. Surviving plants are well developed.

2.3.3 Identification of *Brachiaria* genotypes resistant to spittlebug

Contributors: C. Cardona, G. Sotelo, and J. W. Miles

Rationale

As in previous years, assessment of resistance to spittlebug received special attention. The correct identification of resistant hybrids is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. In 2002, we screened more than 1,400 genotypes under greenhouse and field conditions. Based on results obtained in 2001 and 2002, we developed a successful strategy for the simultaneous but independent screening for resistance to three key species.

A synthetic sexual tetraploid population is being subjected to cyclic selection to improve levels of spittlebug resistance (and other desirable attributes) on a 2-yr cycle. Following 8-10 months of observations of field grown plants from each cycle of the segregating population, a manageable number (~1000) of selections are propagated for assessment of spittlebug reaction. Owing to differential reaction of *Brachiaria* host genotypes to different spittlebug species, screenings are now conducted with three or four species.

Materials and Methods

Approximately 800 individual clones were pre-selected on the basis of general performance in field trials in CIAT-Quilichao and Matazul (Puerto López, Meta) during 2001. These were propagated to produce three propagules per clone. One propagule of each clone was infested with one of three spittlebug species (*A. varia, A. reducta,* or *Z. carbonaria*), so that the entire set of 800 clones was assessed for reaction to three species simultaneously. Based on this first, unreplicated screening, 84 clones were selected for a more refined, replicated evaluation, again simultaneously against all three spittlebug species individually. Standard artificial infestation procedures were used. Resistance was assessed firstly based on plant damage (on a 1 = no damage to 5 = dead plant scale). Nymphal survival was then determined for clones with low mean damage scores. Three hundred seventy-nine SX x AP hybrids (series BR01) were established in field trials (as single, spaced plants). Of these, 98 were “pre-selected” based on visual assessment, for preliminary screening with three spittlebug species.

Screenings for resistance were conducted with *Aeneolamia varia*, *A. reducta*, *Zulia carbonaria*, *Z. pubescens* and *Mahanarva trifissa*. These are the most important species affecting *Brachiaria* in Colombia and some other countries in Latin America. Test materials were compared with 4-6 checks fully characterized for their resistance or susceptibility to *A. varia*. Plants were infested with a known number of eggs (usually 6 or 10) of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs reached the fifth instar stage or adult emergence occurred. Plants (usually 6-10 per genotype) were scored for symptoms using the damage scale (1, no damage; 5, plant dead) developed in previous years.

Percentage nymph survival was calculated. Materials were selected on the basis of low damage scores (<2.0 in the 1-5 scale) and reduced percentage survival (<30%). Large-scale nurseries are initially selected on the basis of unreplicated tests. All those materials rated as resistant or intermediate are reconfirmed in replicated tests. All those susceptible are discarded. All data are statistically analyzed.

Results and Discussion

The most important activity in 2002 was the evaluation for resistance to *A. varia*, *A. reducta* and *Z. carbonaria* of approximately 800 individual clones pre-selected by J. Miles on the basis of general performance in field trials at Quilichao (Cauca) and Matazul (Meta). One plant of each clone was independently but simultaneously evaluated for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. Selection was based on damage scores. None of 83 apomictic x sexual hybrids showed a useful level of resistance to any of the species tested, the mean for this group being statistically the same as the mean for the susceptible checks. In contrast, the new sexual hybrids (SX01) showed, as a group, a level of resistance comparable to that of the resistant checks (Table 26).

Table 26. Damage scores in sexual x apomictic and sexual (SX01) clones evaluated for resistance to three spittlebug species in 2002

Group	*Aeneolamia varia*		*Aeneolamia reducta*		*Zulia carbonaria*	
	No. tested	Mean damage scores	No. tested	Mean damage scores	No. tested	Mean damage scores
Susceptible checks[a]	2	4.8a	2	4.9a	2	4.5a
Sexual x apomicitic hybrids	83	3.9a	82	4.0a	82	3.7a
Hybrid checks[b]	2	3.6ab	2	2.6b	2	2.3b
Sexual hybrids (SX01)	725	2.6b	728	2.8b	689	3.1ab
Resistant checks[c]	2	1.3b	2	2.2b	2	3.2ab

[a] CIAT 0606 and BRX-44-02
[b] BR99NO/4132 and FM95NO/4624
[c] CIAT 36062 and CIAT 6294
Means within a column followed by the same letter are not significantly different ($P < 0.05$) by Scheffe's *F* analysis of arbitrary contrasts

Based on this unreplicated screening, 86 clones were selected for reconfirmation in replicated nurseries. Again, independently but simultaneously evaluated for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. We used six replications per genotype, each plant infested with six eggs. Resistance was assessed first on the basis of damage scores. Nymphal survival was then determined for clones with low mean damage scores. Thirty SX01 clones were selected for resistance to three spittlebug species and were subsequently used by the breeder to establish a recombination block. On average, the 30 hybrids showed damage scores that did not differ from those recorded on the most resistant check, CIAT 36062. As a group, the selected hybrids showed levels of antibiosis (reduced nymph survival) to *A. varia, A. reducta,* and *Z. carbonaria* that were significantly better than the one found in the commercial resistant check, CIAT 6294 ('Marandú'). Antibiosis to *A. varia* and *A. reducta* was high but significantly lower than in CIAT 36062, the most resistant check. Antibiosis to *Z. carbonaria* was the same as in CIAT 36062 (Table 27).

Table 27. Damage scores and percentage nymphal survival in 30 sexual clones selected for resistance to three species of spittlebug

Group	Damage scores[a]			Percentage nymph survival		
	A. varia	*A. reducta*	*Z. carbonaria*	*A. varia*	*A. reducta*	*Z. carbonaria*
30 selected SX01 hybrids	1.9b	1.6c	2.1c	16.8c	17.0c	38.1b
CIAT 36062[b]	1.4b	1.2c	1.6c	2.8d	0d	30.6b
CIAT 6294[b]	1.6b	2.9b	3.5b	33.3b	33.4b	69.4a
BRX-44-02[c]	5.0a	5.0a	4.6a	61.1a	80.6a	47.2ab
CIAT 0606[c]	4.7a	4.8a	4.4a	77.8a	72.2a	50.1ab

[a] On a 1-5 scale (1, no damage; 5, plant killed)
[b] Resistant check
[c] Susceptible check
Means within a column followed by the same letter are not significantly different ($P < 0.05$) by Scheffe's *F* analysis of arbitrary contrasts.

A list of those sexual hybrids selected for resistance to 1, 2 or 3 species of spittlebug is presented in Table 28. In Figure 21 we illustrate the very high levels of antibiosis resistance to the three species detected in SX01NO/ 0102 and SX01NO/0233.

Table 28. Sexual *Brachiaria* (SX01) hybrids selected in 2002 for high antibiosis (<30% nymphal survival) and intermediate antibiosis resistance (31-50% nymphal survival) to one or more species of spittlebug

Combining high resistance to *Aeneolamia varia, A. reducta*, and *Zulia carbonaria*	Combining high resistance to *A. varia* and *A. reducta*	Combining resistance to *A. reducta* and *Z. carbonaria*
SX01NO/ 0102	SX01NO/0067	SX01NO/0263
SX01NO/0233	SX01NO/0159	SX01NO/0760
	SX01NO/0446	SX01NO/1186
	SX01NO/0465	SX01NO/2209
	SX01NO/0878	SX01NO/2420
	SX01NO/1647	SX01NO/2722
	SX01NO/2017	SX01NO/4099
	SX01NO/2619	
	SX01NO/2683	
	SX01NO/3168	
	SX01NO/3615	
	SX01NO/4423	
	SX01NO/4506	
	SX01NO/4785	

Others selected:
SX01NO/1090: for combining intermediate resistance to *A. varia* and *A. reducta*
SX01NO/1111: for combining intermediate resistance to *A. varia* with high resistance to *A. reducta*
SX01NO/1175: combining intermediate resistance to *A. varia* and *A. reducta* with high resistance to *Z. carbonaria*
SX01NO/3390 and SX01NO/4467: for high resistance to *A. reducta* only
SX01NO/3439: for having intermediate resistance to *A. reducta*
SX01NO/4861: for high resistance to *Z. carbonaria* only

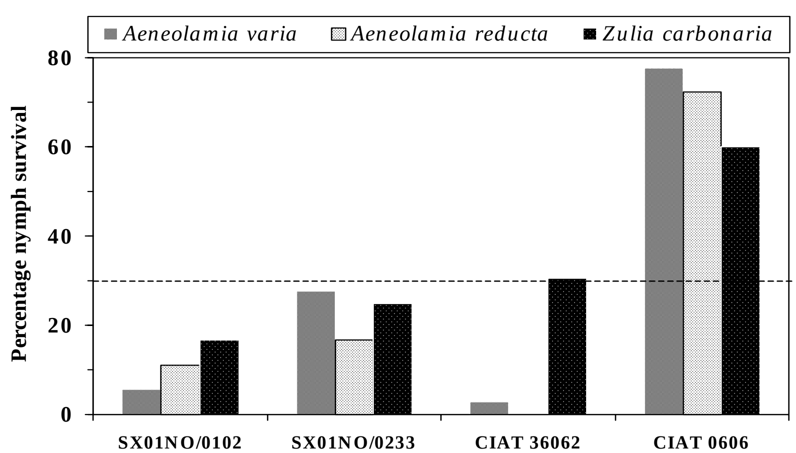


Figure 21. Levels of antibiosis resistance to three species of spittlebug in two SX01 *Brachiaria* hybrids selected in 2002. CIAT 36062 is an apomictic hybrid used as standard resistant check. CIAT 0606 is the susceptible cultivar *B. decumbens* widely grown in Latin America. The dotted line represents the cut-off point for antibiosis resistance rating.

The continuos work on resistance to spittlebug has allowed us to detect significant progress in the incorporation of resistance. To illustrate this, we used data obtained with *Aeneolamia varia*, the species most intensively studied since the beginning of the breeding for resistance program (Figure 22).

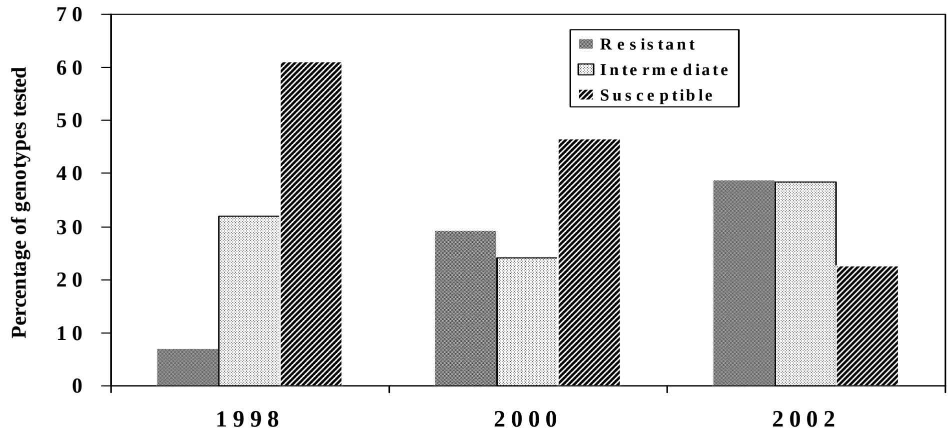


Figure 22. Progress in the incorporation of resistance to *Aeneolamia varia* in *Brachiaria*. Note the steady increase in the frequency of resistant genotypes and the decline in the frequency of susceptible genotypes as a result of continuous cycles of selection.

2.3.4 Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

Contributors: C. Cardona, G. Sotelo, J. W. Miles, and W. Mera

Field screening for resistance to spittlebug continued in 2002. The methodologies have been described in previous reports. We have also reported on the reliability of the system as judged by the high correlation between greenhouse and field resistance ratings. We have also shown in previous reports that the methodology can be used with all spittlebug species. Nine major screening trials (four with *Aeneolamia varia*, two with *Mahanarva trifissa*, and three with *Zulia pubescens*) were conducted in Caquetá in 2002.

In Table 29a we highlight the results of evaluating 44 sexual (SX99) hybrids, four resistant checks, and two susceptible checks. In general, resistance to *A. varia* was high. The mean damage score for resistant hybrids was slightly higher than that for the resistant checks but significantly lower than the mean for susceptible checks. Tiller ratios (number of tillers per plant at the end of the infestation process divided by the number of tillers per plant at the beginning of the infestation process) for resistant genotypes were significantly higher than in the susceptible checks.

This means that tiller mortality did not occur in resistant hybrids or in the resistant checks CIAT 6294 ('Marandú') and CIAT 36062. In contrast, the susceptible checks CIAT 0606 and CIAT 0654 lost 80% and 86% of their tillers, respectively. There was a significant, negative correlation ($r = -0.774$; $P < 0.001$) between damage scores and tiller ratios.

A similar field study (Table 29b) was conducted in Caquetá with a set of BR/ hybrids rated resistant to *A. varia* in greenhouse conditions in Palmira. In three consecutive, identical trials, we compared 32 hybrids with four resistant checks and two susceptible checks. On average, hybrids bred for resistance to *A. varia* performed as well as the resistant checks in terms of damages scores (Table 29b). The mean tiller ratio for selected hybrids was significantly higher than that for resistant checks reflecting not only resistance levels but also the good agronomic performance of the new hybrids. Again, there was a negative and significant correlation (r = - 0.678; *P*< 0.05) between damage scores and tiller ratios, meaning that severe foliage damage reflects a lower capacity of the plant to recover from spittlebug damage and produce new growths.

Table 29a. Field resistance to *Aeneolamia varia* in selected sexual *Brachiaria* hybrids and checks. Means of 10 replicates per genotype

Genotype	Mean damage scores[a]	Tiller Ratio[b]
	Best hybrids	
SX99NO/2927	2.0	1.50
SX99NO/2115	2.0	1.43
SX99NO/1215	2.0	1.41
SX99NO/3690	2.0	1.34
SX99NO/2200	2.2	1.32
SX99NO/2173	2.0	1.31
SX99NO/2030	2.0	1.26
SX99NO/3770	2.0	1.19
SX99NO/0246	2.0	1.18
SX99NO/3564	2.0	1.16
Mean	2.0ab	1.31a
	Susceptible checks	
CIAT 0606	4.7	0.20
CIAT 0654	4.6	0.14
Mean	4.6a	0.17b
	Resistant checks	
CIAT 6133	2.0	1.08
FM9503/4624	1.7	1.16
CIAT 36062	1.0	1.56
CIAT 6294	1.4	1.22
Mean	1.5b	1.25a

Table 29b. Field resistance to *Aeneolamia varia* in selected sexual *Brachiaria* hybrids and checks. Means of one trial, 10 replicates per genotype

Genotype	Mean damage scores[a]	Tiller Ratio[b]
	Best hybrids	
BR00NO/0587	1.5	4.99
BR00NO/0019	1.4	4.85
BR00NO/0425	1.4	4.67
BR00NO/0022	1.6	4.60
BR00NO/1392	1.5	4.44
BR00NO/0595	1.4	4.22
BR00NO/1076	1.4	4.15
BR00NO/1032	1.5	4.01
BR00NO/0106	1.5	3.83
BR00NO/1501	1.4	3.81
BR00NO/1281	1.6	3.65
Mean	1.47b	4.29 a
	Susceptible checks	
CIAT 0606	3.8	0.56
CIAT 0654	4.0	0.34
Mean	3.9 a	0.45c
	Resistant checks	
CIAT 6133	1.3	4.26
FM9503/4624	1.3	2.68
CIAT 36062	1.3	2.74
CIAT 6294	1.2	2.02
Mean	1.27b	2.92b

[a] On a 1-5 damage score scale (1, no damage; 5, severe damage, plant killed).
[b] Ratio of tillers per plant at the end of the infestation process/ tillers per plant at the beginning of the infestation process.
Means within a column followed by the same letter are not significantly different ($P < 0.05$) by Scheffe's *F* analysis of arbitrary linear contrasts.

In yet another set of trials we compared the field resistance of 38 selected hybrids exposed to *Mahanarva trifissa* and *Zulia pubescens*. Results are shown in Table 30.

On average, resistant hybrids exposed to *M. trifissa* performed as well as the resistant checks both in terms of damage scores and tiller ratios. The same hybrids exposed *to Z. pubescens* showed damage scores significantly higher and tiller ratios significantly lower than the resistant checks.

This means that the levels of resistance to *Z. pubescens* are not as high as those already developed for *M. trifissa*, the latter being the species for which antibiosis in resistant genotypes is highest among all spittlebug species studied at CIAT.

Table 30. Field resistance to *Mahanarva trifissa and Zulia pubescens* in selected *Brachiaria* hybrids and checks. Means of 3 trials with *M. trifissa* and two trials with *Z. pubescens*. Ten replicates per genotype per trial.

Genotype	Mean damage scores[a]		Tiller Ratio[b]	
	M. trifissa	*Z. pubescens*	*M. trifissa*	*Z. pubescens*
		Best hybrids		
BR00NO/1501	1.9	2.2	1.90	0.92
BR00NO/1733	1.8	2.2	1.85	0.96
BR00NO/1494	1.8	2.0	1.67	0.94
BR00NO/1392	1.9	2.1	1.66	0.93
BR00NO/1032	1.8	2.0	1.66	1.05
BR00NO/1372	1.7	2.2	1.56	0.96
BR00NO/0106	1.8	2.2	1.26	1.06
BR00NO/0604	1.9	2.3	1.47	0.98
BR00NO/0019	1.8	2.1	1.63	0.79
BR00NO/0078	1.8	2.3	1.60	0.89
BR00NO/0595	1.6	2.1	1.58	0.89
BR00NO/0587	1.7	2.1	1.56	0.90
Mean	1.8b	2.1b	1.62a	0.93b
		Susceptible checks		
CIAT 0606	2.9	3.7	0.80	0.42
CIAT 0654	2.8	4.1	0.90	0.30
Mean	2.8a	3.9a	0.85b	0.36c
		Resistant checks		
CIAT 6133	1.9	1.7	1.54	1.03
FM9503/4624	1.8	1.2	1.46	1.17
CIAT 36062	1.7	1.3	1.71	1.36
CIAT 6294	1.5	1.0	1.40	1.15
Mean	1.7b	1.3c	1.52a	1.17a

[a]On a 1 - 5 damage score scale (1, no damage; 5, severe damage, plant killed)
[b]Ratio of tillers per plant at the end of the infestation process/ tillers per plant at the beginning of the infestation process.
Means within a column followed by the same letter are not significantly different ($P < 0.05$) by Scheffe's F analysis of arbitrary linear contrasts.

Activity 2.4 Identify host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- Detected that under field conditions infestations by two or more spittlebug species may occur on a given *Brachiaria* plant

- Identified and characterized a new insect problem affecting grasses in the Cauca Valley of Colombia.

2.4.1 The effect of mixed infestations on resistance expression

Contributors: C. Cardona, G. Sotelo, A. Pabón, P. Fory, and J. Miles.

Rationale

For years, it was assumed that resistance to one spittlebug species applied without reservations to other spittlebug species. We have shown in previous recent reports that this is not the case and that the mechanisms underlying resistance to one species do not necessarily apply to other species. Hence the importance of studying the mechanisms for each species and the need for screening with as many spittlebug species as possible, in order to ascertain that resistant *Brachiaria* genotypes will perform well under varying growing conditions, even in areas in which two or more species of spittlebug coincide. At the same time, we found that detecting mixed infestations when adults are not present is quite difficult because it is virtually impossible to differentiate among species at the nymphal stage. So, in order to study resistance expression when mixed spittlebug populations occur, we first had to develop reliable techniques to properly identify species.

Materials and Methods: Samples were taken periodically (every month or so for one full year) in one commercial *Brachiaria decumbens* field in Caquetá, at an altitude of 300 meters. Nymphs were collected from individual plants and sent to CIAT for laboratory analysis.

Once we had reliable techniques to properly identify immature stages of different spittlebug species, we established a series of trials aimed at measuring how different species combinations affect resistance expressions in selected resistant or susceptible genotypes well known for their response to several spittlebug species. We measured the effect of single species infestation as opposed to combinations of two or three species in different proportions using a split-plot design (spittlebug species was the main plot; the different genotypes were the sub-plots).

Results and Discussion: Best isozime electhrophoretic patterns were obtained with the use of α-ß esterases (Figure 23). Excellent differentiation among species was also obtained by means of RAPD-PCR analysis (Figure 24).

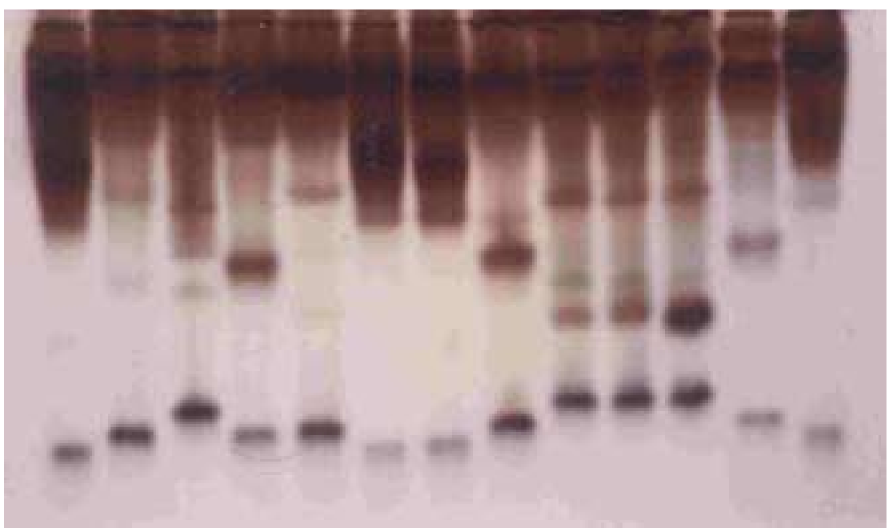


Figure 23. α-ß esterase electrophoretic patterns for fifth instar nymphs of spittlebug. Lanes 1-3, nymphs collected from existing mass rearings of *Aeneolamia varia*, *Zulia pubescens*, and *Mahanarva trifissa*, respectively; all other samples collected from individual *Brachiaria decumbens* plants in Caquetá: 4-5, *Z. pubescens*; 6-7, *A. varia*; 8, *Z. pubescens*; 9-11, *M. trifissa*; 12, *Z. pubescens*; 13, *A. varia*.

This allowed us to detect that mixed infestations occur in commercial fields. Figure 25 shows that individual plants in Caquetá may be affected by one, two or three different species attacking simultaneously.

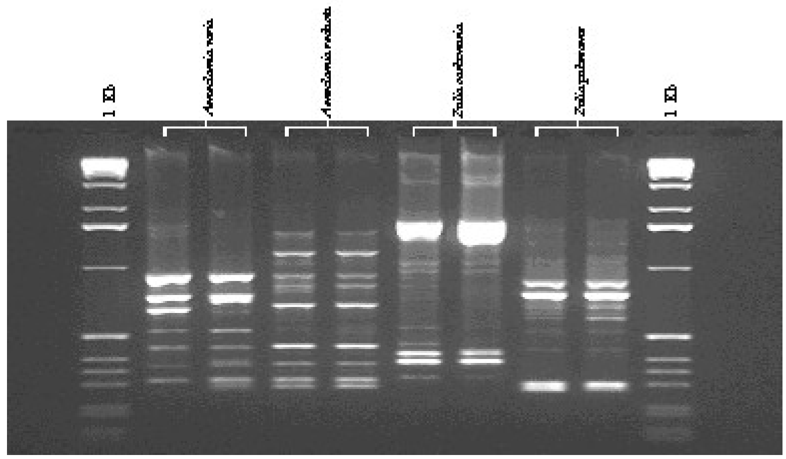


Figure 24. RAPD-PCR (2 ng/µl, OPA-10) patterns obtained with fifth instar nymphs of four spittlebug species (Kb, molecular marker).

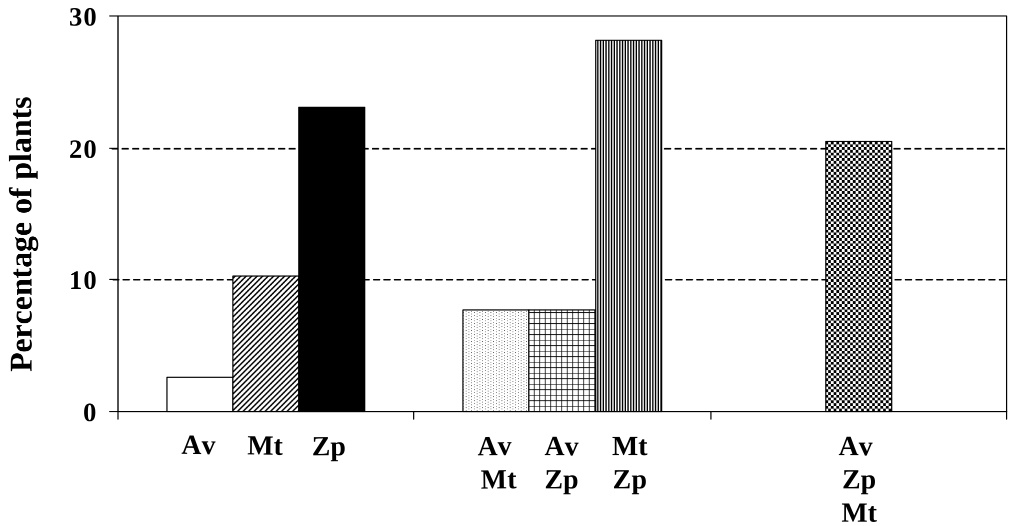


Figure 25. Spittlebug species composition in one commercial *Brachiaria decumbens* field in Caquetá. As shown, individual plants can be infested with pure or mixed stands of nymphs of *Aeneolamia varia* (Av), *Mahanarva trifissa* (Mt) and *Zulia pubescens* (Zp). Note that a significant proportion of plants (21%) can be attacked by as many as three different species.

The possibility of identifying species with precision also allowed us to measure percentage survival of different species when infestations were made with two or more species. The work on resistance expression as affected by simultaneous attacks is still in progress and will be reported in full in 2003. However, we would like to highlight some of the results in order to show how important it is to ascertain that resistance is broad and that the resistance work takes into account the existence of spittlebug species other than *A. varia*.

As shown in Figure 26, when the resistant genotype CIAT 36062 is exposed to *Z. carbonaria* alone or to *Z. carbonaria* in combination with *A. varia* in different proportions, damage scores increase so that the genotype is classified as intermediate resistant rather than resistant. When it is exposed to *Z. pubescens* alone or to *Z. pubescens* in combination with *A. varia*, CIAT 36062 is rated as resistant and damage scores are significantly lower than those obtained with *Z. carbonaria* alone or in combination with *A. varia*. From previous work (see Annual Reports 2002 and 2001) we know that antibiosis to *A. varia* is highest, followed by antibiosis to *Z. pubescens*. There is not an important level of antibiosis to *Z. carbonaria* in CIAT 36062. This may explain the significant differences in terms of damage scores shown in Figure 26.

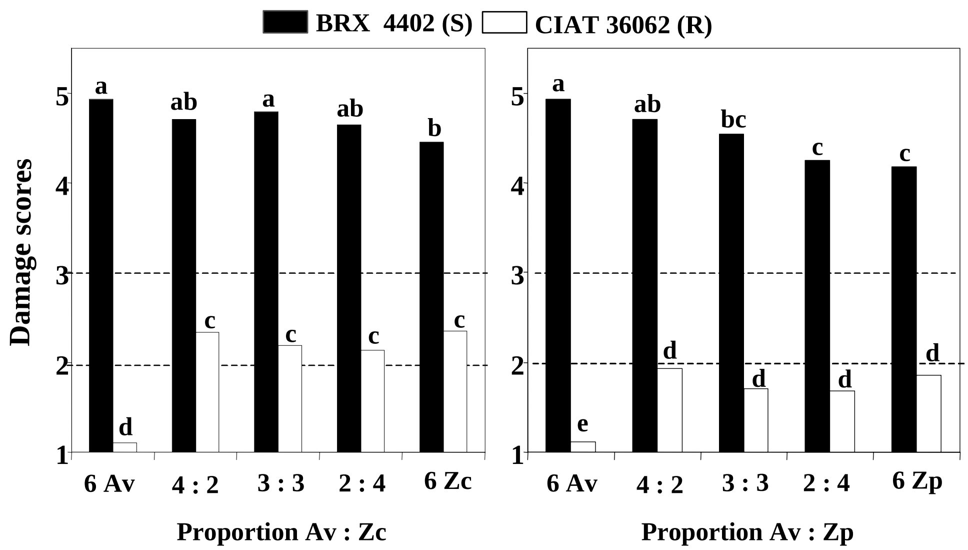


Figure 26. Damage scores recorded on susceptible (BRX-4402) and resistant (CIAT 36062) *Brachiaria* genotypes exposed to individual or simultaneous attack by nymphs of *Aeneolamia varia* (Av), *Zulia carbonaria* (Zc) or *Zulia pubescens* (Zp). Dotted lines represent cut-off points for resistance (< 2) and intermediate ratings (2 - 3) in a 1 - 5 damage score scale. Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.

Most important, we detected significant and differential antibiosis effects when mixed populations of *A. varia* and *Z. carbonaria* in different proportions were used to infest plants of the resistant genotype CIAT 36062. At all levels of infestation, survival of *A. varia* on CIAT 36062 was significantly reduced to levels below the cut-off point for resistance rating (< 30% percentage survival) (Figure 27).

On the contrary, the survival of *Z. carbonaria* nymphs was significantly higher, well above the 50% level used to classify genotypes as susceptible. When *A. varia* and *Z. pubescens* attack simultaneously, the antibiosis mechanism present in CIAT 36062 affected both species and survival was below the cut-off point for resistance rating (Figure 28).

These findings support the need to characterize resistance to as many spittlebug species as possible. If, for example, an improved *Brachiaria* cultivar that has antibiosis resistance to one species but not to others is released in areas where two spittlebug species coexist, antibiosis will reduce populations of one of the species. The species not affected by antibiosis will be freed of competition and may become more abundant and aggressive. Little would have been accomplished in terms of efficient plant protection.

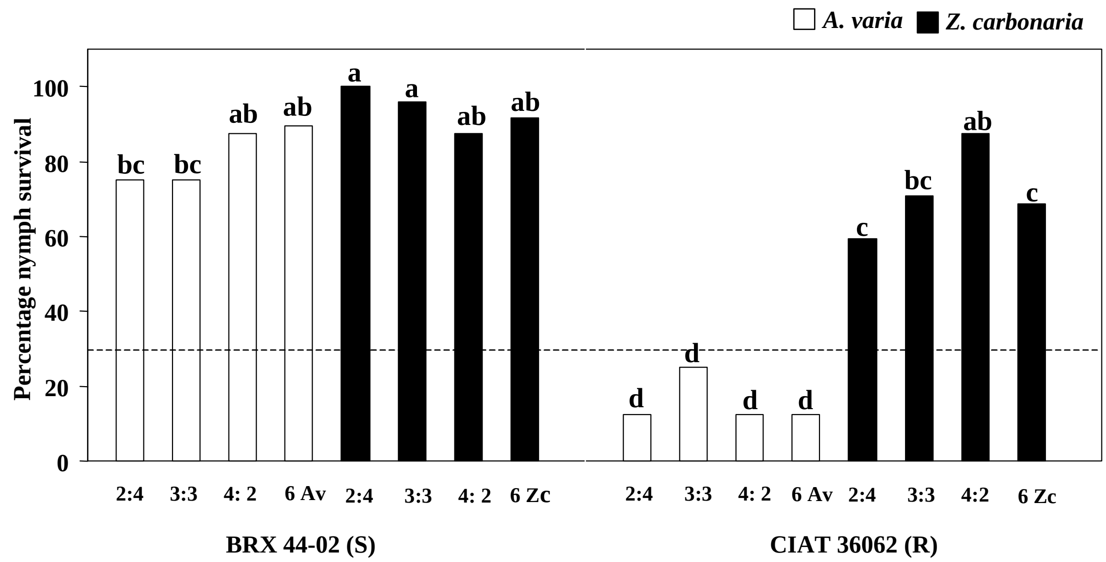


Figure 27. Levels of antibiosis (reduced percentage nymph survival) detected when plants of susceptible (BRX-4402) and resistant (CIAT 36062) *Brachiaria* genotypes were infested with *Aeneolamia varia* or *Zulia carbonaria* or combinations thereof (*A. varia*: *Z. carbonaria*). The dotted line represents the cut-off point for resistance rating (< 30.0% percentage survival). Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.

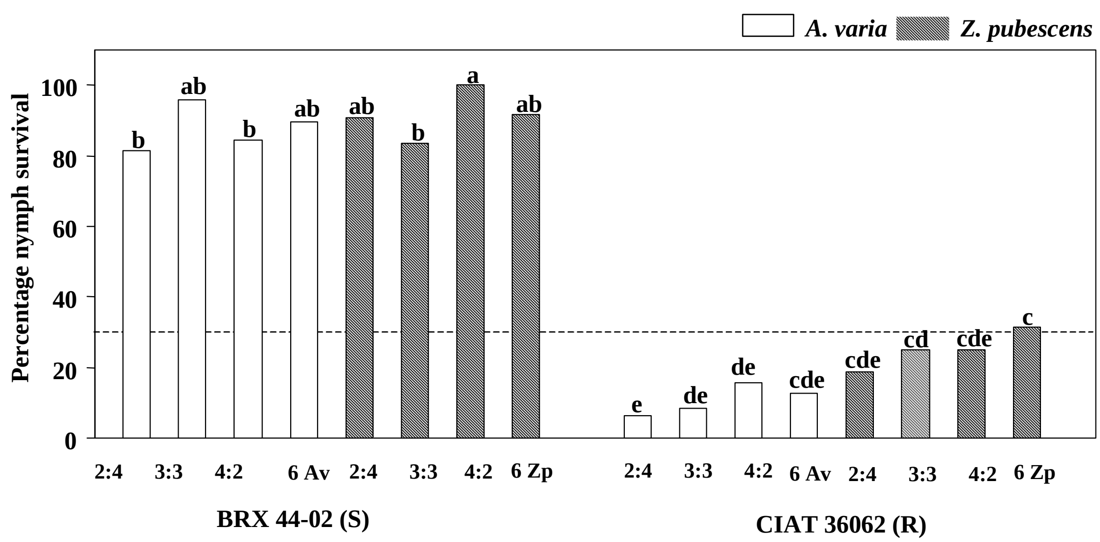


Figure 28. Levels of antibiosis (reduced percentage nymph survival) detected when plants of susceptible (BRX-4402) and resistant (CIAT 36062) *Brachiaria* genotypes were infested with *Aeneolamia varia* or *Zulia pubescens* or combinations thereof (*A. varia*: *Z. pubescens*). The dotted line represents the cut-off point for resistance rating (< 30.0% percentage survival). Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.

2.4.2 Diagnostic survey of a 'new' pest affecting pará grass in Colombia.

Contributors: C. Cardona, G. Sotelo, A Pabón, J. Miles

A new insect problem affecting pará grass (*Brachiaria mutica*) was detected in the Cauca Valley of Colombia. The insect is a stem borer that attacks well-developed plants of pará grass. It first appeared in the Buga region in January 2002 and has spread to other areas such as Yumbo and Bugalagrande along the Cauca River. It is a weevil that belongs to the Order Coleoptera, Family Curculionidae. It was identified by the British Museum of Natural History as *Apinosis* sp. It is not clear if this is a true new pest or if this is the *Apinosis anacentrinus* recorded as a pest of sugar cane many years ago.

The larvae are white and small (2-3 mm in length) and tunnel through the first internode of the stem (Photo 5) causing severe damage. Pupae are found within the stem. Adults are black and small (3-4 mm in length). The damage caused by the larva interrupts the flow of nutrients through the vascular system, thus causing the death of affected stems, and eventually of the entire plant. Damage has been so severe that entire grassland areas have been completely destroyed (Photo 6). The levels of infestation detected so far are very high: 84% of stems damaged in Buga, 75 and 88% in Bugalagrande and Yumbo, respectively.

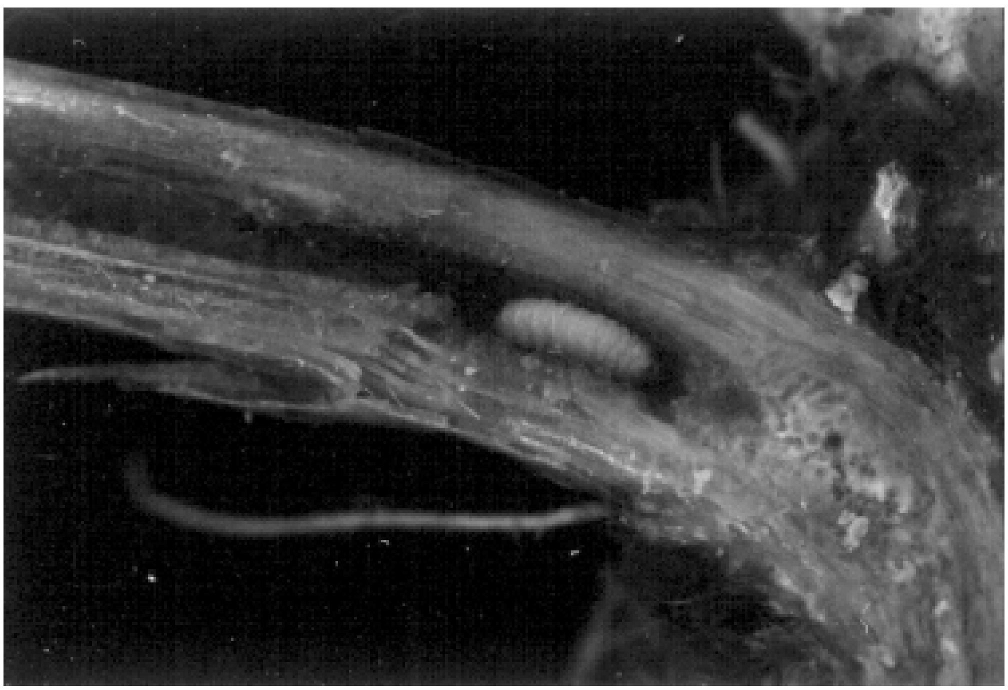


Photo 5. Larva of *Apinosis* sp. in stem of pará grass (*Brachiaria mutica*). Note the tunneling at the level of the first internode.

So far, this insect has been found attacking pará and brachipará (*Brachiaria radicans*) grasses. The weed *Eleusyne indica* is an alternate host. It has not been found affecting 'Pasto Estrella' (*Cynodon nlemfuensis*) or 'Pasto Alemán' (*Echinochloa polystachya*). If these observations are confirmed, the latter grasses could become alternatives for those farmers in areas where the incidence of the stem borer is high.

Photo 6. Severe damage and pasture degradation caused by the pará grass stem borer, *Apinosis* sp., in Buga, Cauca Valley, Colombia. Note grass plants killed and weed proliferation in areas devastated by the insect.

Activity 2.5 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria*

Highlights

- Localized programmed cell death, a common task to arrest the propagation of pathogens, could be important in the resistance of *Brachiaria* to spittlebug

- Agreement between progeny test results and the putative marker of apomixis (PCR marker N14) was good for 107 2000 progenies (94.4% agreement). However, for 13 2001 progenies where we had data on N14, agreement was less than 50%.

2.5.1 Isolation and characterization of sequences associated with resistance to spittlebug in *Brachiaria*

Contributors: C. Romero, I.F. Acosta and J. Tohme- SB-2 Project

Rationale

The spittlebug is the most harmful pest of *Brachiaria* in America. One of the methods of controlling it is through the use of cultivars that are naturally resistant. We are interested in elucidating the molecular basis of resistance to spittlebug in *Brachiaria*. At the genomic level, we isolate sequences bearing strong similarities with resistance genes (R-genes) named RGAs (Resistance Gene Analogs; BRU Annual Report, 2001). Given the need to establish the expression profile of the genes implicated in resistance, the resistant genotype is challenged with larvae of the insect, following two approaches: a) look specifically for elements probably involved in the recognition of the insect and the activation of the resistance and, b) seek information about the defense response itself.

Mapping of RGAs from *Brachiaria brizantha*. A candidate gene approach was developed to characterize Quantitative Trait Loci (QTL) for resistance to spittlebug in *Brachiaria*. Based on the strong structural similarities between R-genes of the NBS-LRR (Nucleotide Binding Site-Leucine Rich Repeat) family, which includes the main part of cloned R-genes, we had isolated RGAs using degenerate primers targeting highly conserved regions (BRU Annual Report, 2001). Now, these RGA sequences have been used as molecular markers to determine whether they are part of the spittlebug resistance QTL.

Isolation of sequences differentially expressed in resistant plants infested with spittlebug. To obtain sequences related with the defense process, we used a differential display technique that allows us to isolate fragments of cDNAs induced by the insect attack in a resistant variety. Sequence information of these fragments is used to hypothesize about its possible function in the defense response in order to get closer to the resistance mechanism.

Materials and Methods

Specific primers were designed for each of 8 RGA classes established previously (BRU Annual Report, 2001) in order to use RGAs as PCR-based molecular markers. Five out of the 8 classes were informative polymorphic markers between the resistant and susceptible varieties (*B. brizantha* CIAT 6294 and *B. ruziziensis* BR4x44-03, respectively). One of these PCR-based markers is a Cleaved Amplified Polymorphic Sequence (CAPS), while the other ones represent dominant molecular markers (Presence/Absence).

These polymorphic classes were evaluated in a population derived from an interspecific cross of *B. ruziziensis* BR4x44-03 X *B. brizantha* CIAT 6294. This population contains 215 individuals that have been scored phenotypically for resistance to one of the most widespread species of spittlebug (*Aenolamia varia*), and have been used to construct a genetic map (BRU Annual Report, 2001). The RGAs were located on this map using Mapmaker (Lander, 1987). The correlation analysis between the segregation of RGAs and the resistance score was done with QGENE (Nelson, 1997).

To isolate sequences differentially expressed in a *Brachiaria*-resistant variety (CIAT 36062) when challenged with spittlebug, we employed a highly sensitive technique known as Differential Subtraction Chain (DSC) (Luo et al., 1999). Expressed sequences from treated and non-treated plants are put in hybridization, and sequences common to both states are suppressed. This is achieved by successive rounds of hybridization with a subsequent enriching of the sequences expressed only in the treated plants.

Two pools of plants from genotype CIAT 36062 grown under similar conditions were used for this assay. One pool was infested with *A. varia* larvae and the other was not treated. Infestation was done in superficial roots according to Cardona (1999) (Photo 5).

These were collected at different stages of the infestation progress (1-30 days) from both pools, and total RNA was extracted from superficial roots with Trizol® reagent. We made two RNA pools that represent two populations of genes expressed in each condition during that time and are denominated "tester" (infested) and "driver" (not infested). Double-strand cDNA was synthesized using the SMART cDNA Synthesis Kit (Clontech) according to manufacturer's instructions. The cDNA was then digested with *Dpn*I and ligated with different adaptor sets for each cDNA pool to make its subsequent amplification by PCR possible.

Photo 7. Infestation system units consist in single steams propagules with profuse superficial roots. This substratum serves as feedings sites for nymphs**.**

We performed two separate hybridization experiments with different hybridization buffers: SSH (Diatchenko, 1996) and DSC (Luo et al., 1999), following the hybridization and PCR parameters suggested by Luo et al. (1999). After each round of hybridization, the level of subtraction was checked by PCR. With the SSH buffer we executed 3 rounds of hybridization; but when the DSC buffer was used, 4 rounds were performed. PCR products from the last round of hybridization were considered to contain differentially expressed sequences from the tester; therefore, they were cloned in batch, and a 96-clone library was constructed for both types of hybridization. Sequences for the complete libraries were obtained, and homologies were searched for in the Gene Bank, using the BLASTN and BLASTX algorithms (Altschul et. al., 1997).

Results and Discussion

Mapping of RGAs from *B. brizantha*. Segregation of 5 RGA markers on the mapping population *B. ruziziensis* BR4x44-03 X *B. brizantha* CIAT 6294 did not show significant association with the trait of interest (resistance to *A. varia*). The highest explicative value for the resistance phenotype was 5%, corresponding to RGA 5B. Some AFLP markers used in the construction of this framework map show much better associations (25%) with resistance to spittlebug (BRU Annual Report, 2001). Despite the small size of such markers (ranging from 100-300 bp), we attempted to sequence them in search for R-gene candidates; however, no apparent homologies were found (data not shown). Moreover, these QTL-containing regions are not saturated in molecular markers; thus they may still contain RGA-type sequences that were not isolated in the set we tried to map. Consequently our next step is to define new classes of RGAs isolated from less complex sources of DNA, such as cDNA or the arrayed genomic library that is currently being constructed in the SB-02 Project. In this way we expect to obtain more diverse and representative RGA sequences that may be included in a genomic region implicated in the resistance of *Brachiaria* to spittlebug.

Isolation of sequences differentially expressed in resistant plants infested with spittlebug: Two "subtraction libraries" were constructed using DSC (see methods). They were supposed to contain sequences being expressed exclusively in resistant *Brachiaria* plants that had been challenged with spittlebug. It is highly probable that such cDNA fragments correspond to genes involved in the resistance response to the insect attack. Sequence analyses of 171 clones (Table 31) revealed 35 sequences with known homology, 4 hypothetical proteins (HP), 2 unknown proteins and 16 sequences with no homology. We isolated sequences that participate in the R-gene-mediated defense response studied in other plant systems (Figure 29) and found Hypersensitive Response (HR)-related sequences (Table 31). This suggested that localized programmed cell death, a common task to arrest the propagation of pathogens, is also important in the resistance to spittlebug. This episode provokes a hormone response that alerts the entire plant; accordingly, we found sequences that are part of the ethylene, jasmonate and salicylic acid pathways triggered during resistance (Cheong, 2002).

This evidence encourages us in the search for R-gene type sequences involved in the resistance mechanism of *Brachiaria*. Additionally, we found other general response elements to feeding, wounding (Reymond, 2002) and stress responses (Table 31), which are well correlated with the type of attack that the insect executes in the plant (mechanical wounding).

Table 31. Functional groups of the differentially expressed sequences, some of which may be part of two groups because some of these pathways have a cross walk**.**

Transcription Factors	Hypersensitive Response Proteins
MADS box	Carbonic anhydrase
PUR alpha 1	Cysteine proteinase
SCARECROW	
	Jasmonate Pathway
Signaling Proteins	LOX 1 (lypoxigenase)
Ca-dependent carrier	LOX 2 (lypoxigenase)
Serine threonine kinase	Phospholipase
Detoxification	Wound-Induced Proteins
Glutathione S conjugate ATPase	Fatty acid desaturase
LOX 1 (lypoxigenase)	Glutathione S conjugate ATPase
LOX 2 (lypoxigenase)	Beta glucanase
Hydrolytic Enzymes	Unclassified Proteins
Beta glucanase	CAB (Chlorophyll a/b binding protein)
	Clone RG 64 (sequence associated to Pi2)
Cell Wall Modifying Enzymes	Developmental protein
Alpha tubulin	dTDP glucose dehydratase
Beta tubulin	Fructose biphosphate aldolase
Methyltransferase	GTP- Dehydratase
O-Methyltransferase	HP 1
Xyloglucan endotransglycolase	HP 2
	HP 3
Stress-Related Proteins	HP 4
Cold-acclimatization and water-stress protein	Safener binding protein
Retrotranscriptase 1	Unknown protein 1
Retrotranscriptase 2	Unknown protein 2
SAMS (S-adenosyl L-methionine synthetase)	

Unfortunately, a high number of redundant clones representing 4 types of rRNA artifact sequences were also isolated (not shown). These sequences are an artifact of the cDNA synthesis even though we used a poly-T primer for that step. One way to prevent this in future experiments is to use mRNA as a template for cDNA synthesis. When we separated the subtraction final products in polyacrylamide gels (data not shown), we were able to see from 50-65 different bands for each hybridization.

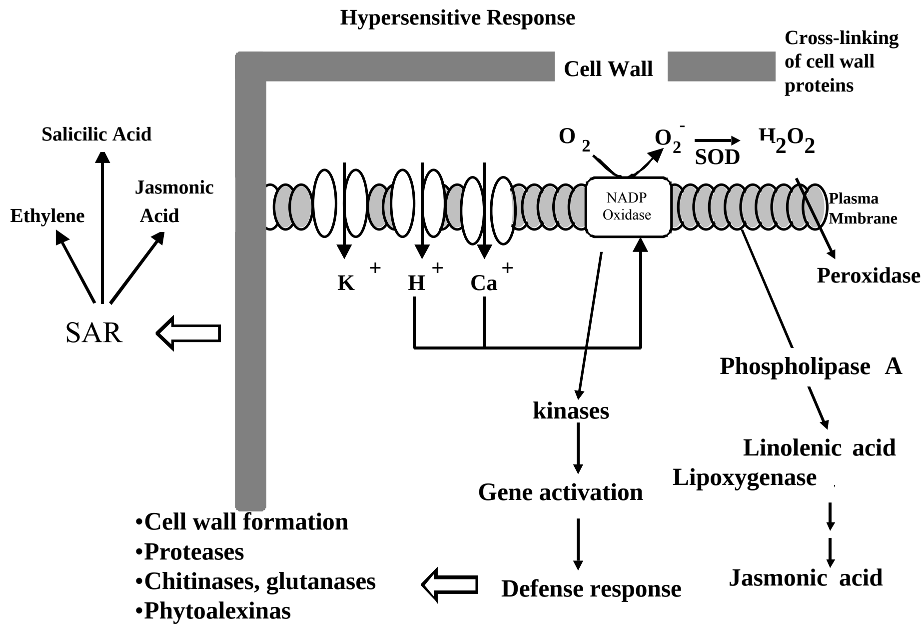


Figure 29. Outline of Hypersensitive response elements. Adapted from: B. F. Matthews, Sept 2002, Expression of Genes in Soybean Roots in Response to the Soybean Cyst Nematode, Soybean Genomics & Improvement, USDA -ARS. In http://www.ndsu.nodak.edu/virtual- genomics/conference_2002.htm

Evidently, the 96-well libraries were too small to represent well the entire set of differential sequences present after subtraction, particularly in this case where the concentration of rRNA-enriched products can "mask" the cloning of less abundant fragments. However, we applied this strategy to make a rapid inspection of the contents and quality of the subtraction. Now our goal is to construct larger libraries that exclude rRNA fragments by cloning smear excisions from polyacrylamide gels. In that way we expect to broaden the light that initial analysis of subtraction products has shed on the mechanisms of resistance to spittlebug in *Brachiaria*.

Future Activities

Isolation and mapping of new RGA sequences from less complex DNA sources and final sequence screening of the subtraction libraries

As found by Cheong et al. (2002), transient-induced expression of genes by feeding and wounding appears in 0.5-6 h after treatment, preceding the sustained expression of other effector genes. This prompted us to design a new assay that will include a subtler infestation method and a new subtraction experiment to cover the first 24 h postinfestation. In this way we will look for regulatory elements upstream of the expressed sequences that we report here. Upstream elements have a particular interest in searching for polymorphism between susceptible and resistant varieties.

The subtractive hybridization performed did not allow detection of constitutive mechanisms of resistance. Therefore we plan to perform a subtractive hybridization of cDNAs from a susceptible and resistant variety, challenging them with the insect to find out whether there is a constitutive mechanism of resistance.

2.5.2 Validation of PCR tag of "apo-locus" and initial application in *Brachiaria* breeding program

Contributors: Olga Giraldo; J. Tohme; J.W. Miles

Rationale

A reliable molecular marker of the apomixis locus would facilitate assessment of reproductive mode in large hybrid populations and improve the efficiency of the breeding program. A RAPD marker that cosegregates with apomictic reproductive mode was identified eight years ago. It is unclear whether it is sufficiently robust for routine use in the breeding program.

Materials and Methods

A total of 123 hybrid (SX x AP) selections were progeny tested. These included 107 BR00 progenies and 16 BR01 progenies. Presence/absence of the N14 marker was determined for all the BR00 progenies and for 13 of the 16 BR01 progenies.

Results and Discussion

Agreement between progeny test results and the putative marker of apomixis was good for the 107 BR00 progenies (6 inconsistencies: 94.4% agreement). However, for 13 BR01 progenies where we had data on N14, agreement was less than 50%. An additional peculiarity of the BR01 progenies is that 14 of 16 (87.5%) were classified as apomictic (or facultatively apomictic) based on uniformity of the OP progeny, rather than the approximately 1:1 segregation expected. We have no explanation for these anomalies. They suggest, however, that use of the N14 marker for routine determinations of reproductive mode is not yet possible.

Activity 2.6 Define interactions between host and pathogen in *Brachiaria*

Highlights

- With the new greenhouse methodology we found that 42 (11%) Brachiaria genotypes had higher or the same level of resistance to *Rhizoctonia solani* than the control (*B. brizantha* CIAT 16320).

- The genetic and pathogenic variability or *Xanthomonas campestris* pv. *Graminis* isolates infecting *Brachiaria* was determined.

2.6.1 Screening of *Brachiaria* hybrids for *Rhizoctonia* foliar blight

Contributors: Carolina Zuleta , John Miles and Segenet Kelemu

Rationale

Rhizoctonia foliar blight, caused by *R. solani*, is an important disease of species of *Brachiaria*. The disease can cause substantial foliar damage on susceptible genotypes. The pathogen has the ability to survive for long periods of time in the soil or on plant debris as sclerotia, which are first seen as white masses on infected plant tissues. These turn brown in color as they mature and become loosely attached. They are subsequently shed on to the soil, thus forming primary sourcesof inoculum. The search for resistant plant materials is a major part of our work in effectively managing this disease.

This year, we have standardized and greatly improved the artificial inoculation method, thus reducing the disease reaction variability among plants within the same genotype.

Materials and Methods

Pathogen isolate and Inoculum preparation. The isolates used in this study was collected from diseased *B. brizantha* accession number CIAT 6780 in the field. The fungus was grown on potato dextrose agar (PDA, Difco) in Petri plates and incubated at 28 °C. The isolate was maintained as dried sclerotia produced in PSY broth (20g peptone, 20g sucrose, 5g yeast extract in 1 L deionized water) at room temperature.

Inoculation method. Plants were propagated vegetatively and artificially inoculated 15 days after later. Inoculation was conducted by placing fresh mycelial disc of *R. solani* grown on potato dextrose agar. Mycelial discs were placed on stems approximately 2 cm above the soil line and wrapped with parafilm (Photo 8). The inoculated plants were then transferred to a humidity chamber (see Photo 9) with applications of one hour mist in the morning and another one in the afternoon. Temperatures were maintained at 30-35°C. Plants were evaluated 15 days after inoculation.

Photo 8. Inoculation of *Brachiaria* with *Rhizoctonia solani*

Photo 9. Humidity chamber for the development of rhizoctonia foliar blight disease

The upward progression of the disease was measured from the inoculation point. Visual rating of the disease was also conducted using the method described by Horsfall and Barratt (1945. An improved grading system for measuring plant diseases. Phytopathology 35:655)

Results and Discussion

Symptoms developed fully within 15 days after inoculation. All inoculated materials uniformly expressed disease symptoms (Photo 10). The upward progressions of symptoms from the inoculation points ranged from 10.33 cm in resistant materials to 37 cm in susceptible ones. Forty- two of the 381 materials evaluated showed either lower or the same disease progression as the resistant control CIAT 16320 (Figure 30).

Figure 31 shows ratings of the genotypes when both the upward disease progression and the Horsfall and Barratt methods were applied. However, these data are preliminary and a more careful analysis is needed. One has to take into consideration that the presence of the endophyte *A. implicatum* is variable among genotypes and among plants of the same genotype. In addition, plants used as recipients of the pollen of CIAT 16320, as well as the pollen donor plants themselves, have to be examined for the presence or absence of endophytes.

We hope to address these questions in the AR-2003.

Photo 10. Rhizoctonia foliar blight symptom in *Brachiaria* 15 days after inoculation with *Rhizoctonia solani.*

The new inoculation method together with the humidity conditions maintained improved homogeneity in symptom expressions within plants of the same genotype. As as result of the 383 genotypes included in the screen, 42 (% showed either higher or the same level of resistance as the control CIAT 16320).

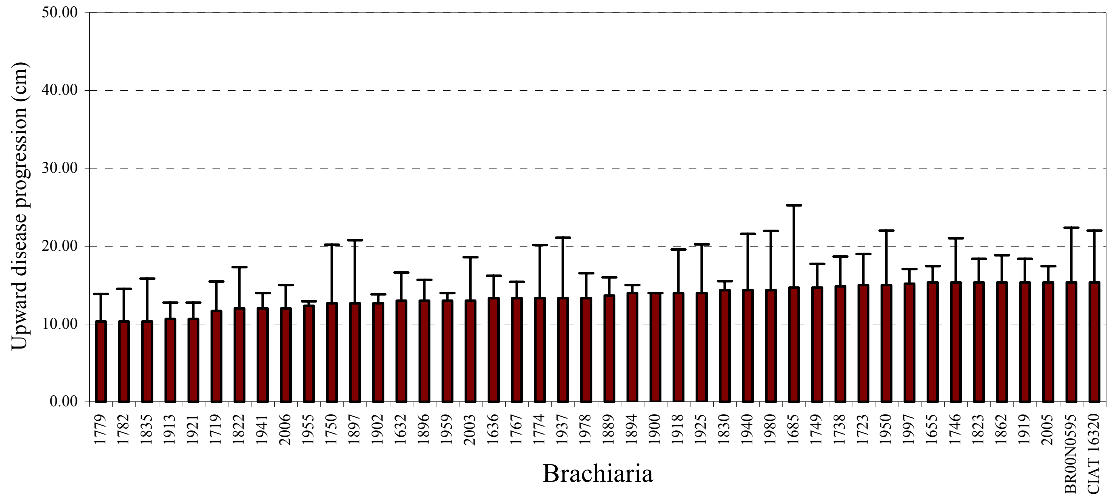


Figure 30. Disease development in resistant genotypes of Brachiaria measured as upward progression of symptoms from the inoculation point of the plant.

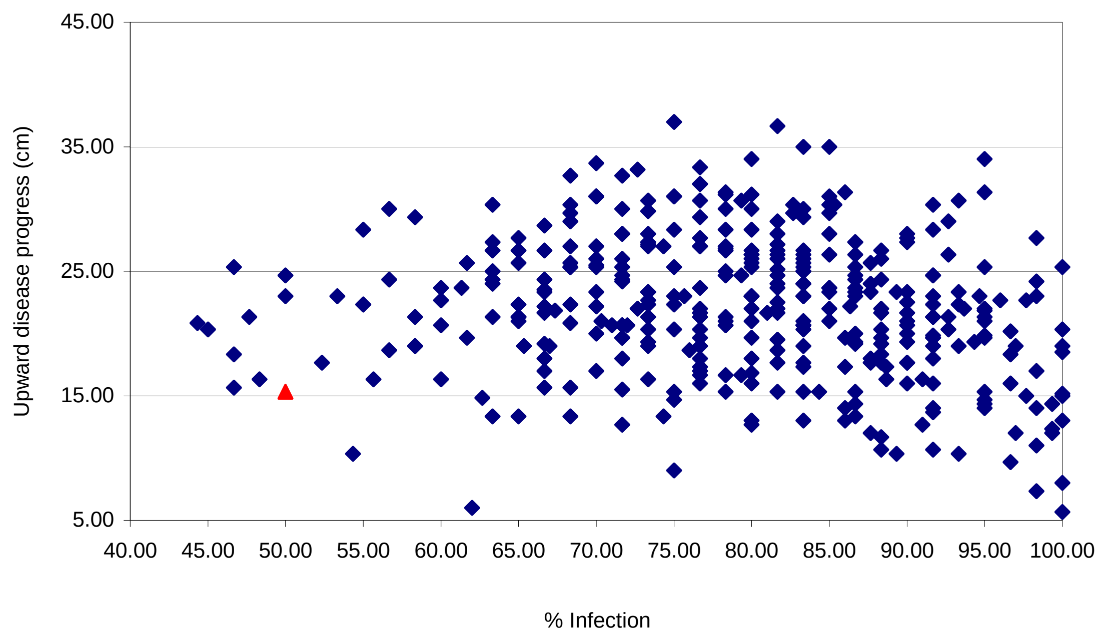


Figure 31. Ratings of *Brachiaria* genotypes using both the upward disease progression measurements and the Horsfall and Barratt methods.

2.6.2 Studies on conditions for sclerotial production and germination of *Rihizoctonia solani*

Contributors: Tomoko Sakai and S. Kelemu

Preparation of sclerotia. Sclerotia of *Rhizoctonia solani* CIAT 6780 were produced on potato dextrose agar (PDA, Difco) at 28°. Sclerotia were collected and separated from the mycelial mats, air-dried in a hood in sterile condition and sorted out by size for further use.

Germination tests. Germination of sclerotia was determined on various media, temperature and pH. Sclerotia were plated on PDA, water agar (WA; 20g agar granulated, 1L distilled water) and Czapek-dox agar (2g $NaNO_3$, 1g K_2HPO_4, 0.3g $MgSO_4$.$7H_2O$, 0.5g KCl, 0.01g $FeSO_4$, 30g sucrose and 20g agar, 1L distilled water plus 1ml of 1% $ZnSO_4$ and 0.5% $CuSO_4$). Each culture medium was adjusted to pH 3, 7 and 9. Three temperature treatments were used for incubation 23°C, 30°C and 35°C). A total of 50 sclerotia were placed on each plate. Plates were checked after 18, 24, 42, 48, 72, 90 hours of incubation to determine germination.

Production of sclerotia. One sclerotium was plated in the center of each plate for each treatment combination of incubation temperature x medium x pH. After 15 days of incubation, all sclerotia produced on each plate were harvested from the mycelial mat with tweezers, air-dried in a hood and weighed. All treatments were replicated twice. Data presented are average values.

Results and Discussion

Production of sclerotia of *R. solani* and their germination were compared on 3 culture media x 3 pH x 3 incubation temperature combinations. The sclerotia of *R. solani* CIAT 6780 germinated on all media x temperature x pH combinations, although germination was slow on media with pH 3 and at 35°C (e.g. Figures 32,A-B). Although mycelial mats were produced from germinating sclerotia on all treatments, no sclerotia were produced on any media x pH combinations at 35°C . On PDA, more sclerotia were produced at lower temperatures than higher ones, but on Czapek agar the results obtained were contrary to those on PDA (Figure 33 A-C).

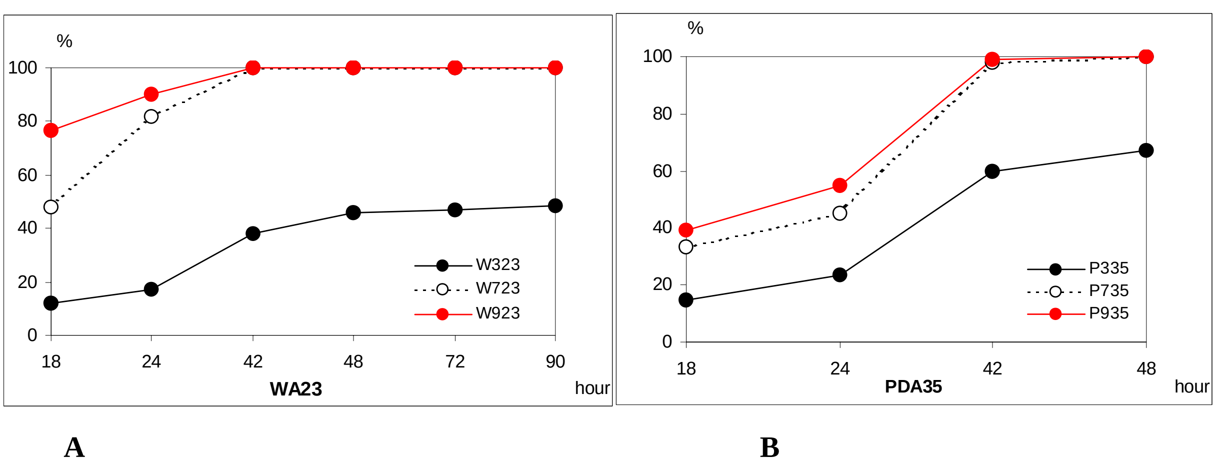
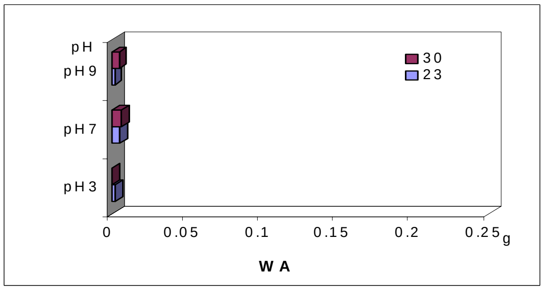
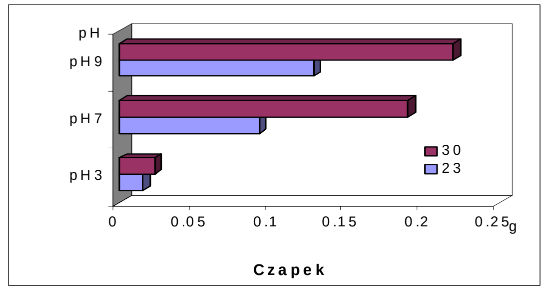


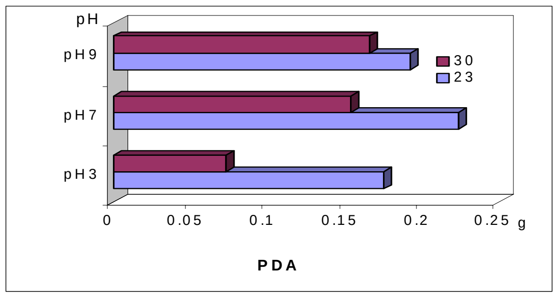
Figure 32 A-B. Percent germination of sclerotia of *Rhizoctonia solani* on three media, temperatures and pH. Key: the letter indicates the media, the number after the letter indicates the pH and the last two numbers indicate for temperature. For example, P335 is for germination of sclerotia on potato dextrose agar at pH 3 and with incubation temperature of 35 °C.



A



B



C

Figure 33 A-C. Sclerotial production by *Rhizoctoni solani* CIAT 6780 on water agar (A), Czapek (B) and potato dextrose agar (C) at three different pH and two temperatures.

The results indicate that germination of sclerotia is unaffected by substrate nutritional status, whereas production of sclerotia is influenced by nutrients. Nutritional status is also known to affect the virulence of sclerotia. Virulence of the hyphae from germinating sclerotia is dependent on the size and condition (eg. age) of the sclerotia and the quality and amount of the food base in the substrate.

2.6.3 Bacterial wilt of *Brachiaria*

Contributors: Martin Rodriguez and Segenet Kelemu

A bacterial wilt disease of *Brachiaria* and its casual agent, have been described in our earlier reports and publications. *Xanthomonas campestris* pv. *graminis* infects a number of cultivated forage grasses. Some of the first symptoms are chlorotic/necrotic stripes along the leaves. As the disease advances, the whole leaf may die. Under severe conditions, the whole plant may turn yellow and die. Another typical symptom is wilting and curling of leaves without any discoloration or lesions, which result in quick plant death.

Isolates of *X. campestris* pv. *graminis* have been collected from sites in Colombia to determine pathogenicity and genetic diversity.

Materials and Methods

Fifty-one independent colonies of X*anthomonas campestris* pv. *graminis* collected from naturally infected species of *Brachiaria* were characterized to determine their genetic variability. Infected samples (with either wilt or chlorotic symptoms along the leaf veins) were collected from fields at CIAT headquarters. Quilichao and Popayan. Leaves were cut into small pieces and surface-sterilized in1% NaOCl solution for 2 min and in 70 % ethanol for 1 min. They were then rinsed with sterile deionized water, and macerated in sterile water. A dilution series of the macerated suspension was plated on nutrient agar for selection of independent bacterial colonies. Their pathogenicity was confirmed by inoculating a susceptible material (hybrid *Brachiaria* CIAT 36062). Selected colonies were grown in nutrient broth with shaking (200 rpm) at 28 ^{o}C. They were stored in 30% glycerol at -20^{o}C. DNA isolations were conducted using standard protocols. PCR amplifications using 8 arbitrary primers (Table 32) were conducted. Conditions are as described by Kelemu et al., 1999. European Journal of Plant Pathology 105:261-272. Statistical analysis was done as described by Kelemu et al., 1999. European Journal of Plant Pathology 105:261-272.

Table 32. List of 10-base oligonucleotide primers used in this study.

Code	Sequence
OPA- 1	CAGGCCCTTC
OPA- 2	TGCCGAGCTG
OPA- 3	AGTCAGCCAC
OPA- 4	AATCGGGCTG
OPAJ- 8	GTGCTCCCTC
OPAJ- 11	GAACGCTGCC
OPC-2	GTGAGGCGTC
OPD- 3	GTCGCCGTCA

Results and Discussion

Amplifications of DNA of 51 *X. campestris* isolates with 8 primers generated a total of 228 RAPD bands. Multiple correspondence analysis generated 4 groups of isolates with an average similarity of 60% (Figure 34).

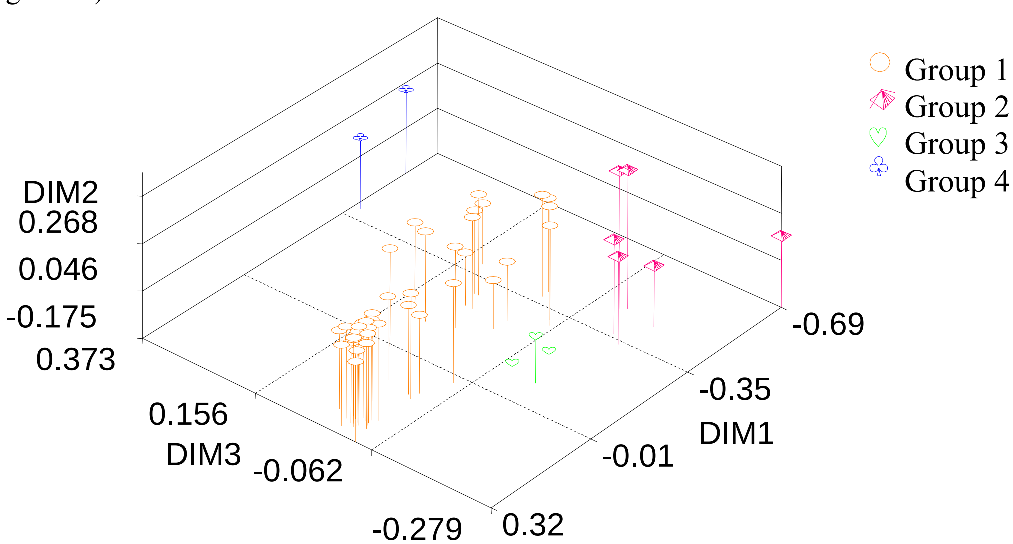


Figure 34. Multiple Correspondence Analysis of isolates of *Xanthomonas campestris* pv. *graminis* collected from Brachiaria.

Although the number of isolates studied is small, it still indicates that the variability among these isolates is great. Most of the isolates were collected at CIAT headquarters in Palmira. We have demonstrated that the pathogen is seed transmitted and also transmitted vegetatively (AR-2001). It is, thus, important to take precautions with planting materials.

Activity 2.7 Elucidate the role of endophytes in tropical forage grasses

Highlights

- A PCR-based endophyte detection was developed and successfully implemented.
- *Acremonium implicatum* is transmitted through seeds of *Brachiaria*
- The presence of *Acremonium implicatum* reduces rhizoctonia foliar blight by up to 20% in the early stages of infection in glasshouse tests.

2.7.1 Endophyte seed transmission studies in *Brachiaria*

Contributors: Huang Dongyi and Segenet Kelemu

Rationale

DNA from isolates of *A. implicatum* was amplified using 10-base random primers. Primer OPAK 10 (Operon Technology Inc.) amplified bands including a 500-bp product common to most of the isolates tested. This fragment has been cloned and sequenced (IP-5 Report 2001). Based on this sequence data, several primers were designed and synthesized. A primer pair designated P1 (5'-TTCGAATGATAAGGCAGATC-3' and P4 (5'-ACGCATCCACTGTATGCTAC-3') amplified a 450-bp product with template DNA from isolates of *A. implicatum* in pure cultures and in tissues of *Brachiaria* infected with *A. implicatum*. No amplification product was detected in plants free from *A. implicatum* or using DNA of non-endophytic fungi or the bacterium *Xanthomonas campestris* pv. *graminis*, a pathogen of species of *Brachiaria*. These primers were used to conduct seed transmission studies in plants with and without *A. implicatum*. The primer pair amplified products with template DNA of seeds harvested from *A. implicatum* infected *Brachiaria* plants, but no amplified products were observed with DNA of seeds from endophyte-free plants.

We report here the development of a PCR-based method for rapidly and specifically detecting the endophyte *A. implicatum* in *Brachiaria* seeds. We also show that the endophyte *A. implicatum* is transmitted through seeds of *Brachiaria*.

Materials and methods

Endophyte elimination. The fungicide Folicur® was used to generate endophyte-free *Brachiaria* clones. Twenty or more plantlets were propagated from a mother plant naturally or artificially infected with the endophyte. Half of these plantlets were soaked in a solution of 0.6 mL/L of Folicur® (250 g a.i./L) for 6 h to eliminate the endophyte, and the other half were left untreated to serve as controls. All plantlets were individually planted in small pots and placed in the greenhouse. Plants were examined 4-6 weeks after treatment for the presence or absence of *A. implicatum*.

DNA isolations. Fresh mycelia of endophyte isolates cultured on PDA plates, endophyte-infected or endophyte-free plant leaves, or seeds were collected and ground in liquid nitrogen for genomic DNA isolation. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

PCR. Amplifications of specific primers P1 (5'-TTCGAATGATAAGGCAGATC-3') and P4 (5'-ACGCATCCACTGTATGCTAC-3') were used in the PCR reactions. Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc.), programmed with 44 cycles for genomic DNA of endophyte pure cultures or plant leaves, and 54 cycles for DNA from *Brachiaria* seeds, of a 30 sec denaturation step at 94°C (3 min for the first cycle), follwed by 1 min at 65°C, and primer extension for 1 min (10 min in the final cycle) at 72°C. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad), stained with ethidium bromide and photographed under UV lighting.

Seed samples were collected from plants confirmed to be endophyte-infected or endophyte-free using the PCR tests with leaf DNA and fungal endophyte isolation on culture media.

Results and Discussion

In order to determine the specificity of the primer pair P1/P4, genomic DNA from isolates of *A. implicatum*, pathogenic (*Drechslera* sp. and *R. solani*) and non-pathogenic fungi (*Colletotrichum gloeosporioides*), and bacterial pathogen *Xanthomonas campestris* pv. *graminis* were used for amplifications. A single band of about 450-bp in all examined isolates of *A. implicatum* was amplified. No amplification product was observed with DNA from *Drechslera* sp., *R. solani*, isolates of *X. campestris* pv. *graminis* or isolates of *C. gloeosporioides* (Figure 35).

This result indicates that the primer pair is useful for quickly and reliably identifying and differentiating *A. implicatum* from non-endophytic fungi and bacterium associated with species of *Brachiaria*. Endophyte-containing and endophyte-free plants were also consistently differentiated using this primer combination (Figure 36).

All seed DNA from endophyte-containing plants had a 450-bp amplified product. No amplification product was detected with seed DNA from endophyte-free plants (Figure 37). These results indicate that *A. implicatum* is transmitted through seeds of Bachiaria.

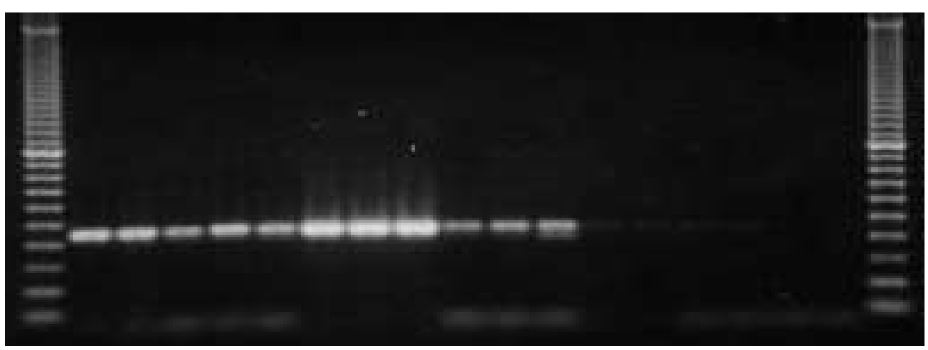


Figure 35. Specific detection of *Acremonium implicatum* isolates EH45, EH19, EH47, EH32a, EH32b, EB606, EB6780.201, EB16845.904, EB6780.501, EB16845.909, EPinedo (lanes 1-11, respectively) using polymerase chain reaction (PCR) with primer pair P1 (5'- TTCGAATGATAAGGCAGATC- 3') /P4 (5'-ACGCATCCACTGTATGCTAC- 3'). Lanes 12 and 13 are template DNA of pathogenic fungi *Drechslera* sp. and *Rhizoctonia solani*, respectively; lanes 14 and 15 are DNA from two isolates of *Xanthomonas campestris* pv. *graminis* hybrid *Brachiaria* plants BR00NO/988 and *B. ruziziensis* x *B. brizantha* CIAT 36061, respectively; lanes 16 and 17 are genomic DNA of *Colletotrichum gloeosporioides* isolates #16663 and # 16712, respectively. Lane M, 100-bp ladder.

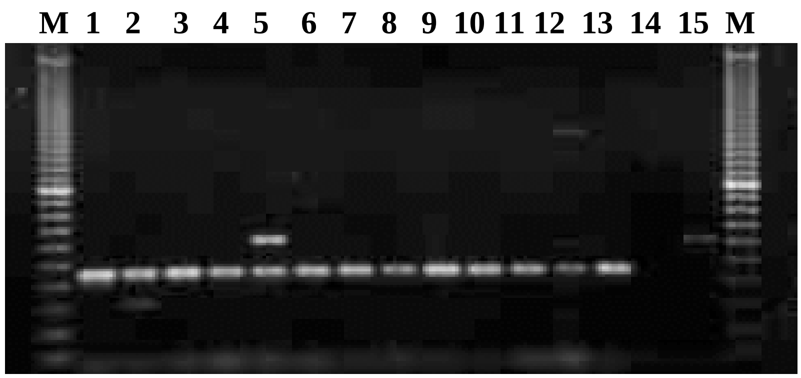


Figure 36. PCR products using primer pair P1/P4. Lanes 1~12, genomic DNA of *Brachiaria* hybrid plants *BROONO(#*7), *BROONO* (#8), *BROONO* (#14), *BROONO* (#22), *BROONO* (#23), *BROONO* (#29), *BROONO* (#37), *BROONO* (#39), *BROONO* (#42), *BROONO* (#44), *BROONO* (#48), *BROONO* (#52), respectively; lane 13, *B. brizantha* CIAT 26110 (#15), artifically inoculated with endophyte isolate EB6780 (201); lanes 14 and 15 *B. brizantha* CIAT 16320 (#32-25) and *B. brizantha* CIAT 16320 (#32-29), respectively. These were plants derived from naturally endophyte infected tillers and whose endophytes were successfully eliminated by treatment with fungicide Folicur® (0.6mL/L) for 5 hr. ; lane M = 100-bp ladder.

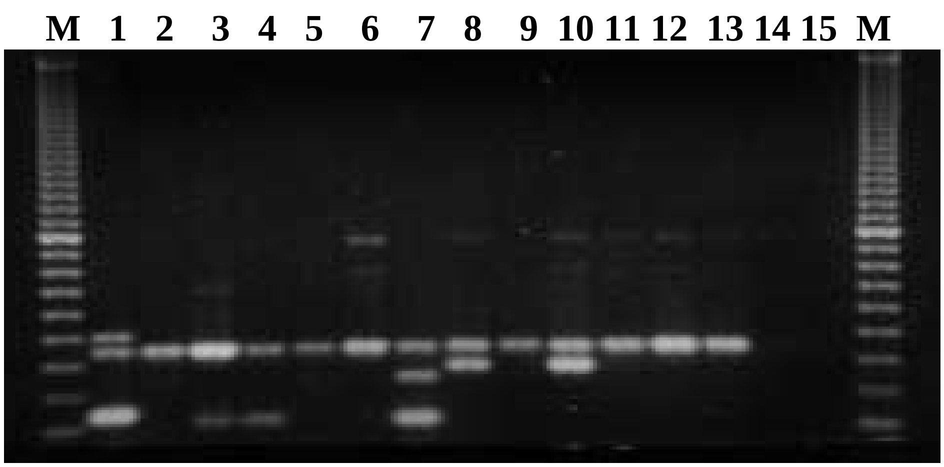


Figure 37. PCR products using primer pair P1/P4. DNA extracted from seeds of, lanes 1~12, hybrid plants *BROONO(#*7), *BROONO* (#)8, *BROONO* (#14), *BROONO* (#22), *BROONO* (#23), *BROONO* (#29), *BROONO* (#37), *BROONO* (#39), *BROONO* (#42), *BROONO* (#44), *BROONO* (#48), *BROONO* (#52), respectively; lane 13, *B. brizantha* CIAT 26110 (#15), artificially inoculated with endophyte isolate EB6780 (201); lanes 14 and 15, *B. brizantha* CIAT 16320 (#32-25) and *B. brizantha* CIAT 16320 (#32-29), respectively. These were plants derived from naturally endophyte infected tillers and whose endophytes were successfully eliminated by treatment with fungicide Folicur® (0.6mL/L) for 5 hr. ; lane M = 100-bp ladder.

2.7.2 PCR analysis and screening of *Brachiaria* genotypes for endophyte presence

Contributors: C. Zuleta, X. Bonilla, H. Dongyi, S. Kelemu, J.W. Miles (CIAT).

Rationale

Endophytic fungi form complex and fascinating associations with their host plants. These associations are often considered to be mutualistic, because these fungi provide their hosts a level of protection from biotic and abiotic stresses and the plants supply the endophytes with nutrients. Grasses with endophyte associations are reported to have better persistence under drought conditions, resistance to insects and pathogens, better vigor and other agronomic traits of applied value than endophyte-free grasses of the same genetic background.

Acremonium implicatum has been reported to have endophytic associations with species of *Brachiaria* (Kelemu and Takayama, 1998; Kelemu et al. 2001). The fungus is slow growing which often took as long as 5 weeks for the appearance of colonies on potato dextrose agar (PDA, Difco). *A. implicatum* provides species of *Brachiaria* protection from fungal pathogens such as *Drechslera* spp. (Kelemu and Takayama, 1998; Kelemu et al. 2001), and from the aphid *Rhopalosiphum maidis* (Kelemu, unpublished results). In addition, endophyte infected *Brachiaria* plants maintained better leaf expansion and produced significantly greater leaf biomass than endophyte-free plants under severe drought stress conditions (Rao and Kelemu, Annual Report 2001).

Endophytic fungi can be detected in plant tissues histochemically and immunologically. Detection of *A. implicatum* in tissues of *Brachiaria* using staining procedures, culturing of tissues on agar media or a combination of the two, is time consuming and not 100% reliable in cases of limited fungal hyphae and/or sparse hyphal distribution in plant tissues. An accurate and fast detection method is essential for determining the distribution of this and related endophytes in *Brachiaria* and for endophyte/*Brachiaria* association studies.

Polymerase chain reaction (PCR)-based detection methods have been effectively used for plant pathogens and in endophyte-plant symbiotic associations. Identifications based on morphological and culture characteristics not only take time involving isolation from infected tissues and culturing the slow-growing fungus, but also require skilled labor. In order to detect the endophyte with enhanced sensitivity and reliability, we developed a PCR-based method that detects *A. implicatum* in species of *Brachiaria*.

The objective of this study was to develop PCR primers highly specific to *A. implicatum* which would enable us to accurately and rapidly screen *Brachiaria* genotypes for the presence or absence of the fungus. We report here the identification, cloning and sequencing of an amplified product generated with random amplified polymorphic DNA (RAPD) – polymerase chain reaction. A primer pair designed based on the sequence was used for successfully detecting *A. implicatum* in *Brachiaria* tissues and for differentiation of the endophyte from other non-endophytic fungi and bacteria. Preliminary results of this work have been reported previously (Kelemu et al. 2002).

It is hypothesized that the outstanding resistance to Rhizoctonia foliar blight in *B. brizantha* accession CIAT 16320 is due to the presence of a fungal endophyte. If the resistance is due to endophytes, it should not be heritable through pollen.

Materials and Methods

Endophytic fungal isolations, and culture maintenance. Fungal isolations and culture maintenance were done as described previously (Kelemu et al. 2001). In short, leaf blades or leaf sheathes were surface sterilized and plated on PDA supplemented with 10µg/ml tetracycline and incubated for 4 to 6 weeks at 28°C. Pure cultures were maintained for long term storage by lyophilization. They were also maintained by growing the fungus on sterilized filter papers overlaid on PDA. The filter papers were removed from the agar and air-dried once the fungus covered them. They were subsequently placed in sterile envelopes and stored at -20°C. Pieces of these filter papers containing the fungus were plated on fresh PDA for DNA extractions and other experiments.

Plant inoculation with endophyte isolates *Brachiaria* seeds were surface sterilized in 3% NaOCl for 20 min, in 50% sulfuric acid for another 20 min, and rinsed three times with sterilized distilled water.

Excess moisture was removed from the samples with sterilized filter papers. Seeds were then germinated on basal MS medium (Murashige & Skoog, 1962). Cultures were maintained under sunlight next to a big glass window at room temperature (about 24°C) for about three weeks. Using a fine entomological needle, a small amount of endophyte mycelia was placed into vertically slit apical meristem of seedlings under a binocular stereomicroscope in a laminar flow hood. The wound was sealed with sterile Vaseline in order to keep the fungus in the plant. The inoculated seedlings were transferred to Magenta vessels (Sigma) containing fresh MS medium, and incubated for 15 days at room temperature with access to sunlight. All surviving plants were then transplanted to pots containing autoclaved soil and placed in a glasshouse. Inoculated plants were examined for the presence of the endophyte 8-12 weeks after inoculation by re-isolating the fungus and/or tissue staining procedures (Kelemu et al. 2001).

Endophyte elimination. The fungicide Folicur® (common name: tebuconazole; chemical name: (RS)-1-p- = chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol) was used to generate endophyte-free *Brachiaria* clones as described by Kelemu et al. (2001). Twenty or more plantlets were propagated from a mother plant already infected with the endophyte. Half of these plantlets were soaked in a solution of 0.1 mL/L of Folicur (250 g a.i./L) for 6 hours (Bacon and White, 1996) to eliminate the endophyte, and the other half were left untreated to serve as controls. All plantlets were individually planted in small pots and placed in the glasshouse. Tissues were examined 4 to 6 weeks later for the presence or absence of the endophyte.

DNA isolations. Fresh mycelia/conidia were collected from PDA plates and ground in liquid nitrogen. For detection tests in *Brachiaria*, endophyte-infected or endophyte-free plant tissues were collected and ground in liquid nitrogen. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, California). DNA concentration was determined using Hoefer DyNA Quant 200 flurometer (Pharmacia Biotech).

DNA amplifications. Eleven arbitrary 10-base oligonucleotide primers from Operon Technologies [5'-CAGGCCCTTC-3' (primer code A-01), 5'-TCGCTGCGGA-3' (AN-07), 5'-GGGAACCCGT-3' (AN-06), 5'-CAAGCGTCAC-3' (AK-10), 5'-AGCCGGGTAA-3' (AN-14), 5'-AGTGTAGCCC-3' (AK-12), 5'-TCGCAGCGAG-3' (AK-19), 5'- ACGGGTCAGA-3' (AJ-01), 5'-GAATGCGACC-3' (AJ-04), 5'-CAGTTCCCGT-3' (AJ-12), and 5'-CAGCCGTTCC-3' (AJ-13)] were used for PCR amplifications. PCR reactions (25 µl) contained 0.2 mM dNTPs, 3.75 mM $MgCl_2$, 0.5 µM primers, I unit of Taq DNA Polymerase, 1X PCR buffer, and 20-60 ng of template DNA. Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc.) programmed with 45 cycles of a 1 min (2 min for the first cyle) denaturation step at 94 °C, annealing for 1 min at 35 °C, and primer extension for 1 min (7 min in the final cycle) at 72 °C. The amplification products were separated by electrophoresis in a 1.2% agarose gel (Bio-Rad), stained with ethidium bromide, and photographed under UV lighting.

Cloning and sequencing of PCR products. An approximately 500 bp product, one of the amplified fragments with arbitrary primer AK10, and common to most of the endophyte isolates tested, was recovered from the agarose gel using Wizard® DNA Clean-Up System (Promega, Madison, WI). This fragment was ligated into linearized pGEM® -T vector (Promega) according to instructions by the manufacturer. The ligation mixture was transformed into competent *Escherichia coli* DH5α, prepared using standard calcium chloride procedure (Sambrook et al. 1989). Mini- plasmid preparations were conducted from ampicillin-resistant blue white colonies (Sambrook et al. 1989) for further confirmation. A positive clone was sequenced using the Dye Terminator Cycle Sequencing Kit and an Applied Biosystems Prism 377 DNA sequencer (Perkin-Elmer Corporation, California), and edited with Sequencher (Genecodes, Ann Arbor, MI).

DNA hybridization An approximately 500-bp RAPD-PCR amplification product was recovered from the gel and digoxigenin (DIG)-labeled using DIG high primer DNA labeling and detection system (Roche, Mannheim, Germany). The system uses digoxigenin to label probes (random primed labeling) for hybridization and chemiluminescence detection by enzyme immunoassay. For dot blot analysis, 5 μg of denatured genomic DNA was placed on nylon membrane (Amersham, Little Chalfont) using a vacuum dot blot microfiltration apparatus (Bio-Rad). Each well was washed with a 1.5 M NaCl solution and the membrane rinsed in 2x SSC (0.3 M NaCl, 0.0.03 M sodium citrate). DNA was fixed to the membrane by UV-crosslinking (UV Stratalinker 1800, Stratagene). All labeling, hybridization and detection were performed according to the manufacturer's instructions.

Synthesis of primers

Based on the sequence data, orders were placed to synthesize 7 primers with the following sequences: 5'- TTCGAATGATAAGGCAGATC- 3' (primer 1), 5'- CGTCATGACAGGAGTATAGG- 3' (primer 2), 5'- CGAGGAGCACTTTGCGGATG -3' (primer 3), 5'- ACGCATCCACTGTATGCTAC- 3' (primer 4), 5'- TGAGAAGACCTCTTGTTATG- 3' (primer 5), 5'- GTCCAGCTGTCTCGTCACTA- 3' (primer 6), 5'-GGAGTACATAGTTTCACCCG-3' (primer 7), and synthesized by QIAGEN Operon Technologies, Inc. (USA).

Results and Discussion

RAPD analysis. After preliminary testing of 3 of the *A. implicatum* isolates with 30 arbitrary 10-base oligonucleotide primers, 11 primers were selected and used to evaluate all of the endophytic isolates from species of *Brachiaria*. A total of 220 bands were generated with the 11 primers. Comparisons of each banding profile for each primer were conducted on the basis of presence or absence (1/0) of RAPD products of the same size. Matrices were generated and analyzed using NTSYS-pc, Dice coefficient and UPGMA clustering method to produce a similarity dendrogram. The similarity clustering showed that isolates EH45, EH19 and EH47 were clonal. The same conclusion was reached using amplified fragment length polymorphism (AFLP) analysis (data not shown). Isolates EH32a and EH32b were identical to each other. The two groups' similarity coefficient was above 0.80. Isolates EB6780 (201) and EB16845 (904) were identical and very similar to isolate EB606 with a coefficient of more than 0.9. Isolates EB6780 (501), EB16845 (909) and EPinedo were distinct from each other and from the rest of the isolates, with less than 40% similarity.

Dot blot analysis. The random 10-mer primer AK-10 produced products that were amplified by all the tested isolates of *A. implicatum*, with a 500-bp product common to most of the isolates. This PCR product was excised and eluted from the gel. In order to evaluate this fragment, dot blot analysis was performed using this fragment as a probe. The results indicated that the fragment gave rise to a strong signal with DNA from all endophyte isolates, but not from non-endophytic fungi associated with *Brachiaria* or with RAPD amplified DNA from *Brachiaria* free of endophytic fungi. The non-endophytic fungi selected and included in the test were *Drechslera* sp. and *Rhizoctonia solani* CIAT 6780. The fungus *Drechslera* sp. causes a leaf spot disease in species of *Brachiaria* in parts of Colombia. *R. solani* is the causal agent of foliar blight disease of *Brachiaria* with wide distribution in many areas of *Brachiaria* production. It is important to include these common pathogens of *Brachiaria* in the test in order to make sure that the DNA fragment of interest does not hybridize with DNA from these pathogens.

Polymerase chain reaction analysis. The selected PCR product was cloned in a pGEM®-T easy vector, and sequenced (Figure 38) generating a clone coded *Ai*AK10-500 (see materials and methods). Based on the sequence of the clone, 7 primers were designed: P1 (5'-

TTCGAATGATAAGGCAGATC-3’), P2 (5’-CGTCATGACAGGAGTATAGG-3’), P3 (5’-CGAGGAGCACTTTGCGGATG-3’), P4 (5’-ACGCATCCACTGTATGCTAC-3’), P5 (5’-TGAGAAGACCTCTTGTTATG-3’), P6 (5’-GTCCAGCTGTCTCGTCACTA-3’), P7 (5’-GGAGTACATAGTTTCACCCG-3’). It is important to note that primers P2, P4, and P6 are complementary sequences to the regions indicated in the clone’s sequence (Figure 38), 5’-CCTATACTCCTGTCATGACG-3’, 5’- GTAGCATACAGTGGATGCGT- 3’, 5’-TAGTGACGAGACAGCTGGAC- 3’, respectively. The seven primers were used, individually and in pairs, for amplifications of genomic DNA from isolates of *A. implicatum* and pathogenic (*Drechslera* spp, *Rhizoctonia solani*) and non-pathogenic fungi (*Hyalodendron* sp., *Colletotrichum gloeosporioides*). The fungus *Hyalodendron* sp. was isolated from *B. brizantha* accession CIAT 26110. *C. gloeosporioides* is non-pathogenic on *Brachiaria* sp., but it is the most important pathogen of *Stylosanthes guianensis*, a forage legume. *Brachiaria* is sometimes grown in mixed stand with legumes such as *S. guianensis* to enhance forage and soil quality. The bacterial pathogen *Xanthomonas campestris* pv. *graminis* was also included in the test. This pathogen causes bacterial wilt disease in species of *Brachiaria*.

CAAGCGTCACGGAGTACATAGTTTCACCCGGTGTTCGAATGATAAGGCAGATCCGAGGAG
CACTTTGCGGATGAGAAGACCTCTTGTTATGCCGGAGGGGGGTTGTTGGAGTTACGCTGA
TCTCTATGAGCCCAGATTATTAGAGGAGGAGACGAGTTGGAAATTTGAGTCCCGTCGCCC
CTTCCCGACTTCTGACCAGACTGTGGTGACCACTTGCGCCTGCCATTGCCACCTGGCGTC
CCGACTGTTGCCAACCCAGTTTTCTTCCATGGCCGGCTATGCTCAGTCTTGCATTTGTGAG
AAAAGAAAGATCCTGCTCTCTATTCGTGCAGCGAAAAATGTTTGCGGAACCCCCTTTCACC
CCTTGGATGCCGATCCATGCGCGACTTCCTCTTGGTTGATCTAGCTGGTGTTATNCACATG
TCCCGATCTCGCCTTCTTGTACTAGTAGTGACGAGACAGCTGGACCGTAGCATACAGTGG
ATGCGTCCTATACTCCTGTCATGACGGTGACGCTTG

Figure 38. Sequence data and primer selection on clone *Ai*AK10-500.
5'- TTCGAATGATAAGGCAGATC- 3' (P1), 5'- CGTCATGACAGGAGTATAGG- 3' (P2), 5'-CGAGGAGCACTTTGCGGATG -3' (P3), 5'- ACGCATCCACTGTATGCTAC- 3' (P4), 5'-TGAGAAGACCTCTTGTTATG- 3' (P5), 5'- GTCCAGCTGTCTCGTCACTA- 3' (P6), 5’-GGAGTACATAGTTTCACCCG-3’ (P7). Note that primers P2, P4, and P6 are complementary sequences to the regions indicated in the clone’s sequence, 5’-CCTATACTCCTGTCATGACG-3’, 5’-GTAGCATACAGTGGATGCGT- 3’, 5’- TAGTGACGAGACAGCTGGAC- 3’, respectively.

Of the primers synthesized and tested, the primer pair (P1/P4) amplified a single band of approximately 500-bp in all examined isolates of *A. implicatum* (see Figure 35 under seed transmission studies). This primer pair amplified no visible band from *Drechslera* sp., *R. solani*, isolates of *X. campestris* pv. *graminis* or isolates of *C. gloeosporioides*. This indicates that the primer pair is useful in identification and differentiation of *A. implicatum* from non-endophytic fungi quickly and reliably.

Detection of *A. implicatum* in infected species of Brachiaria. The validity of the polymerase chain reaction (PCR) with primer pair P1 (5'- TTCGAATGATAAGGCAGATC- 3') /P4 (5'-ACGCATCCACTGTATGCTAC- 3') for detection of *A. implicatum* in *Brachiaria* plants was further tested. DNA isolated from plants naturally infected with *A. implicatum* was amplified with the primer pair. Results showed that samples from all plants with *A. implicatum* had a 500-bp amplified product.

No amplification product was detected with DNA extracted from *Brachiaria* tillers which originated from a mother plant whose endophytic fungus was removed with the fungicide folicur (Figura 39). This 500-bp fragment was also consistently amplified with DNA extracted from *Brachiaria* plants successfully inoculated with the endophyte (Figure 40).

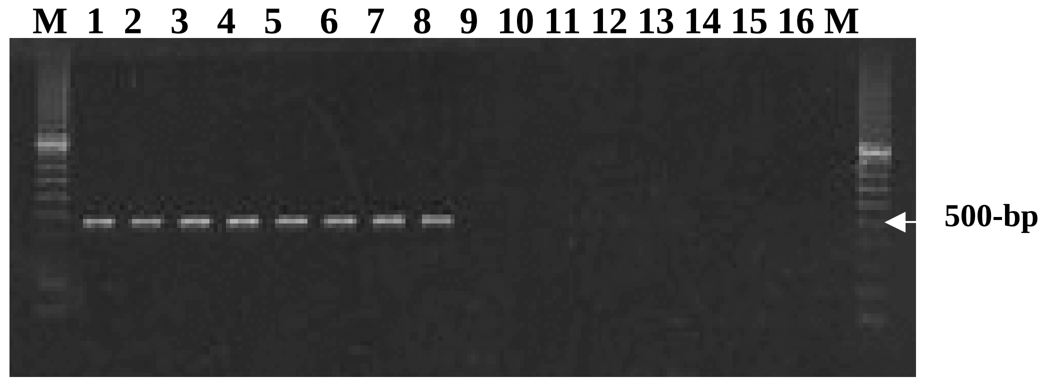


Figure 39. Detection of *Acremonium implicatum* in *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1 (5'- TTCGAATGATAAGGCAGATC- 3') /P4 (5'- ACGCATCCACTGTATGCTAC- 3'). Lanes 1-8, DNA from endophyte-containing plants; lanes 9-16, DNA from endophyte-free plants. Endophyte-containing plants were treated with the fungicide folicur in order to eliminate the endophyte and create endophyte-free clones. Lane M, 100-bp ladder.

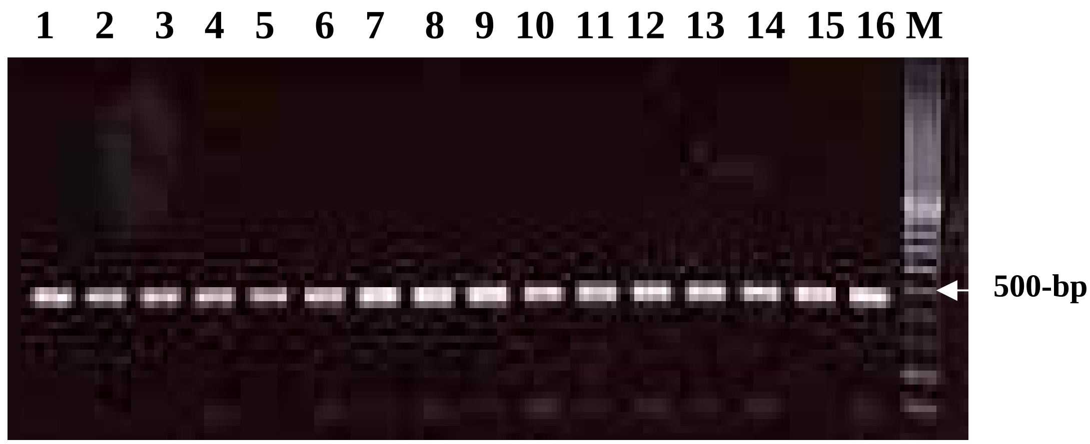


Figure 40. Detection of *Acremonium implicatum* in naturally infected or artificially inoculated *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1 (5'- TTCGAATGATAAGGCAGATC- 3') /P4 (5'- ACGCATCCACTGTATGCTAC- 3'). DNA was isolated from both leaf sheaths (lanes 1,3,5,7,9,11,13,15) and leaf blades (2,4,6,8,10,12,14,16). Lanes 1 and 2, DNA extracted from hybrid plant FM9503/S046/024 (19); lanes 3 and 4, from *B. brizantha* CIAT 16320 (32a); lanes 5 and 6, from hybrid plant FM9503/S046/024 (45); lanes 7 and 8, hybrid SX99/2341 (47); lanes 9 and 10, from *B. brizantha* CIAT 26110 (15); lanes 11and 12, from *B. brizantha* CIAT 6780 (56); lanes 13 and 14, from *B. brizantha* CIAT 6780 (63); lanes 15 and 16, *B. brizantha* CIAT 6780 (111); lane M, 100-bp ladder. Plants *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (63), and *B. brizantha* CIAT 6780 (111) were artificially inoculated with *A. implicatum*. All others were naturally infected.

It appears that the endophyte is relatively more concentrated in leaf sheaths than in leaf blades of *Brachiaria*. As a result, we have had more successes of isolating the fungus from leaf sheaths than from leaf blades of infected plants, indicating that isolations are not always successful from any parts of infected plants as the endophyte is unevenly distributed in the plant. This problem was solved by using the primer pair P1/P4 in this PCR-based detection method. The 500-bp product was consistently generated from both leaf blades and leaf sheaths of various genotypes of *Brachiaria* infected with *A. implicatum* (Figure 40). These results were verified using tedious and repeated culturing and tissue staining methods.

The results presented in this paper clearly demonstrate that this PCR-based method is a reliable technique to specifically detect genetically distinct *A. implicatum* isolates in *Brachiaria* as well as in pure cultures. It also effectively differentiates *A. implicatum* from important *Brachiaria* pathogens such as *R. solani*, *Drechslera* sp., and *X. campestris* pv. *graminis* and other non-pathogenic fungi associated with *Brachiaria*, thus providing an accurate and quick identification of the endophytic fungus *A. implicatum* because no amplified products were detected in these major fungi associated with *Brachiaria*, thus reducing the need for skilled labor in fungal taxonomy.

This PCR-based method has several advantages: It is quick, enabling one person to screen more than 100 samples per day. It is sensitive which uses only small amount of sample from either leaf blades or leaf sheaths. It is simple to apply and one does not have to have a special mycological taxonomic skill to identify the presence of the endophyte. It is either the presence or absence of an amplified product which determined the presence or absence of the endophyte.

Conclusion

An endophytic association of *Acremonium implicatum* with species of *Brachiaria* has been identified. DNA from isolates of *A. implicatum* was amplified using the random amplified polymorphic DNA (RAPD) technique with arbitrary 10-mer primers. A 500-bp polymerase chain reaction (PCR) product amplified with primer OPAK10 and common to most of the isolates of *A. implicatum* was selected for further evaluation. The fragment was digoxygenin-labeled and used to probe a dot blot containing genomic DNA of isolates of *A. implicatum*, non-endophytic fungi and species of *Brachiaria*. Strong signals were obtained with DNA from *A. implicatum* isolates, but not with non-endophytic fungi or species of *Brachairia* free from endophytes. This fragment was cloned and subsequently sequenced. Based on this sequence data, two specific primers, P1 (5’-TTCGAATGATAAGGCAGATC-3’) and P4 (5’-ACGCATCCACTGTATGCTAC-3’) were synthesized. The primer pair amplifies a single fragment of about 500-bp from DNA of isolates of *A. implicatum* whether from pure culture or in association with *Brachiaria* plants. No amplification product was detected using DNA from endophyte-free plants, pathogenic fungi, *Xanthomonas campestris* pv. *graminis* and non-pathogenic fungi associated with *Brachiaria*. This assay allows precise and rapid detection of endophytes in *Brachiaria* plants and permits differentiation between endophytic and non-endophytic fungi.

2.7.3 Effect of *Acremonium implicatum* on fungal pathogens

Contributors: Huang Dongyi (South China University of Tropical Agriculture), S. Kelemu (CIAT)

Isolates of *Acremonium implicatum* have endophytic associations with species of *Brachiaria*. One such association resulted in resistance in *Brachiaria* to a leaf spot disease caused by Drechslera sp. (Kelemu et al. 2001. An endophyte of the tropical forage grass *Brachiaria brizantha*: Isolating, identifying, and characterizing the fungus, and determining its antimycotic properties. Canadian Journal of Microbiology 47:55-62). The reactions of endophyte-containing and endophyte free plants to *Rhizoctonia solani,* causal agent to rhizoctonia foliar blight disease of *Brachiaria* were studied.

Materials and Methods

Fungal culture maintenance, growth conditions, and creation of endophyte-free plants are as described by Kelemu et al. 2001 (Canadian Journal of Microbiology 47:55-62.) Genetically identical endophyte-containing (45 plants) and endophyte-free (45 plants) plants were inoculated with mycelial discs of *Rhizoctonia solani* (see exact method in this report on “Screening for resistance to rhizoctonia foliar blight”).

Disease evaluations were done at 7, 14 and 21 days after inoculations. The upward progressions of the disease were measured in cm from the inoculation point.

Results and Discussion

In i*n vitro* inhibition tests, most of the endophyte isolates inhibited the growth of *Drechslera sp.* and *R. solani* inhibition zone of up to 13 mm, depending on the endophyte isolate. *A. implicatum* isolates EB 6780(501) and EH 32a had the strongest growth inhibiting capacity to both *Drechslera sp.* and *R. solani*. Some of the isolates have stronger inhibitory effects on the slower growing fungus *Drechslera* than on *R. solani*.

Endophyte-containing and endophyte-free Brachiaria plants showed significant differences in their reactions to *R. solani* at 7 days after inoculation with differences diminishing at 14 and 21 days after inoculation (Figure 41) when plants were kept continuously in humidity chambers. The presence of endophytes in *Brachiaria* reduces infection by up to 21% at the early stages of interaction. This may be quite significant under field conditions where humidity conditions are not as optimum as were kept in the glasshouse. It is important to indicate that there was correlation between *in vitro* and in vivo tests, in that the isolates with strong *in vitro* inhibitory effects showed strong plant protection.

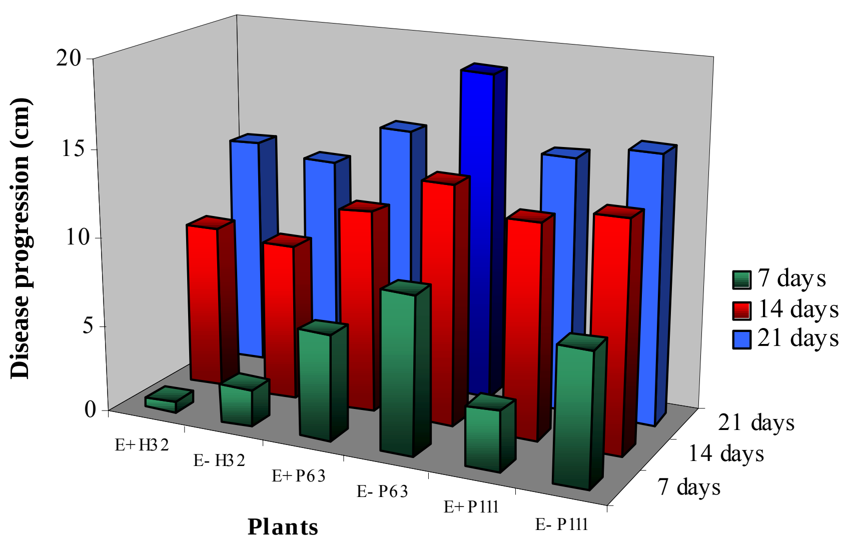


Figure 41. Disease progression measured in endophyte-infected and endophyte-free plants of *Brachiaria brizantha* inoculated with *Rhizoctonia solani* CIAT 6780. *E+* H32: *B. brizantha* CIAT 16320 infected with *Acremonium implicatum* isolate EH32a, *E-* H32: endophyte-free *B. brizantha* CIAT 16320; E+ P63: *B. brizantha* CIAT 6780 infected with *A. implicatum* isolate EB 6780 (201), E- P63: endophyte-free *B. brizantha* CIAT 6780; E+ P111: *B. brizantha* CIAT 6780 infected with *A. implicatum* isolate EB 6780(501), E- P111: endophyte-free *B. brizantha* CIAT 6780.

Endophyte-containing plants had fewer and smaller lesions caused by *Drechslera* sp. than endophyte-free plants (Kelemu et al. 2001. Canadian Journal of Microbiology 47:55-62). *Drechslera* grows slower and it is a lot less aggressive than *Rhizoctonia solani*. The infection processes implemented by the two fungi are also different. The difference between host resistance and susceptibility is the speed and the quantity of defense arsenals produced. The defense produced by the endophyte in *Brachiaria* may be sufficient enough to significantly reduce infection at early stages, but probably not at enough pace as the aggressive growth of *R. solani*.

As we indicated above, under field conditions it may be unlikely to have optimum conditions (continuous high humidity, pathogen contact with every plant, etc) for such a prolonged time and as a result the protection provided by *A, implicatum* may have a bigger effect than what was observed under optimal glasshouse conditions. Field trials are in progress to examine the level of protection under field conditions.

2.7.4 Drought tolerance in endophyte-infected plants under field conditions

Contributors: S. Kelemu, I. M. Rao, X. Bonilla, C. Zuleta, C. Plazas and J. Ricaurte (CIAT)

Rationale

Under severe drought stress conditions with soil-grown plants in the greenhouse, we showed that endophyte infected plants maintained better leaf expansion and produced significantly greater leaf biomass (IP-5 Annual Report, 1999; 2000). This year, to validate the findings from the greenhouse study, we initiated a field study in the Llanos of Colombia to quantify the impact of endophytes in improving drought tolerance and persistence in *Brachiaria*.

Materials and Methods

A field trial was established at Matazul farm in May of 2002. The trial included 2 accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). Plantlets were propagated from the original mother plant containing the endophyte *Acremonium implicatum* (J. Gilman and E. V. Abbott) W. Gams. Half of these plants were treated with the fungicide (Folicur) to eliminate the endophyte (method described in Kelemu et al. 2001. Canadian Journal of Microbiology 47:55-62) while the remaining half was left untreated. The trial was planted as a randomized block in split-plot arrangement with the presence or absence of endophytes as main plots and two accessions as subplots with 3 replications. The plot size was 5 x 2 m.

The trial was established with low levels of initial fertilizer application (kg/ha: 20 P, 20 K, 33 Ca, 14 Mg, 10 S) that are recommended for establishment of grass alone pastures. A number of plant attributes including forage yield, green leaf production, dry matter distribution and nutrient uptake will be measured during wet and dry season for the next 3 years.

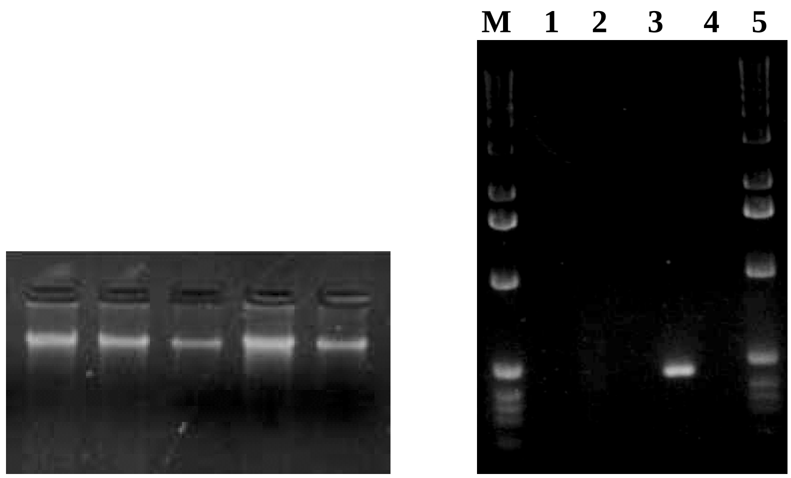
Results from this activity will be reported next year.

2.7.5 Search for new endophytes in *Brachiaria* and characterization.

Contributors: X. Bonilla and S. Kelemu

Slow-growing fungi were isolated from seeds of *Brachiaria* in an attempt to look for new isolates of endophytic fungi. Seeds were surface sterilized using standard methods and plated on potato dextrose agar (PDA). Three slow-growing fungi were isolated from seeds of CIAT 16809, CIAT 16457 and CIAT 26745. DNA was isolated from these isolates using a protocol described by Doyle and Doyle (1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15). (Figure 42).

The results showed that none of the three slow-growing fungi tested positive with the specific primers used.



A B

Figure 42. A) DNA extracted from slow-growing fungi asscociated with seeds of *Brachiaria*; B) PCR amplifications using primers developed for specific detection of *Acremonium.implicatum*. Lanes 1-5, fungi isolated from seeds of CIAT 16809, CIAT 164573, CIAT 26745, *Acremonium implicatum* 6780, negative control, respectively. M, 1Kb ladder.

Activity 2.8 Define interactions between host and pathogen in *Arachis* and *Stylosanthes*

Highlights

- One hundred sixty isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes guianensis* were characterized.

2.8.1 Biodiversity studies on the anthracnose pathogen of *Stylosanthes*

Contributors: Martin Rodriguez and Segenet Kelemu

Rationale

Anthracnose is a disease of economic importance on many economically important plants. The pathogen, *Colletotrichum gloeosporioides*, is both complex and heterogeneous. After several years of research on the anthracnose disease and the pathogen, which resulted in numerous publications, training of several young scientists, and the identification of several anthracnose resistant materials, we decided to conclude this work and shift our focus on other problems. What follows is a summary of more findings.

Materials and Methods

We have included and analyzed data on characterization of 160 isolates of *Colletotrichum gloeosporioides* (more than 200 isolates characterized last year) bringing the total number of isolates to 366. The genetic variability of these isolates was determined by random amplified polymorphic DNA (RAPD) and AFLP. The isolates were collected mostly in Colombia. Methods used for fungal culture maintenance, DNA isolations and PCR amplification conditions, plant inoculations and evaluations, and data analysis were as described earlier (IP-5 annual report 2001; Kelemu et al. 1996. Virulence spectrum of South American isolates of *Colletotrichum gloeosporioides* on selected *Stylosanthes guianensis* genotypes. Plant Dis. 80:1355-1358; Kelemu et al. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105:261-272). Eleven differential hosts were used to determine pathogenic variability. Comparisons of banding profiles for each primer were conducted on the basis of presence or absence (1/0) of amplified products of the same size, scoring bands of the same size as identical (eg. Figure 43). An example of AFLP gel picture (6 % polyacrylamide gel) is shown in Figure 44.

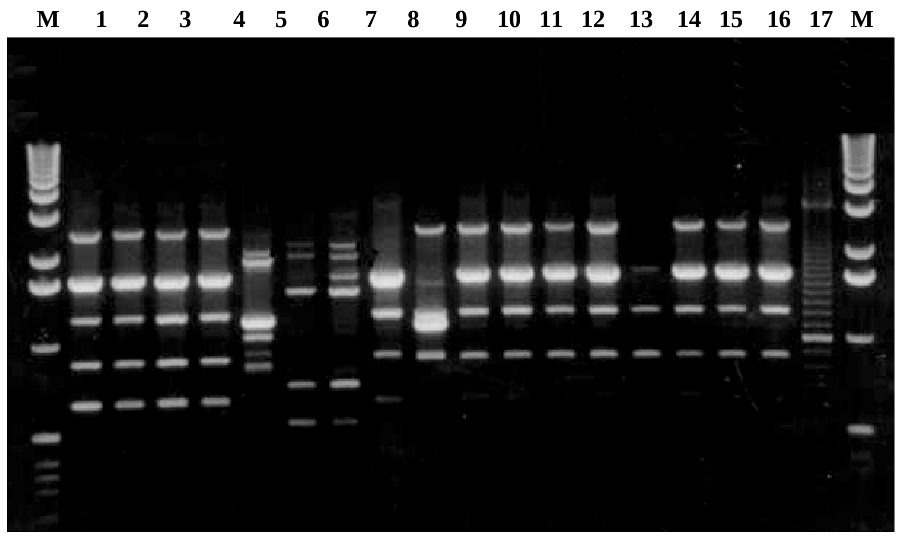


Figure 43. DNA from isolates of *Colletotrichum gloeosporioides* amplified with arbitrary primer AJ-4 (5'- GAATGCGACC –3'), lanes 1-17; M, 1kb DNA ladder (GIBCO); N , 100- bp EZ load marker (BIO-RAD).

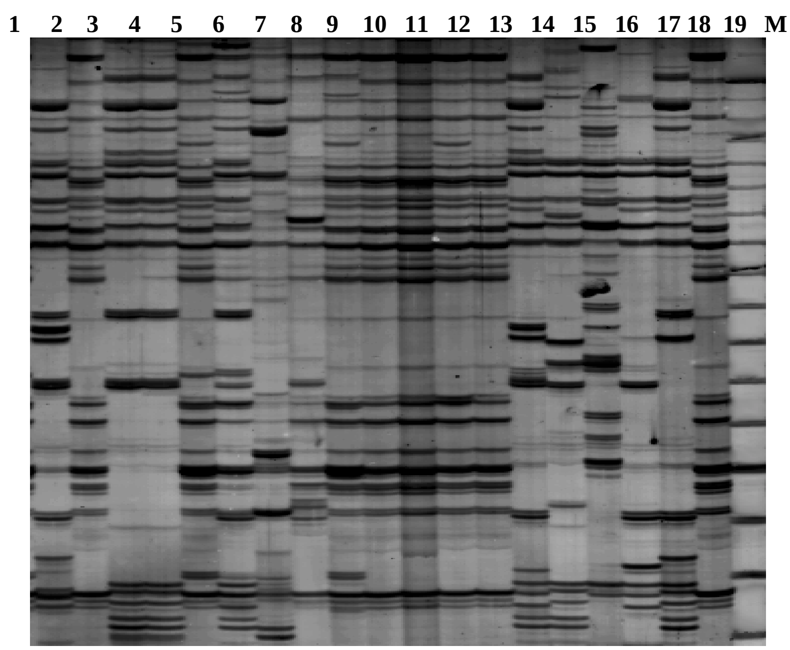


Figure 44. AFLP profile, of isolates of *Colletotrichum gloeosporioides*, generated with combination of primers **E**-AC **M**-A, lanes 1-19; M, size marker 30-330 AFLP DNA ladder (GIBCO).

Results and Discussion

Based on their interactions with the differential hosts, the isolates of *C. gloeosporioides* were grouped into 183 races. Eight of the isolates tested were incompatible with all of the 11 differentials. Isolates 16066 (Carimagua), 16405 (Brazil), 16097 (Caquetá) and 16095 (Caquetá) infected all the differentials, with *S. guianensis* accession CIAT 184 being the least susceptible. The same trend was observed with Caquetan isolates 16114, 16166, 16098 and 16112, an isolate from Quilichao (16755), and Carimaguan isolates 16212 and 16211 with the exception that the least susceptible of the differentials was accession CIAT 18750.

Five isolates from Caqueta and two from Quilichao were pathogenic on all the differentials. The most susceptible differential was CIAT 2312 followed by cultivar Endeavour. CIAT 2340 was the most resistant followed by CIAT 1507.

No correlation between pathogenicity groups and groups based on AFLP and RAPD data was observed. However, a high degree of variability was evident in all cases in the pathogen population studied. There was a high degree of correlation (r = 80.7%) between AFLP and RAPD data.

Multiple correspondence analysis of the AFLP data produced 13 groups with an average similarity of 63% (Figure 45). Group 1 is the largest of the groups with 194 isolates with an average similarity of 35%. Group 2 has 32 isolates and it is a group consistently clustered together both with AFLP and RAPD data and independent of the method of data analysis. All the isolates in this group with the exception of one were collected from Quilichao. However, the average similarity of this group is only 49%. Groups 3 and 4 also consist of large numbers of isolates, whereas group 5 has only 5 isolates all from Palmira and collected from one accession, but with a relatively low average similarity of 43%. The rest of the groups, with the exception of group 9 (20 isolates), each contains only a few isolates and with a tendency to be grouped by geographic origin.

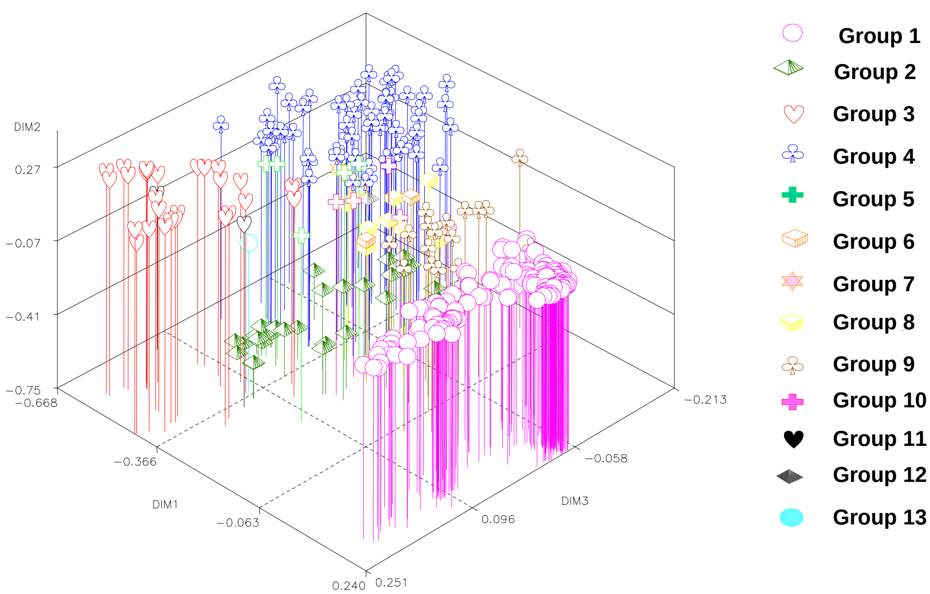


Figure 45. Clusters of isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes guianensis* based on multiple correspondence analysis of AFLP data.

RAPDs. Multiple correspondence analysis of RAPD data clustered the isolates into 10 groups with an average similarity of 65% (Figure 46). Group one, containing 205 isolates, is the largest of the groups with an average similarity of 43%. Members of this group, with a few exceptions, correspond to those of group 1 of the AFLP data. Group 2 consists of 38 isolates with an average similarity of 59%. This group has also (with a few exceptions) the same isolates as group 2 of the AFLP data. Groups 3 and 4 consist of 11 and 71 isolates with average similarities of 73% and 67%, respectively. Groups 5-10 contain only a few isolates each.

The majority of the isolates were collected in Quilichao from a field with a wide range of *Stylosanthes* genotypes. A wide genetic variability was observed among the isolates.

We separated the isolates based on the location they came from and analyzed the data. There were 18 isolates collected in Palmira, and interestingly these isolates formed groups based on collection year (those collected in 1993 and those in 1994). All of these isolates with the exception of one were collected from *S. guianensis* CIAT 184. The 40 isolates collected in Caquetá showed the same trend, isolates collected in 1994 grouping separately from those of 1995. The 162 isolates collected from Quilichao were also grouped by collection year, especially those collected between 1994 and 1999.

In summary, there was no correlation between pathogenicity and AFLP or RAPDA data. This finding is in agreement with our previous findings (Kelemu et al. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105:261-272). The results of this study also further confirm our previous findings that South American contains a diverse population of *C. gloeosporioides*. The isolates collected in Caqueta showed the least variability in contrast to those collected in Quilichao.

This study concludes our research on anthracnose of *Stylosanthes* and a manuscript is in preparation.

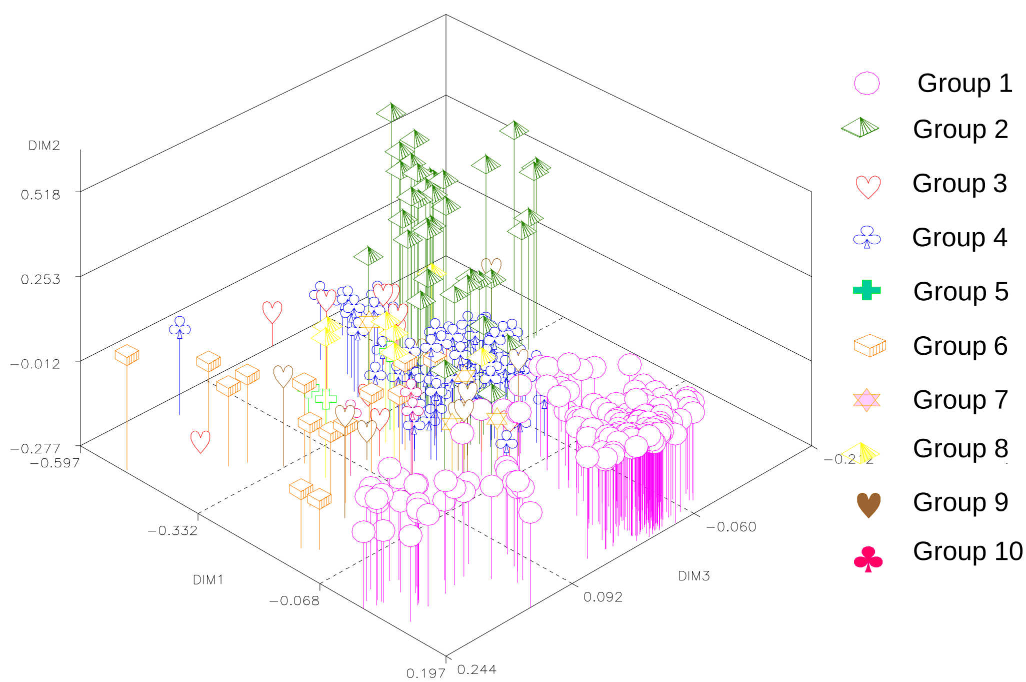


Figure 46. Clusters of isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes guianensis* based on multiple correspondence analysis of RAPD data.

2.8.2 Characterization of transgenic *Stylosanthes* plants with chitinase gene—T-3 generation

Contributors: Gustavo Segura and Segenet Kelemu

Rationale

Foliar blight disease, caused by *Rhizoctonia solani* Kühn affects *Stylosanthes* spp. in regions where the annual rainfall is more than 1500 mm. Under prolonged humidity, rotting and extensive foliar death can result. No resistance is known in *S. guianensis*. We reported (IP-5 Annual Report 2001) the introduction of a rice-chitinase gene into *S. guianensis* accession CIAT 184 in order to enhance foliar blight disease resistance. We evaluated segregants in T3 generation descending from a transgenic plant.

Materials and Methods

Seeds collected from transgenic T2 plants with enhanced levels of resistance and with no resistance (segregants with no chitinase gene) and planted in small pots. Twenty-five seeds collected from each plant were planted separately maintaining individuality of the origin of the seeds. Plants were grown in the greenhouse for 46 days, after which they were inoculated with agar discs containing fresh mycelia of *R. solani*. Agar discs were placed at the bottom part of stems just above the soil line and wrapped with parafilm (Photo 11) and placed in a himidity chamber (Photo 11).

Photo 11. Incoulation of *Stylosanthes guianensis* plants with mycelial discs of *Rhizoctonia solani* (A), and incolated plants in a humidity chamber (B).

Results were not available at the time of this report writing and will be reported in IP-5 Annual Report 2003.

Output 3: Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed

Activity 3.1 Genotypes of *Brachiaria,* other grasses and *Arachis* with adaptation to edaphic factors

Highlights

- Showed for the first time that the surface charge density of root apices of aluminum resistant *Brachiaria decumbens* acts as a generic mechanism to avoid uptake of phytotoxic aluminum and other cationic toxicants.

- Phenotypic characterization of *Brachiaria decumbens* x *Brachiaria ruziziensis* population resulted in identification of few hybrids superior to *B. decumbens* in production of fine roots in the presence of toxic level of aluminum in solution.

- Using screening procedure to evaluate aluminum resistance, 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) were identified with greater level of Al resistance than that of the sexual parent, BRUZ/44-02.

- Showed that the *Brachiaria* hybrid, FM9503-S046-024 performed well into the third year after establishment in the Llanos and its superior performance at 28 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.

- Collaborative research conducted under controlled environmental conditions in a growth chamber in Goettingen, Germany indicated that the *Arachis pintoi* accession CIAT 22159 is better adapted to very low P supply in an oxisol when compared with the commercial cultivar, CIAT 17434.

- Results from a field study in the Piedmont showed that the *Arachis pintoi* accession CIAT 22159 is superior to the commercial cultivar (CIAT 17434) in terms of rapid establishment as well as regrowth potential.

3.1.1 Edaphic adaptation of *Brachiaria*

Contributors: P. Wenzl, A.L. Chaves, M.E. Recio, J. Tohme and I.M. Rao

Widespread adoption of *Brachiaria* cultivars depends on their efficiently acquiring nutrients, particularly P, N, and Ca, from the soil and using them for growth. Identifying plant attributes that confer adaptation to low-fertility acid soils is needed to rapidly develop cultivars through agronomic evaluation and genetic improvement. Plant attributes appear to be linked to different strategies to acquire and use nutrients. Understanding these linkages is fundamental in integrating plant attributes into selection indices, which can then be used to develop rapid and reliable screening procedures for germplasm evaluation and/or improvement.

As part of the restricted core project funded by BMZ-GTZ of Germany, we continued our efforts to dissect the physiological and genetic components of aluminum resistance in *Brachiaria*.

– **Exploring surface charge density of root apices as a potential factor contributing to aluminum resistance of *Brachiaria decumbens***

Rationale

There is a pronounced difference in aluminum (Al) resistance between *B. decumbens* and *B. ruziziensis*. Previous research has indicated that *B. decumbens* might exclude phytotoxic Al ions from root apices using a mechanism that does not rely on external detoxification by organic acids (Wenzl et al., 2001). Based on these results we hypothesized that a less negative (or even positive) surface charge density of root apices could be a highly effective mechanism of non-metabolic Al exclusion. According to this hypothesis, *B. decumbens* also should be more resistant to other toxicants with different physicochemical properties (ion radius, etc.) as long as they are cations. Here we report on experiments to evaluate the susceptibility of the two *Brachiaria* species to lanthanum (La^{3+}) and other lanthanide ions using relative root elongation (RRE) of seedlings as a measure of resistance.

Materials and Methods

Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 µM $CaCl_2$ (pH 4.2) for 4 – 5 days. Homogeneous seeds, with root lengths between 2 and 3 cm, were transferred to continuously aerated solutions containing 200 µM $CaCl_2$ (pH 4.2) plus a lanthanide salt (chlorides of lanthanum, cerium or ytterbium). The seedlings were left to grow in the glasshouse for 3 days. At harvest, root lengths were measured and root elongation was calculated by subtracting the root length at transfer.

Results and Discussion

The results obtained with La^{3+} show that Al-resistant *B. decumbens* is more resistant to La^{3+} ions than Al-sensitive *B. ruziziensis* (Figure 47). In all other plant species tested so far, genotypes differing in their resistance to Al^{3+} ions are equally sensitive to La^{3+}. Other lanthanide ions such as Yb^{3+} and Ce^{3+} triggered a similar differential response (Figure 48).

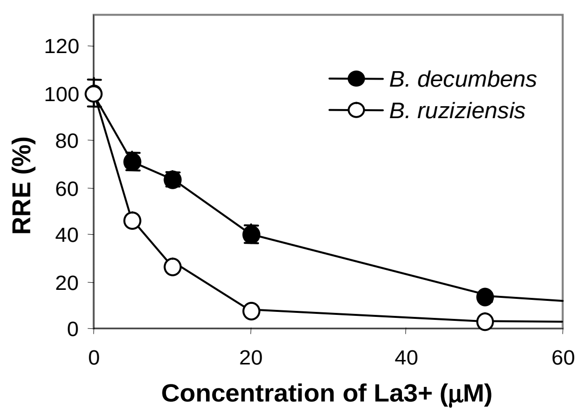


Figure 47. Relative root elongation (RRE) of *Brachiaria decumbens* and *Brachiaria ruziziensis* as affected by La^{3+} in the nutrient solution. The values shown are means ± SE of 12 seedlings.

This is the first time that a general resistance to trivalent cationic toxicants has been observed in plants. These findings are consistent with the possibility that the surface charge density of root apices — the principal site of Al injury — acts as a generic mechanism to avoid uptake of phytotoxic cationic toxicants. Yet the possibility remains that this 'cross resistance' is due to other factors. Further work is in progress to evaluate the significance of a surface-charge-based Al-resistance mechanism compared with other mechanisms.

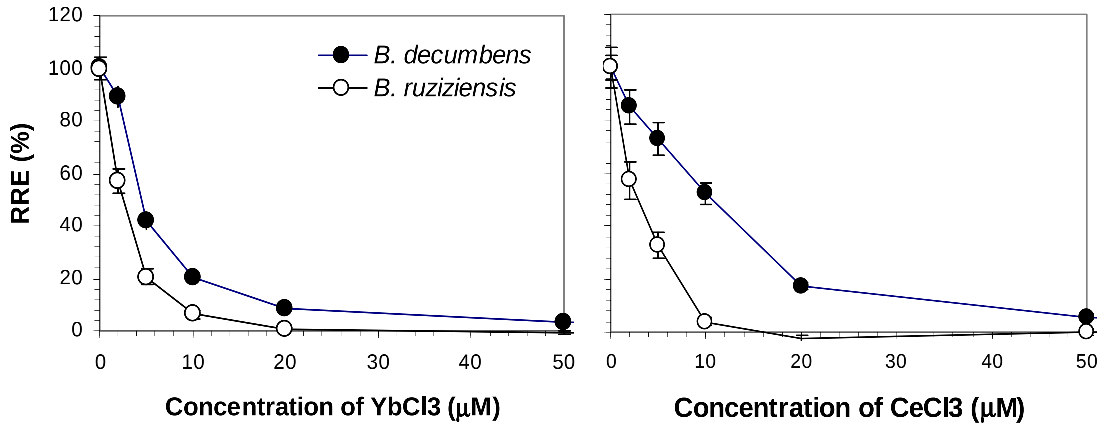


Figure 48. Relative root elongation (RRE) of *Brachiaria decumbens* and *Brachiaria ruziziensis* as affected by two different lanthanide cations in the nutrient solution. The values shown are means ± SE of 12 seedlings.

Results from these experiments may provide insights to dissect Al resistance of *B. decumbens* into physiological mechanisms. This may be particularly useful if genetic control of the trait turns out to be complex (which may indeed be the case; see the section on "Identifying individuals with contrasting aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrid population").

– **Evaluating traits contributing to acid-soil adaptation in a population of 274 *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrids**

Contributors: M.E. Buitrago, M.E. Recio, A.L. Chaves, P. Wenzl, J. Tohme, J.W. Miles and I.M. Rao

Rationale

In a pilot experiment we found that hybrids of a *Brachiaria ruziziensis* × *Brachiaria decumbens* population segregated for root-related characters that are relevant for the adaptation to acid soils. These include 'root growth potential' (the ability to initiate and elongate roots in the absence of externally supplied nutrients) and aluminum (Al) resistance (see the section on "Identifying Individuals with Contrasting Aluminum Resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* Hybrid Population"). We therefore extended the ongoing phenotypic evaluation to the complete hybrid population available at CIAT.

The results of this experiment are going to be useful in two ways. First, they will enable us to refine a single-step screening procedure to rapidly discard genotypes not adapted to acid soils. Second, the phenotypic data will provide a basis for the mapping of QTLs controlling physiological and morphological characters associated with acid soil adaptation. The latter should provide an avenue towards implementing marker-assisted selection for acid soil adaptation in the future.

Materials and Methods

Stem cuttings from hybrids of a *Brachiaria ruziziensis* × *Brachiaria decumbens* population were rooted in a low ionic strength nutrient solution in the glasshouse for 9 days. Rooted stem cuttings from each genotype were grouped into homogeneous pairs. One cutting of each pair was used to measure root growth in the absence of Al (in 200µM $CaCl_2$; pH 4.2), while the other was used to measure growth in an identical solution, to which 200µM $AlCl_3$ had been added. Treatment solutions were changed every second day to minimize pH drifts. At harvest on day 21 after transfer to treatment solutions, stems were separated from roots and dried to determine their dry weight. Roots were put into a solution containing neutral red and methylene blue and left to stain for at least 24 h. After rinsing with water, they were scanned on a flatbed scanner. The resulting images were analyzed with WinRHIZO image analysis software to determine root length and average root diameter. This experiment is still ongoing. So far, three growth cycles, each comprising up to three pairs of stem cuttings per genotype (more for the two parents) have been fully analyzed.

Results and Discussion

An extensive root system that explores a large soil volume can take up relatively immobile nutrients such as phosphorus more efficiently. It is an important trait permitting plants to grow on nutrient-deficient acid soils. Adapted genotypes, therefore, should have long thin roots and cluster in the lower right corner of a plot of root diameter (y axis) versus root length (x axis) (Figure 49). To produce such a plot, root length and thickness data were log-transformed and adjusted for the effect of stem cutting dry weight as described in the following section on “Identifying Individuals with Contrasting Aluminum Resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* Hybrid Population”. The two sets of data were then plotted against each other, both for stem cuttings grown in absence and presence of Al (Figure 49).

In the absence of Al, stem cuttings of hybrids had root lengths between 0.6 and 7.0 m. While the poorly adapted parent (*B.ruziziensis*) was at the low end of the range, a significant number of hybrids produced longer roots than well adapted *B. decumbens* (Figure 49, left panel). The two parents were close to the two extremes of the range of root diameters, however (Figure 49, left panel). As expected, in the presence of Al, root elongation was inhibited and roots became thicker (see differences in population means in right vs. left panel of Figure 49).

Growing rooted stem cuttings in the Al treatment and choosing genotypes with long and thin roots (Figure 49, right panel) could be an effective screen to discard non-adapted segregants in the *Brachiaria* breeding program. It is, however, important to keep in mind that such a test simultaneously screens for several desirable physiological attributes (root growth potential in the absence of external nutrients + Al resistance + inherently thin roots). The genetic components controlling these characters, however, may be scattered throughout different genotypes and could be lost if the test is applied too stringently.

For these reasons, it would be desirable to isolate ‘physiological components’ contributing to root growth in the Al treatment. Performing the same screen in the absence of Al provides access to the component that allows plants to initiate and elongate roots in the absence of all nutrients but Ca in the growth medium and to the component that is responsible for inherently thin roots (Figure 49, left panel). A comparison, for each genotype, between root length in the presence and absence of Al provides indirect access to the physiological component that governs Al resistance (see the section on “Identifying individuals with contrasting aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrid population”).

We are continuing to accumulate data from additional growth cycles to increase the precision with which we will be able to 'dissect' root growth into these physiological components. Subsequently, the set of physiological data will be combined with genetic data to identify QTLs associated with these traits.

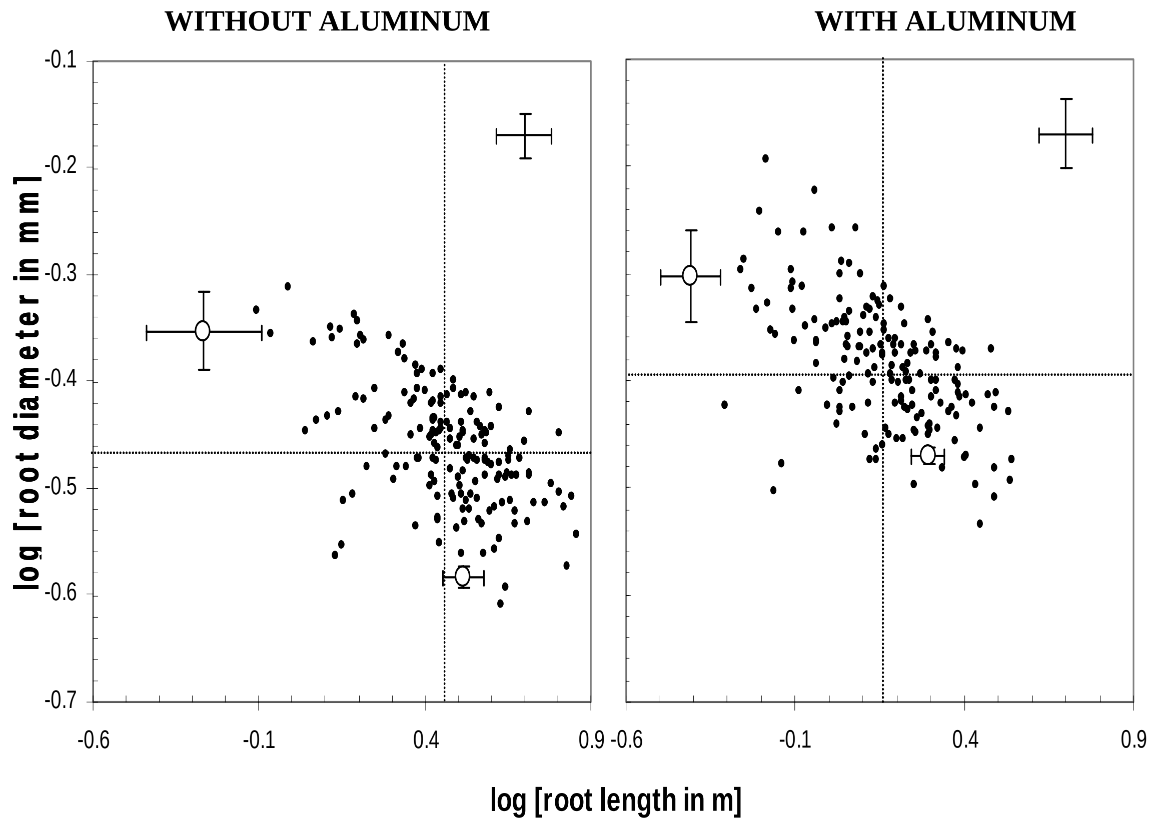


Figure 49. Comparison of root length and root diameter of rooted stem cuttings from a *B. ruziziensis* x *B. decumbens* hybrid population grown in the absence (left panel) and presence of Al (right panel) in solution. The values of the two pare nts are shown with large symbols (upper left, *B. ruziziensis*; lower right, *B. decumbens*). The values of the hybrids are shown with small symbols. The error bars in the upper right corner designate average standard errors for the two variables. Only hybrids for which at least two independent measurements had been taken were included in this figure.

– Identifying individuals with contrasting aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrid population

Contributors: A. Arango, P. Wenzl, J. Tohme, J.W. Miles and I.M. Rao

Rationale

Previous experiments have established that there is a significant difference in aluminum (Al) resistance between *B. decumbens* (resistant) and *B. ruziziensis* (sensitive) (Wenzl et al., 2001). Because apomictically reproducing plants tend to be highly heterozygous, alleles conferring Al resistance in apomictic *B. decumbens* are expected to segregate in a *B. ruziziensis* × *B. decumbens* hybrid population. The main objective of this work is to use this population to isolate genes whose expression is associated with Al resistance.

Here we report on a physiological evaluation of 38 individuals of this hybrid population. They had been selected, using data from preliminary experiments, to comprise both Al-resistant and sensitive hybrids. Based on the results of this evaluation, two groups of contrasting individuals will be formed to compare gene expression patterns in root apices, the presumed site of action of Al-resistance mechanisms.

Materials and Methods

Rooted stem cuttings of each genotype were grouped into homogeneous pairs. One stem cutting of each pair was used to measure the root-elongation potential in a solution containing 200 µM $CaCl_2$ (pH 4.2), the other was used to measure growth in an identical solution, to which 200 µM $AlCl_3$ had been added (for more details, see the above section on "Evaluating Acid-Soil Adaptation of 274 *Brachiaria ruziziensis* × *Brachiaria decumbens* Hybrids"). The experiment comprised ten independent growth cycles, each with up to nine pairs of stem cuttings per genotype.

Results and Discussion

Inhibition of root elongation is a well-known symptom of Al toxicity. Addition of Al to the basal solution containing only $CaCl_2$ had a pronounced effect on root elongation in most genotypes. Initial analyses, however, indicated that the effect of Al was highly inconsistent, both within and between blocks.

Several factors may have contributed to this variability. First, root elongation in a solution lacking all nutrients but Ca largely depends on reserves (sugars, nutrients) present in stem cuttings. Those that have a greater biomass, therefore, are expected to show superior root elongation in both treatments. Second, previous results suggest that a good nutritional status increases the Al-resistance level of *Brachiaria* species (Wenzl et al., 2002). This may enhance the positive effect of the stem cutting's biomass on root elongation in the Al treatment. Third, stem cuttings taken from plants with a good nutritional status are likely to contain more nutrients per unit of biomass, thus potentially amplifying the effect of the stem cutting's biomass on root elongation in both treatments. These considerations highlight the importance of adjusting root elongation data for stem cutting biomass and the nutritional status of the donor plants — the latter presumably varying in an unpredictable manner among different growth cycles.

All data were log-transformed because growth data tend to be log-normally distributed (Causton and Venus, 1981). This improved normality in most cases, as judged by the Kolmogorov-Smirnov test. Linear regression of root length and diameter on the dry weight of stem cuttings indicated a highly significant effect for the former ($P<10^{-25}$) but not the latter. At least 13 % (basal treatment) and 20 % (Al treatment) of the variance of root length data could be accounted for by the variable dry weight of stem cuttings. Root length data, therefore, were adjusted for the effect of the dry weight of stem cuttings (using separate linear regressions for individual growth cycles, which slightly increased the percentage of explained variance).

Adjusted root lengths of most genotypes fell within the range of 2.5 to 6.3 m per stem cutting (log values between 0.4 and 0.8; Figure 50). Only *B. ruziziensis* (1.0 m) and hybrid No. 114 (0.7 m) had much shorter roots. Interestingly, about half of the hybrids had longer roots than acid-soil adapted parent *B. decumbens*. Given this inherent variability in root elongation potential, root elongation in the presence of Al is expected to be the result of a superimposition of two physiological components: the ability to elongate roots in the absence of externally supplied nutrients plus Al resistance. While this might be advantageous for rapidly discarding non-adapted genotypes in a breeding program, it makes it necessary to isolate the component of overall performance in the Al treatment that is attributable to Al resistance. This is because the planned experiments to isolate genes rely on the assumption that sensitive genotypes do not express genes that confer Al resistance. Not dissecting the two components, however, could result in a misclassification of genotypes expressing 'Al-resistance' genes as 'sensitive' because they lack the (presumable unrelated) ability to elongate roots in the absence of nutrients.

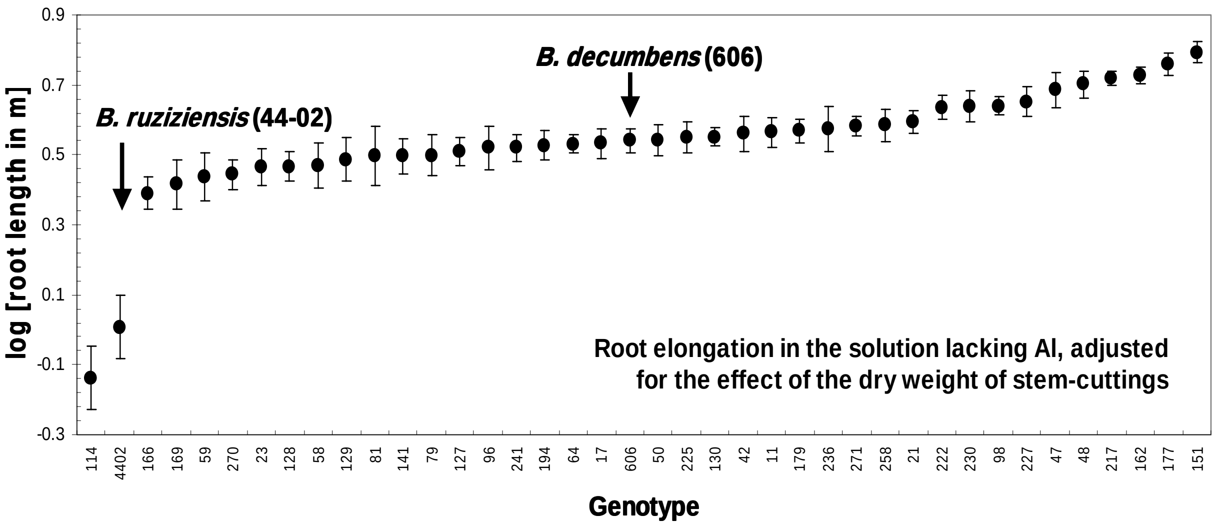


Figure 50. Log-transformed root lengths adjusted for the variable dry weight of stem cuttings.

Aluminum resistance, therefore, was quantified by comparing root elongation in the Al treatment with root elongation in the basal treatment. For each pair of stem cuttings that had been split between the two treatments, the difference between the log-transformed root lengths in the two treatments was calculated (this is equivalent to computing the ratio of untransformed root lengths). The two poorly elongating genotypes (114, 44-02) were excluded from this analysis because a too large fraction of their final root length had already been present at the time of transferring stem cuttings into treatment solutions. The average effect of Al on root elongation varied among growth cycles (33 to 60 % inhibition), perhaps because of the differences in the nutrient status of the stem cuttings (see discussion above). An adjustment for growth cycle-specific effects, which accounted for 13 % of the variance of the difference between the log-transformed root lengths, was achieved by subtracting the average difference in a particular growth cycle (i.e. the average effect of Al) from the individual values computed for pairs of stem cuttings. The resulting value was called 'Al resistance index'.

If Al resistance and the ability to elongate roots in the absence of external nutrients are separate physiological components that segregate independently, then there should be no correlation between Al resistance indices and the root elongation potential (the log-transformed root lengths in the absence of Al).

Genotypes were organized according to their Al resistance index (Figure 52). Two independent findings seem to confirm the validity of our approach to quantify Al resistance of stem cuttings. First, the Al-resistant parent (*B. decumbens*) had the highest Al resistance index (the Al-sensitive parent could not be scored due to its extremely poor root elongation in the basal treatment). Second, evaluation of a different Al toxicity symptom — the lateral swelling of roots — demonstrated that roots of genotypes classified as Al resistant tended to swell less than those of Al-sensitive genotypes ($r = -0.88$; Figure 52).

Figure 51 appears to confirm this assumption ($r^2 = 0.02$) and underscores the need to dissect the two components for the purpose of associating gene expression patterns with either of the two component traits.

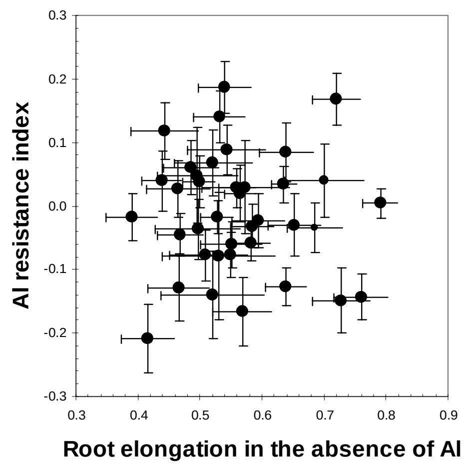


Figure 51. Lack of relationship between Al resistance and root elongation potential. Al resistance index = {log[root length in Al solution] – log[root length in basal solution]} – [average difference for a particular growth cycle]. Root elongation in the absence of Al = log[root length in basal treatment]. All log-transformed root length had been adjusted for the effect of stem cutting dry weight.

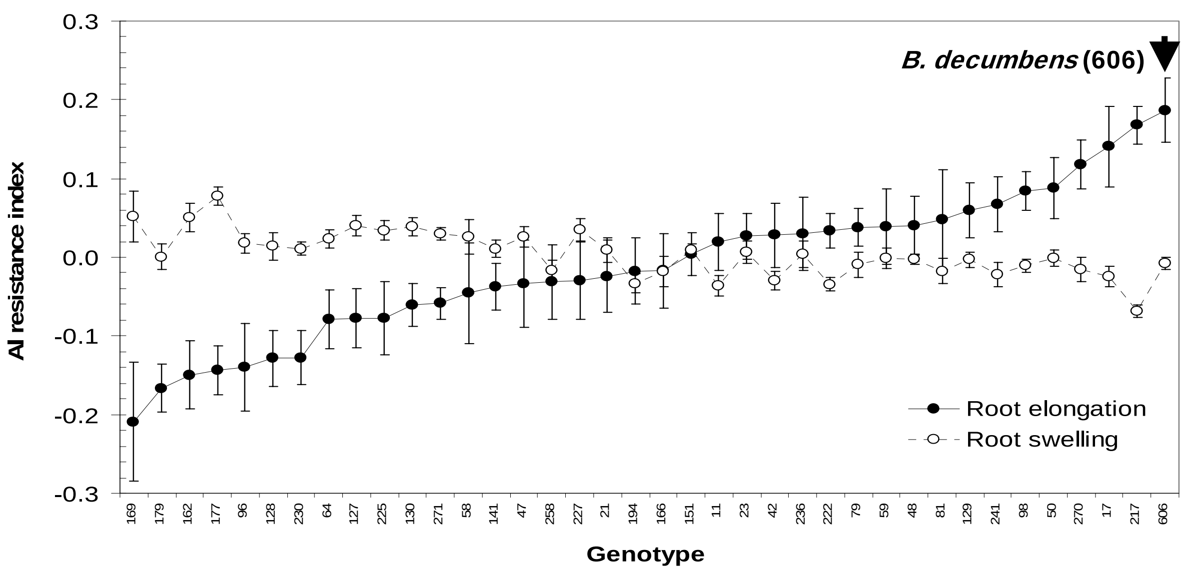


Figure 52. Genotypes ordered according to their Al resistance index computed from root length data. A similar index was computed using root thickness data and is included for comparison.

Two groups of contrasting genotypes (4 – 6 each) will be chosen from the upper and lower end of the range of Al resistance indices for subsequent experiments to associate gene expression patterns with an Al-resistant phenotype.

- **Screening of improved tetraploid, sexual and apomictic *Brachiaria* hybrids for resistance to aluminum**

Contributors: I. M. Rao, J. W. Miles, R. Garcia and J. Ricaurte (CIAT)

Rationale

For the last two years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. Last year, we have identified 2 sexual hybrids (SX 2349 and SX 497) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. With the partial support of BMZ-GTZ of Germany and Papalotla (seed company) of Mexico to the *Brachiaria* improvement project, this year we evaluated Al resistance of the most promising sexual *Brachiaria* hybrids that are resistant to spittlebug.

Materials and Methods

A total of 90 genotypes including 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were selected for evaluation of Al resistance. Among the 87 hybrids selected for screening, 84 were sexuals and three were apomicts (CIAT 36062, BR99NO/4132, FM9503-S046-024). All the hybrids were resistant to spittlebug infestation and some sexuals were resistant to 3 species of spittlebug (C. Cardona, personal communication; also see Output 2 and Activity 2.3 of this report). Stem-cuttings were rooted in a low ionic strength nutrient solution containing $CaCl_2$ (200µM), selected for uniformity and transferred to a solution containing 200 µ*M* $CaCl_2$ (pH 4.2) and exposed to 2 levels of $AlCl_3$ (0 and 200µM). The solution was replaced every third day, and total root length and root biomass were measured after 21 days of Al treatment. Root architecture was measured using WINRHIZO software program (Figure 53). Results reported are mean values from 3 experiments. Data from 2 additional experiments are being processed.

Results and Discussion

As observed before, results on total root length per plant after exposure to 21 days with or without toxic level of Al in solution indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figure 54). Among the 87 hybrids tested, 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and 1 apomictic hybrid (BR99NO/4132) showed greater level of Al resistance based on total root length per plant (Figure 54). Among the two sexual hybrids, SX01NO3178 exhibited coarse root system while SX01NO7249 had fine root system similar to that of the Al-resistant parent, CIAT 606.

The level of Al resistance of these two sexuals was outstanding compared with that of the sexual parent, BRUZ/44-02 (Figure 53). Another sexual hybrid SX01NO2025 also showed greater level of Al resistance but in the absence of Al in solution, its total root length was moderate. It is important to note that the total root length of the sexual hybrid, SX01NO3178 was superior to the parent CIAT 606 in the absence of Al in solution. Exposure to Al decreased the mean value of total root length of the 90 genotypes from 189 to 69 cm $plant^{-1}$.

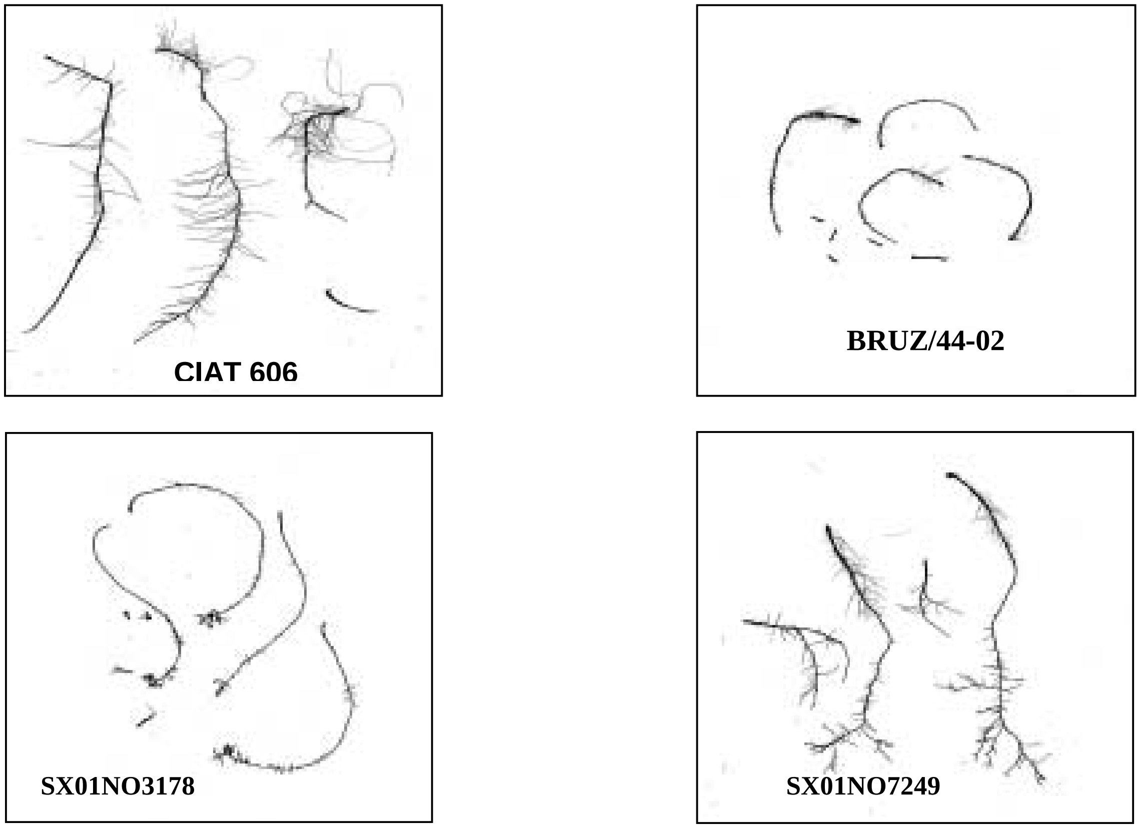


Figure 53. Root images of (a) aluminum resistant apomictic parent (CIAT 606) with fine root system; (b) aluminum sensitive sexual parent (BRUZ/44-02) with coarse root system; (c) aluminum resistant sexual hybrid (SX01NO3178) with coarse root system; and (d) aluminum resistant sexual hybrid (SX01NO7249) with fine root system. Roots were scanned after 21 days of exposure to 200 µM aluminum chloride with 200 µM calcium chloride (pH 4.2) in solution.

One of the apomictic hybrids, FM9503-S046-024, that performed well in the field in the Llanos showed less root elongation and produced coarse root system in the presence or absence of Al in solution (Figures 54 and 55). This suggests that some vigorous hybrids may also adapt well to acid soils with coarse root system similar to that observed with cultivar Mulato due to their greater ability to acquire Ca and Mg from acid soils. But this type of hybrids may function better during dry season and may require moderate level of soil fertility. The lack of fine root system in these hybrids may limit their adaptation to highly infertile acid soil conditions.

Results on mean root diameter showed that the parent CIAT 606 had the lowest values under both with and without Al treatments (Figure 55). Two sexual hybrids, SX01NO7249 and SX01NO4099, were closer to the values of CIAT 606. One of the sexual hybrids, SX01NO2192, showed very high values of mean root diameter of about 1 mm (coarse root system) under with and without Al in solution.The mean values of root diameter decreased from 0.59 to 0.47 mm with the exposure to Al in nutrient solution. The relationship between root length and root diameter without Al in nutrient solution indicated that several sexual hybrids have mean root diameter values similar to CIAT 606 but total root length of one sexual hybrid (SX01NO3178) and CIAT 606 were greater than the rest of the genotypes tested (Figure 56). But in the presence Al in solution, CIAT 606 was outstanding in combine total root length with lower values of mean root diameter (Figure 57). Several sexual hybrids showed greater level of Al resistance than the three apomictic hybrids (CIAT 36062, BR99NO/4132, FM9503-S046-024).

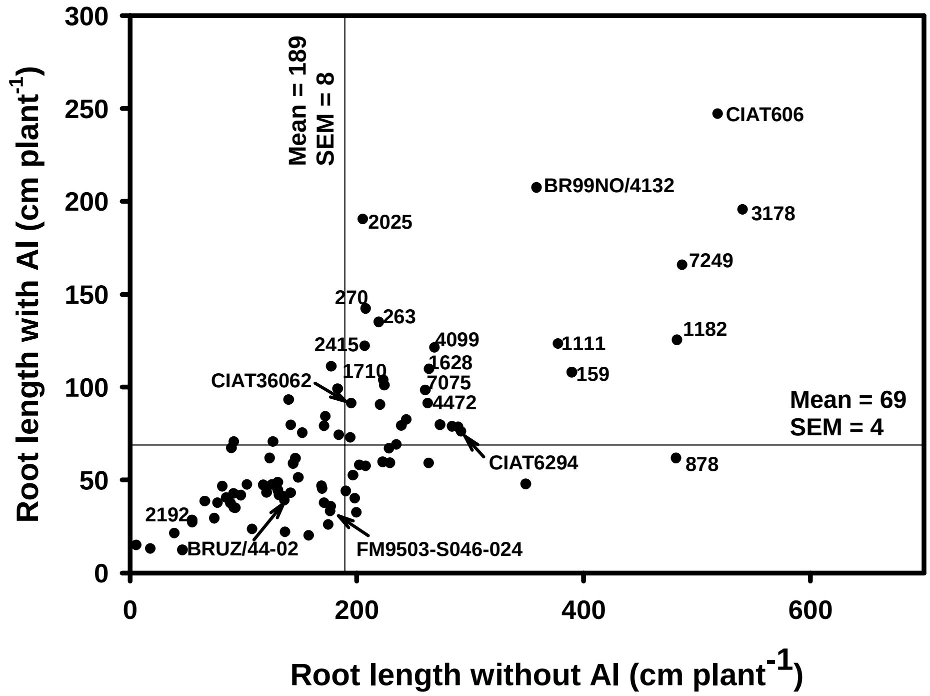


Figure 54. Identification of Al resistant sexual hybrids of *Brachiaria* based on total root length . Hybrids that were superior in root length with no or high Al in solution were identified in the upper box of the right hand side. Total root length was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days. SEM = standard error of the mean.

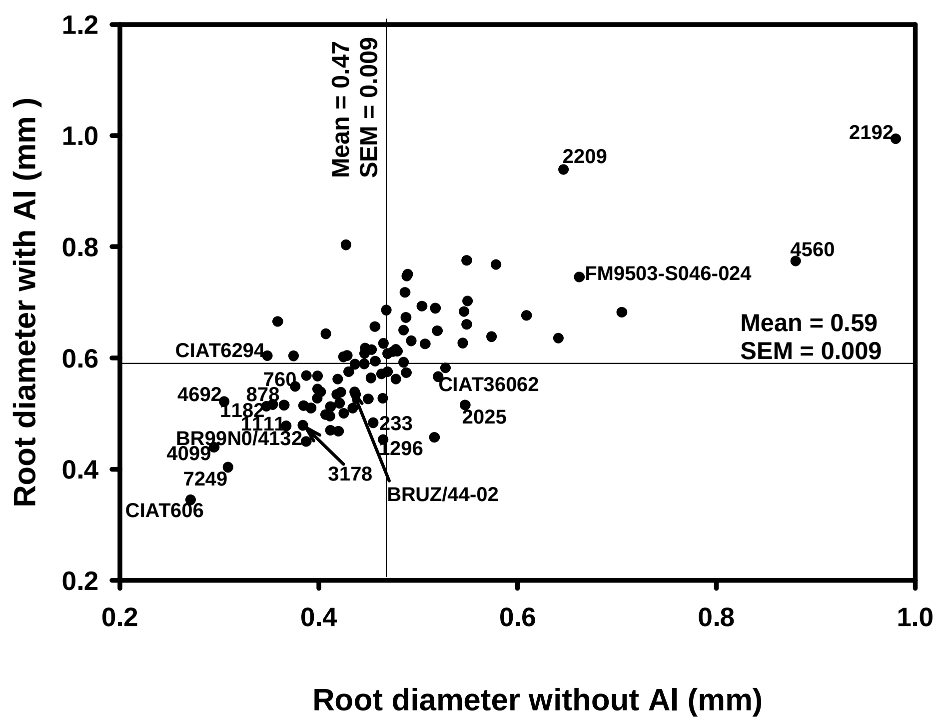


Figure 55. Identification of Al resistant sexual hybrids of *Brachiaria* based on mean root diameter. Hybrids with lower values of mean root diameter (fine roots) with no or high Al in solution were identified in the lower box of the left hand side. Root diameter was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days. SEM = standard error of the mean.

Based on the results obtained from screening for resistance to 3 species of spittlebug and from this study, a group of 22 sexuals (SX01NO67, 102, 159, 233, 263, 446, 465, 878, 1090, 1175, 1186, 1710, 2017, 2420, 2619, 3168, 3178, 3390, 3439, 3615, 4506, 4785, 4861) were used in the crossing block to generate superior hybrids that combine desirable attributes.

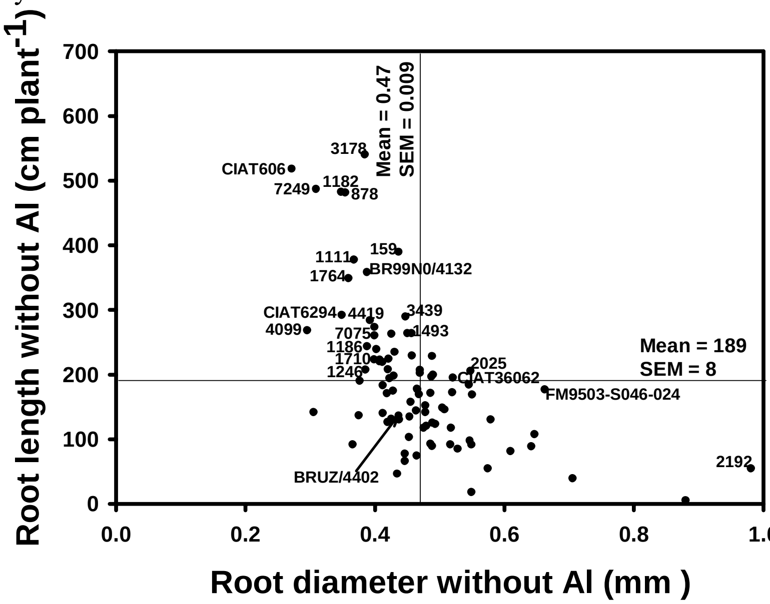


Figure 56. Relationship between total root length and mean root diameter of 90 genotypes of *Brachiaria* without the presence of aluminum in solution. Hybrids that develop finer root system in the absence of Al in solution were identified in the upper box of the left hand side. Total root length and mean root diameter were measured with exposure to 0 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days. SEM = standard error of the mean.

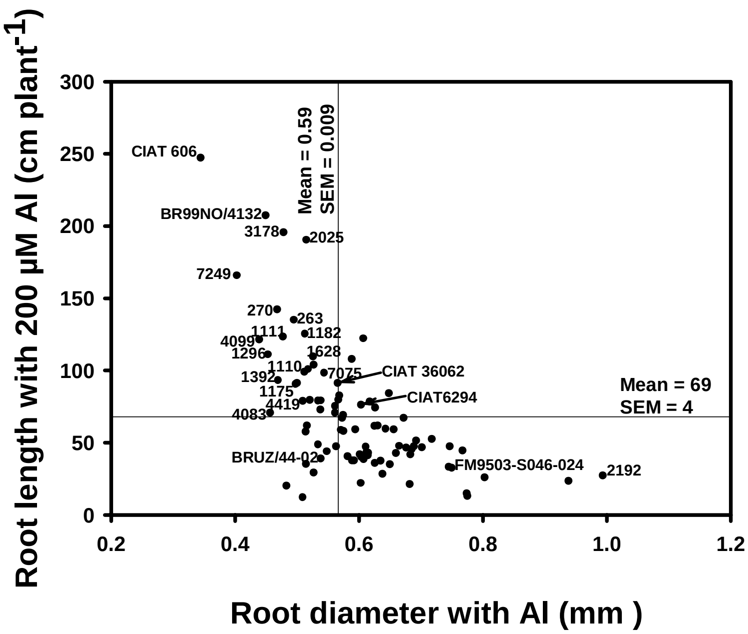


Figure 57. Relationship between total root length and mean root diameter of 90 genotypes of *Brachiaria* with the presence of aluminum in solution. Hybrids that develop finer root system in the presence of Al in solution were identified in the upper box of the left hand side. Total root length and mean root diameter were measured with exposure to 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days. SEM = standard error of the mean.

We have identified 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02.

– Identification of plant attributes for persistence with low nutrient supply in hybrids and accessions of *Brachiaria*

Contributors: I. M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. Garcia (CIAT)

Rationale

A field study is in progress at Matazul Farm in the Llanos of Colombia. The main objective was to identify genetic recombinants of *Brachiaria* with tolerance to low nutrient supply and evaluate plant attributes that contribute to superior adaptation. Results obtained from this field study at 15 months after establishment indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 was associated with its ability to acquire greater amounts of nutrients from low fertility soil. This year we continued our work to monitor the performance of genetic recombinants in terms of their growth and persistence under low fertility acid soil conditions.

Materials and Methods

A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, and shoot nutrient uptake were measured at the end of the wet season (November 2001), i.e., at 28 months after establishment. Maintenance fertlizer (half the levels of initial application) was applied in July 2001.

Results and Discussion

As expected, application of high amounts of maintenance fertilizer at 2 years after establishment improved forage yield of most of the genotypes compared with low fertilizer application (Table 33). At 28 months after establishment, live forage yield with low fertilizer application ranged from 404 to 2264 kg/ha and the high values of forage yield were observed with three germplasm accessions (CIAT 26110, 26318 and 6294) and one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 33). This spittlebug resistant genetic recombinant not only showed rapid establishment but also maintained greater level of forage production over time with low initial fertilizer application. It also was very responsive to higher fertilizer application as revealed by live shoot biomass and total forage yield. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants. These results are similar to those observed at 15 months after establishment (IP-5 Annual Report, 2001). The values of leaf to stem ratio were markedly superior with the genetic recombinants than the parents and other germplasm accessions with both levels of fertilizer application (Table 33). This is an important attribute for improving animal production through *Brachiaria* breeding activities.

Among the genetic recombinants, FM9503-S046-024 was outstanding in production of green leaf biomass with both low and high fertilizer application (Figure 58).

Table 33. Genotypic variation as influenced by fertilizer application in live shoot biomass, leaf to stem ratio and total forage yield of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001) LSD values are at the 0.05 probability level.

	Live shoot biomass		Leaf to stem ratio		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Genotype	(kg/ha)					
Recombinants:						
BR97NO-0082	659	872	3.58	3.29	1079	1477
BR97NO-0383	907	795	1.85	3.88	1434	1445
BR97NO-0405	824	956	2.34	2.02	1542	1798
FM9201-1873	1144	1575	2.29	1.94	2451	2266
FM9301-1371	940	1139	4.53	3.54	1501	2328
FM9503-S046-024	1514	2112	1.96	1.93	2370	3257
Parents:						
CIAT 606	812	1132	1.86	1.67	1272	1905
CIAT 6294	1787	1777	1.82	2.17	3087	3083
BRUZ/44-02	404	898	1.97	0.43	968	1284
CIAT 26646	1352	2092	2.07	1.98	1959	2655
Accessions:						
CIAT 26110	2264	2473	2.38	2.26	3041	3201
CIAT 26318	1968	2423	1.84	1.78	2684	3120
Mean	**1190**	**1483**	**2.37**	2.24	**1877**	**2265**
LSD *(P=0.05)*	734	710			1173	1232

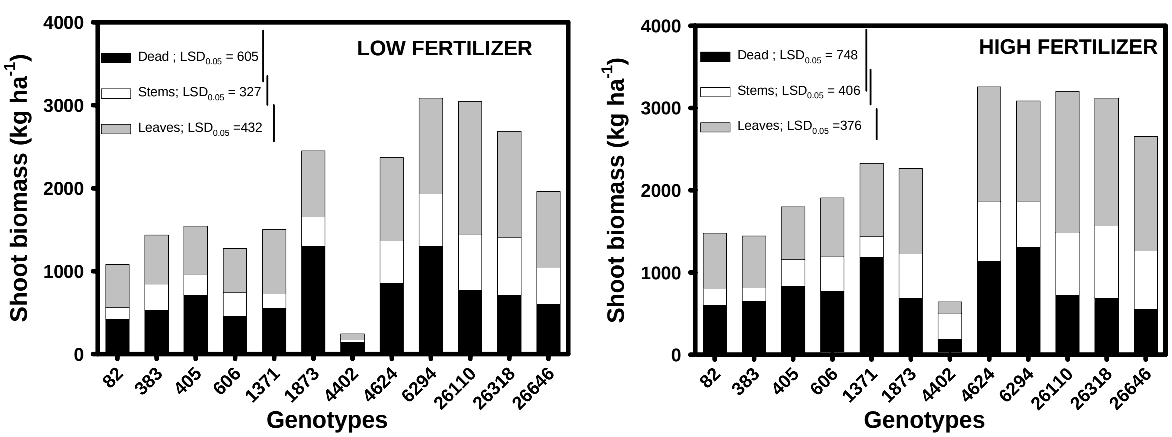


Figure 58. Genotypic variation as influenced by fertilizer application in dry matter distribution among leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001) LSD values are at the 0.05 probability level.

One of the germplasm accessions, CIAT 26110 was outstanding in its leaf biomass production and its production was almost 2 times greater than that of the mean value of 12 genotypes tested. Results on leaf and stem N content indicated that BRUZ/44-02 had greater amount of N per unit dry weight but its ability to acquire N (shoot N uptake) was lowest compared with other parents and genetic recombinants (Table 34).

Table 34. Genotypic variation as influenced by fertilizer application in leaf N content, stem N content and shoot N uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001). LSD values are at the 0.05 probability level.

Genotype	Leaf N content (%)		Stem N content (%)		Shoot N uptake (kg/ha)	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Recombinants:						
BR97NO-0082	1.25	1.26	0.96	0.95	7.83	9.96
BR97NO-0383	1.28	1.36	0.96	1.13	10.62	8.53
BR97NO-0405	1.30	1.32	0.87	0.91	9.54	11.51
FM9201-1873	1.22	1.22	0.89	0.94	12.94	16.87
FM9301-1371	1.37	1.21	0.94	0.83	11.97	12.83
FM9503-S046-024	1.31	1.20	0.81	0.78	16.76	22.29
Parents:						
CIAT 606	1.27	1.23	0.95	1.03	9.36	12.60
CIAT 6294	1.36	1.26	0.88	0.82	21.38	19.75
BRUZ/44-02	1.52	1.17	1.39	0.89	5.96	8.59
CIAT 26646	1.29	1.33	0.93	1.06	15.47	25.22
Accessions:						
CIAT 26110	1.45	1.26	0.94	0.81	29.84	27.43
CIAT 26318	1.28	1.33	0.92	0.87	22.82	28.45
Mean	**1.31**	**1.27**	**0.92**	**0.92**	**15.16**	**17.37**
LSD *(P=0.05)*	0.19	NS	0.16	0.23	10.83	7.81

NS = not significant.

Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318), one parent (CIAT 6294) and one genetic recombinant (FM9503-S046-024). This genetic recombinant was also outstanding in its ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer application when compared with other genetic recombinants (Tables 35 and 36; Figure 59). Among the parents, two accessions of *B. Brizantha*, CIAT 6294 and CIAT 26646 were superior in nutrient acquisition from low and high fertilizer application (Figure 59). Shoot nutrient uptake by CIAT 26110 was outstanding with both low and high fertilizer application (Figure 59).

Again the genetic recombinant, FM9503-S046-024 was outstanding in its ability to acquire greater amounts of Ca from low and high fertilizer application. This ability to acquire greater amounts of Ca from acid soil (Table 36) could not only contribute to its superior persistence on acid infertile soils but also could contribute to greater forage quality and animal production.

It is important to note that live forage yield was associated with lower contents of stem Ca and K with both low and high fertilizer application (Table 37). Live forage yield with low fertilizer application showed a highly significant negative relationship (-0.53***) with stem Ca content. This observation indicates that efficient mobilization of Ca from stems to leaves and efficient utilization of Ca for the production of green forage are important physiological mechanisms for superior performance with low fertilizer application. Results from this field study indicated that the *Brachiaria* hybrid, FM9503-S046-024 was not only rapid in its establishment but also performed well into the third year after establishment. Its superior performance at 28 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.

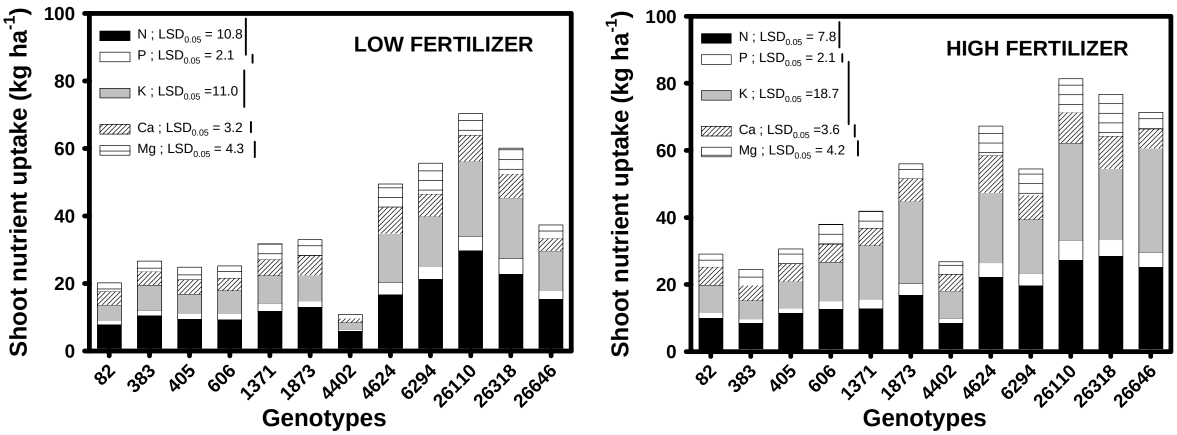


Figure 59. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001) LSD values are at the 0.05 probability level.

Table 35. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.183	0.174	0.149	0.141	1.12	1.60
BR97NO-0383	0.178	0.146	0.145	0.14	1.39	1.13
BR97NO-0405	0.193	0.158	0.137	0.119	1.50	1.49
FM9201-1873	0.174	0.216	0.146	0.195	1.92	3.55
FM9301-1371	0.226	0.270	0.165	0.202	2.12	2.90
FM9503-S046-024	0.239	0.217	0.195	0.153	3.49	4.22
Parents:						
CIAT 606	0.208	0.194	0.219	0.212	1.70	2.37
CIAT 6294	0.224	0.229	0.178	0.184	3.79	3.64
BRUZ/44-02	0.142	0.168	0.128	0.142	0.55	1.31
CIAT 26646	0.210	0.215	0.174	0.180	2.58	4.29
Accessions:						
CIAT 26110	0.188	0.234	0.168	0.231	4.18	5.82
CIAT 26318	0.222	0.207	0.217	0.196	4.66	4.88
Mean	**0.203**	**0.204**	**0.172**	**0.176**	**2.56**	**3.18**
LSD *(P=0.05)*	0.08	0.077	0.063	0.086	2.07	2.13

Table 36. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 28 months after establishment (November 2001). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	4.69	8.21	3.97	5.47	2.51	3.82
BR97NO-0383	7.53	5.51	4.03	4.67	3.09	4.59
BR97NO-0405	5.82	7.99	4.24	5.17	3.68	4.47
FM9201-1873	7.62	24.40	5.81	6.77	4.72	4.37
FM9301-1371	8.31	15.91	4.70	5.16	4.63	5.03
FM9503-S046-024	14.53	20.86	7.83	11.07	6.84	8.81
Parents:						
CIAT 606	6.86	11.67	3.72	5.34	3.56	5.97
CIAT 6294	14.76	16.02	6.60	7.27	9.10	7.76
BRUZ/44-02	1.90	8.23	1.33	4.95	1.06	3.68
CIAT 26646	11.61	31.17	3.68	5.68	4.02	5.04
Accessions:						
CIAT 26110	22.23	28.97	7.73	9.11	6.42	10.08
CIAT 26318	17.96	21.22	7.05	9.76	7.58	12.40
Mean	**10.95**	**17.05**	**5.29**	**6.78**	**5.02**	**6.45**
LSD *(P=0.05)*	10.96	18.72	3.18	3.61	4.29	4.20

Table 37. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.95***	0.89***
Dead shoot biomass (t/ha)	0.69***	0.38**
Leaf biomass (t/ha)	0.98***	0.96***
Stem biomass (t/ha)	0.97***	0.91***
Leaf N content (%)	0.18	-0.21
Leaf P content (%)	0.28	0.41**
Leaf K content (%)	0.22	0.27
Leaf Ca content (%)	-0.15	-0.35**
Leaf Mg content (%)	0.06	-0.11
Leaf Al content (%)	-0.22	-0.39**
Stem N content (%)	-0.26	-0.40**
Stem P content (%)	-0.24	-0.36*
Stem K content (%)	-0.44**	-0.36**
Stem Ca content (%)	-0.53***	-0.38**
Stem Mg content (%)	-0.12	-0.38**
Stem Al content (%)	-0.33*	-0.33*

– Field evaluation of most promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Greenhouse and field screening of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising brachiaria hybrids. We selected 4 of these hybrids for further field-testing in comparison with their parents. The main objective is to evaluate growth and persistence with low nutrient supply in soil at Matazul farm of the altillanura.

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at 4 months after establishment (September 2001).

Results

With high initial fertilizer application, one of the two parents *B. brizantha* cv. Marandu (CIAT 6294) was outstanding in shoot biomass production (Figure 60). This result is expected because this parent is very demanding in terms of its nutrient requirements and in general responds well to high initial fertilizer application. Among the 4 hybrids tested, 4015 was outstanding in its adaptation to low intial fertilizer application. It is important to note that one of the parents, *B. decumbens* was outstanding in its level of adaptation to low fertilizer application (Figure 60).

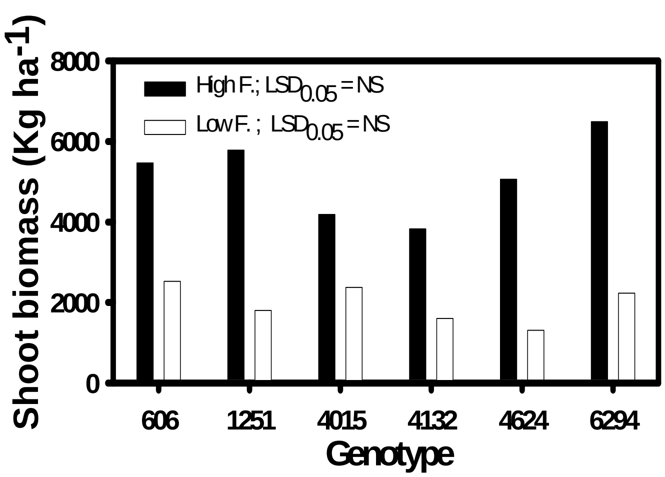


Figure 60. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 4 months after establishment (September 2001). LSD values are at the 0.05 probability level. NS = not significant.

The performance of the 4 hybrids will be monitored for the next 2 years in comparison with the two parents in terms of forage yield and nutrient acquisition.

– **Participatory evaluation of *Brachiaria* hybrids in comparison with commercial cultivars**

Contributors: P. Argel, A. Schmidt, M. Peters, J. Miles and I. M. Rao

Rationale

As part of the BMZ-GTZ project on developing aluminum resistant *Brachiaria* hybrids, this year we initiated field studies in Costa Rica, Nicaragua and Honduras for evaluation of new hybrids of *Brachiaria* along with commercial Brachiaria cultivars in participation with farmers. The main objective of participatory evaluation was to expose the promising hybrids to farmers and generate information on farmer selection criteria. This information is highly useful *Brachiaria* improvement program to incorporate farmer perspectives on *Brachiaria* ideotypes for multiple use in crop-livestock systems.

Materials and Methods

Costa Rica - A field trial was established on 30 August 2002 in the area of Puriscal (Cantón Mora) in a farm called El Rodeo. National staff responsible for managing the trial are Ing.Beatriz Sandoval and Marco Lobo from MAG of Costa Rica. The design is a complete randomized block with 3 repts. The plot size is 2 m long X 4 m wide. The texture of the soil is loamy with the following chemical characteristics: pH 4.6, Al (0.8 ppm), Ca (6.2 cmol/kg), Mg (1.76.2 cmol/kg), K (0.46.2 cmol/kg), Zn (0.6 mg/kg), Cu (5.0 mg/kg), Fe (52 mg/kg); OM (5.7%). The *Brachiaria* species planted had the following CIAT numbers: 606, 26110, 26990, 26318, 26646, 6133, 36061, 6789, 36062, 26124 and Mixe (no CIAT No). One month after planting this was respectively the number of plants per square m: 3.1, 6.6, 1.3, 3.5, 3.5, 1.3, 4.9, 8.0, 0.9, 1.8 and 5.3. Among the 11 genotypes planted, CIAT 36061 and CIAT 36062 are apomictic hybrids. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake will be measured at the end of both wet and dry seasons of each year.

The trials were well established and the results will be reported next year.

3.1.2 Edaphic adaptation of *Arachis pintoi*

Determine genotypic variation in *Arachis pintoi* for tolerance to low P supply

Contributors: N. Castañeda, N. Claassen (University of Goettingen, Germany) and I. M. Rao (CIAT)

Rationale

Phosphorus (P) supply in soil limits forage production in acid soils such as oxisols and ultisols, where P is strongly fixed and largely unavailable for plant uptake. These soils may not be low in total P, but most of it is present in a form of extremely low solubility (Fe/Al or Ca phosphate). This may result in low P uptake and poor plant growth. The problem of low P uptake could be alleviated both by identifying and/or developing plant species that are able to absorb P from low available P pools and with application of small amounts of P fertilizer. Plant species and genotypes differ in their ability to grow under low P supply conditions, i.e. they differ in their P efficiency.

The P efficiency of one species or genotype relative to another may change depending on the binding form of P in soil. Differences in P efficiency can arise in three ways: 1) efficiency with which P is utilized in the plant to produce final yield. This is often called internal P requirement and is the P concentration in the plant to produce for example 90% of maximum yield; 2) the uptake efficiency is the ability of the root system to acquire P from soil and accumulate it in the shoots. This is given by the amount of roots and the uptake per unit of root i.e. the influx; and 3) the shoot growth rate may affect the P efficiency, i.e. a plant with a high potential growth rate may put a higher demand on the root system for supplying the shoot with nutrients or may have a lower P concentration in the shoot than a plant with low growth potential even though the uptake efficiency wase the same. Thus there could be different causes for differences in P efficiency among species and genotypes.

Several researchers have proposed that differences in P uptake efficiency are based on size and type of root system i.e. root length, root radius, root hair density as well as on rate of shoot growth, since the latter imposes the demand for P on the root. Phosphorus uptake may also be influenced by chemical changes in the rhizosphere due to root exudates, for example pH changes, organic acid etc.. The solubilizing effect will depend on the binding form of P in soil. Al and Fe phosphate solubility increases with increasing pH while the solubility of Ca phosphates, including phosphate rocks decreases. Thus a plant species efficient to mobilize for example Fe/Al-P may not be efficient if Ca-P predominates in the soil. Furthermore, the association of plants with vesicular-arbuscular mycorrhizae improves P uptake form soil.

Field studies conducted in Caquetá, Colombia and greenhouse studies conducted at CIAT-Palmira indicated significant genotypic variation in P acquisition and utilization in *A. pintoi*. In order to identify P-efficient *Arachis pintoi* genotypes and to define specific mechanisms contributing to P efficiency, a Ph. D. theis work is in progress at the University of Goettingen in collaboration with CIAT. Two growth chamber studies were conducted and results from the first study to determine genotypic differences among ten accessions of *A. pintoi* in P-acquisition and utilization from low P soil are reported below. Results from the second study are being processed and will be reported next year.

Material and Methods

A growth chamber trial was conducted at Institut for agricultural chemistry – Goettingen, Germany. An Oxisol (clay 50%, organic carbon 0.35%, pH_{CaCl2} 5.1, pH_{H2O} 5.2, P-CAL 0.4 mg /100 g soil and P-Bray II 1.4 mg/100 g soil, Fe/Al-P 788 mg kg^{-1} and Ca-P 330 mg kg^{-1}; in soil solution pH 5.2 and 13 µg P L^{-1}) was used for evaluating the phosphorus efficiency of 10 accessions of *A. pintoi* (CIAT 17434, 18747, 22172, 18744, 18748, 22160, 22155, 18751, 18745, 22159) in a pot experiment. Treatments were arranged in a randomised block design with a split-plot arrangement with 3 replications. Three levels of P supply were used as main plots and 10 accessions of *A. pintoi* as sub-plots. For high P supply treatment, only 4 accessions of *A. pintoi* (CIAT 17434, 18747, 22172, 18744) were included. Among the 10 accessions tested, CIAT 17434 is a commercial forage cultivar in Latin America.

Plastic pots of 4 L capacity were filled with 2.7 kg air-dry soil at a bulk density of 1.2 g cm^{-3}. Three levels of P supply (0, 10 and 400 mg kg^{-1}) as $Ca(H_2PO_4)_2.H_2O$ were given. Basal nutrients were applied (mg kg^1) to each pot: 100 N as $Ca(NO_3)_2$, 50 K as K_2SO_4, 40 Mg as $MgSO_4$, 0.2 B as H_3BO_3, 0.1 Mo as $(NH_4)_6Mo_7O_{24}$. Two weeks before sowing, water was added to get a moisture content of 25% w/w. Seeds were sown directly in the pots. One pot for each P treatment was kept unplanted to measure soil moisture evaporation losses from the pots. Plants were thinned at the earliest to maintain 4 plants in each pot. The soil surface in each pot was covered with a layer of quartz sand (1 to 2 cm) to avoid the formation of a superficial crust due to the watering. The pots were watered daily and water was added to maintain the soil with 60% of its water holding capacity. Pots were kept in a growth chamber, maintained at 25°C, with a photon flux density of 300 µmol m^{-2} s^{-1} and 80% relative humidity during 16 h day and at 20 °C and 70% relative humidity during 8 h night.

At harvest (90 days), quartz sand was removed form the soil surface and shoots were cut above the soil surface. The dry weight of shoot was recorded. Roots were carefully separated by washing the soil on a sieve with 200 µm mesh width. Roots were cleaned of any foreign material, surface moisture was removed by centrifuging and root fresh weight was determined. Subsamples of around 0.5 g were preserved in 20% ethanol for root length measurements using line intersection method.

To determine P concentration in plant tissue, plant samples were wet digested in HNO_3 and P was determined with Molybdate-Vanadium method. Shoot P uptake, P acquisition efficiency (mg of P uptake in shoot biomass per unit root length), and P use efficiency (g of forage production per g of total P acquisition) were determined. Data were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an F-test. A probability level of 0.05 was considered statistically significant.

Results and Discussion

With no external supply of P (P-0) to an infertile oxisol, the commercial cultivar (CIAT 17434) was least productive in terms of shoot biomass compared with the other 9 accessions (Figure 61). Among the 10 accessions, CIAT 22159 was outstanding in shoot biomass production. This observation is consistent with the field observations made at Carimagua on a very infertile oxisol. At low level of P supply (P-10), CIAT 22159 and 18744 were superior to other accessions. But at very high P supply (P-400), the commercial cultivar (CIAT 17434) was outstanding indicating its greater demand for P supply in soil.

Results on total root length showed that the superior performance of CIAT 22159 with no P supply was related to its ability to produce fine root system (Figure 62). At low P supply, the differences among 10 genotypes in total root length were small. But with high P supply, total root length of CIAT 18744 was markedly lower than that of CIAT 18747 and CIAT 17434 indicating its relative less dependence on high P supply on soil and also greater efficiency in acquiring P per unit root length. This was evident from the results shown in Figure 63 on shoot P uptake.

The differences in shoot P uptake between P-0 and P-10 treatments were small and CIAT 18744 was outstanding in acquiring greater amounts of P from P-0 treatment. Shoot P uptake by CIAT 18744 was greater at high P supply (P-400) and this was achieved with lower value of total root length indicating its greater efficiency in acquiring P per unit root length (Figure 64). At low level of P supply (P-10), CIAT 22159 showed greater ability to acquire P per unit root length.

This is an important attribute that could contribute to its superior field performance observed in Carimagua in field trials.

P use efficiency, which is a measure of g of shoot biomass produced per mg of total P uptake, of the commercial cultivar (CIAT 17434) with P-0 treatment was markedly lower than the other 9 accessions (Figure 65). As expected, P use efficiency decreased markedly with increase in P supply to soil. The greater values of P use efficiency were observed with CIAT 18751 and CIAT 22160 with P-0 and P-10 treatments, respectively.

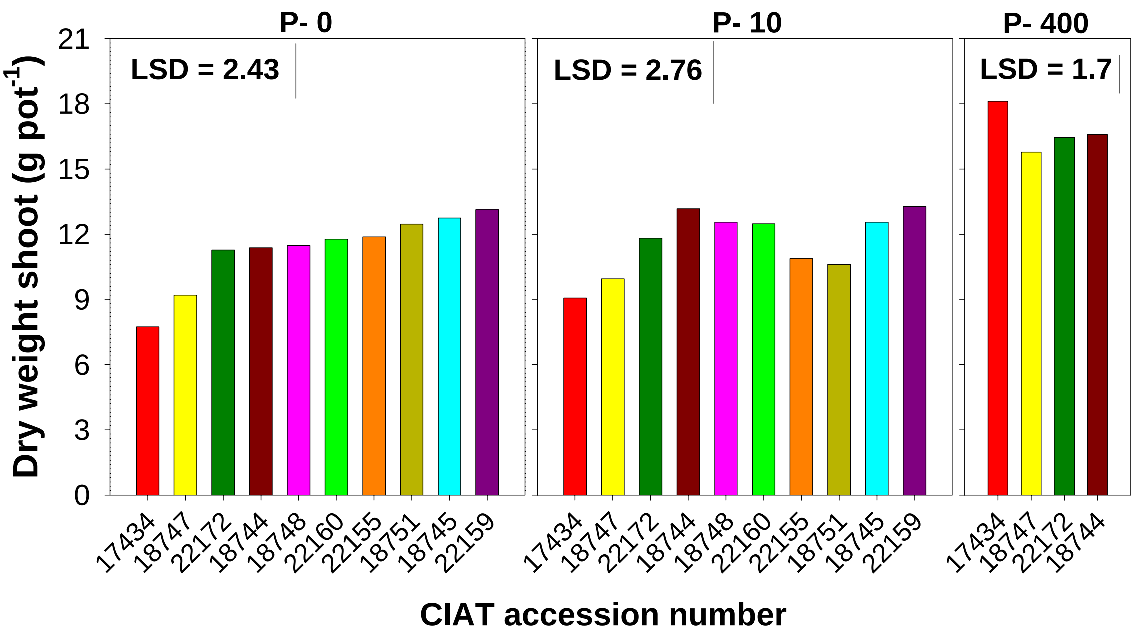


Figure 61. Genotypic variation as influenced by phosphorus supply (P-0, P-10 and P-400) in shoot biomass (forage) production of 10 accessions of *Arachis pintoi* grown for 90 days in a clay loam oxisol in a growth chamber. LSD values are at the 0.05 probability level.

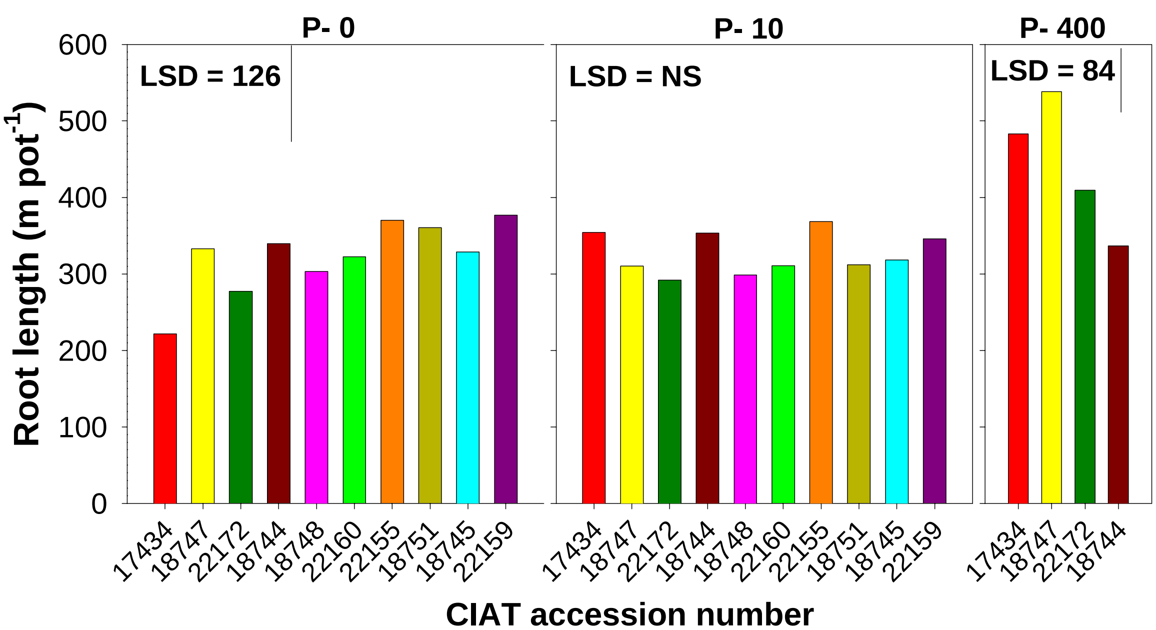


Figure 62. Genotypic variation as influenced by phosphorus supply (P-0, P-10 and P-400) in total root length of 10 accessions of *Arachis pintoi* grown for 90 days in a clay loam oxisol in a growth chamber. LSD values are at the 0.05 probability level.

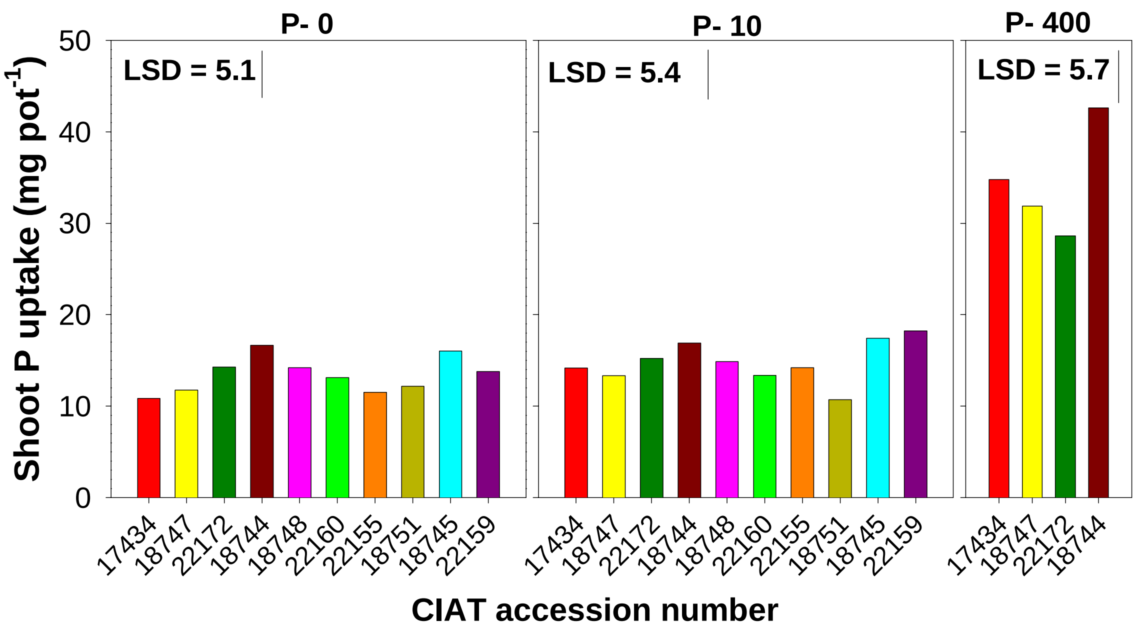


Figure 63. Genotypic variation as influenced by phosphorus supply (P-0, P-10 and P-400) in shoot P uptake of 10 accessions of *Arachis pintoi* grown for 90 days in a clay loam oxisol in a growth chamber. LSD values are at the 0.05 probability level.

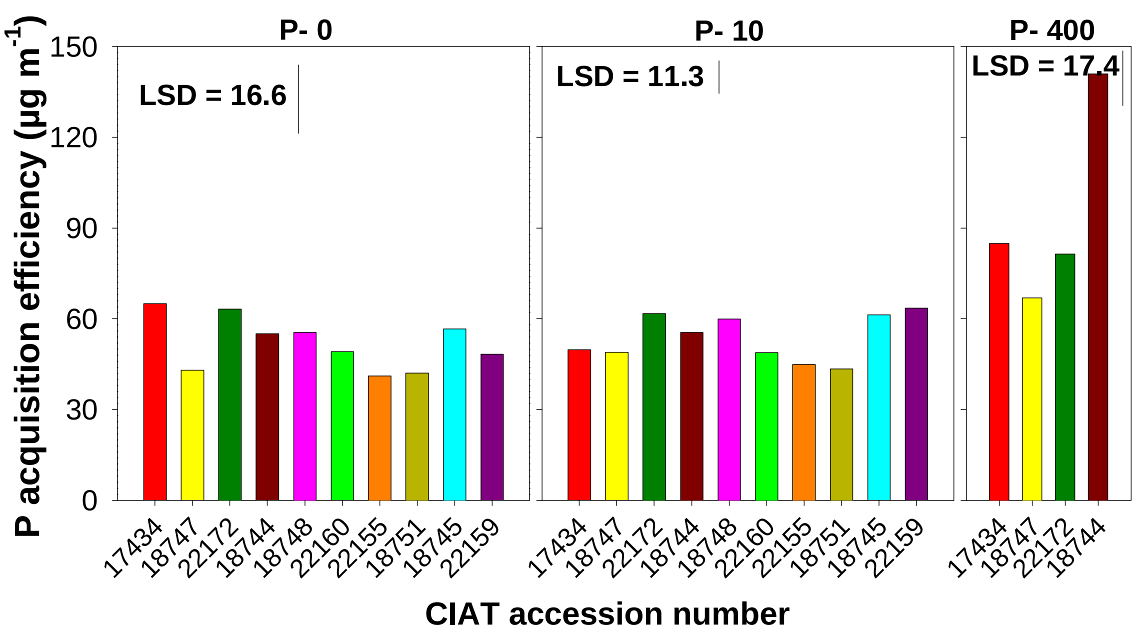


Figure 64. Genotypic variation as influenced by phosphorus supply (P-0, P-10 and P-400) in P acquisition efficiency of 10 accessions of *Arachis pintoi* grown for 90 days in a clay loam oxisol in a growth chamber. LSD values are at the 0.05 probability level.

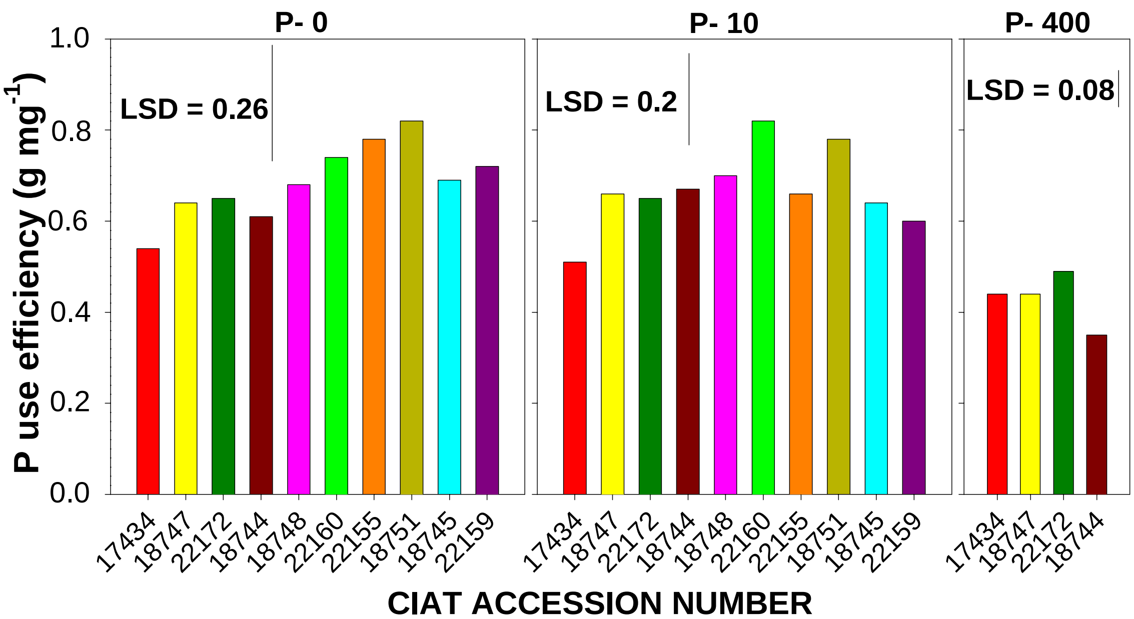


Figure 65. Genotypic variation as influenced by phosphorus supply (P-0, P-10 and P-400) in P use efficiency of 10 accessions of *Arachis pintoi* grown for 90 days in a clay loam oxisol in a growth chamber. LSD values are at the 0.05 probability level.

This study conducted under controlled environmental conditions in a growth chamber indicated that the commercial cultivar (CIAT 17434) is relatively less adapted to low P supply in soil. Results from this study also confirmed the previous results obtained under field conditions that the accession CIAT 22159 is better adapted to very low P supply in oxisols.

– Field evaluation of most promising accessions of *Arachis pintoi* in the Llanos of Colombia

Contributors: I. M. Rao, M. Peters, C. Plazas and J. Ricaurte

Rationale

Based on field studies conducted in Caqueta, Colombia and the data collected from multilocational evaluation, we have assembled a set of 8 genotypes for further testing at two sites (Piedmont and Altillanura) in the Llanos of Colombia. The site in Piedmont is close to La Libertad (CORPOICA Experimental Station) and the soils in this region are relatively more fertile than in the Altillanura. The site in Altillanura is at Matazul farm where the soils are relatively infertile (sandy loam). The main objective of this work was to identify plant attributes related to superior adaptation of the most promising accessions for the llanos of Colombia.

Materials and Methods

A field trial was established in May, 2001. The trial was planted in Piedmont as monoculture. We expect multiple use for this legume in the Piedmont area (e.g., cover legume in plantations). The trial included 8 accessions of *Arachis pintoi* (CIAT 17434; 18744; 18747; 18748; 18751; 22159; 22160 and 22172). The trial was planted as randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications (Photo

12). Genotypic differences in establishment were determined at 12 months after establishment and the plots were standardized to evaluate regrowth potential at low and high initial fertilizer application.

High initial fertilizer **Low initial fertilizer**

Photo 12. Genotypic variation among 8 accessions of *Arachis pintoi* in response to initial level of high or low amounts of fertilizer application.

Results

A visual assessment of vigor and soil cover was carried out at 140 days after establishment (Table 38). Under both low and high initial fertilization, CIAT 18751 showed rapid establishment and vigor. But the soil covering ability of the commercial check CIAT 17434 was greater than the other accessions with high fertilizer application. Under low fertlizer application treatment, CIAT 18741 and CIAT 18751 were superior in soil covering ability than the other accessions.

Table 38. Differences among 8 accessions of *Arachis pintoi* in vigor (%) and soil cover (%) at 140 days after establishment as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

Germplasm	High Fertilizer		Low fertilizer	
accession	Vigor	Soil cover	Vigor	Soil cover
17434	4.0	93	3.6	90
18744	3.6	75	3.6	98
18747	3.6	75	3.6	100
18748	4.0	83	3.0	83
18751	4.3	90	4.0	100
22159	3.3	68	3.0	73
22160	3.3	45	3.0	60
22172	3.0	75	3.0	82
Mean	**3.6**	**76**	**3.4**	**86**

Vigor: 1 = poor; 5 = excellent.

At 12 months after establishment of the trial, the response to initial fertilizer application was greater with the commercial check, CIAT 17434 (Figure 66). But the 7 out of the total 8 accessions performed better than the commercial check with both low and high initial fertilizer application. Three months after standardization of plots (at 12 months after establishment), two genotypes CIAT 18744 and

CIAT 22159 were outstanding in their potential for regrowth with low initial fertilizer application (Figure 67).

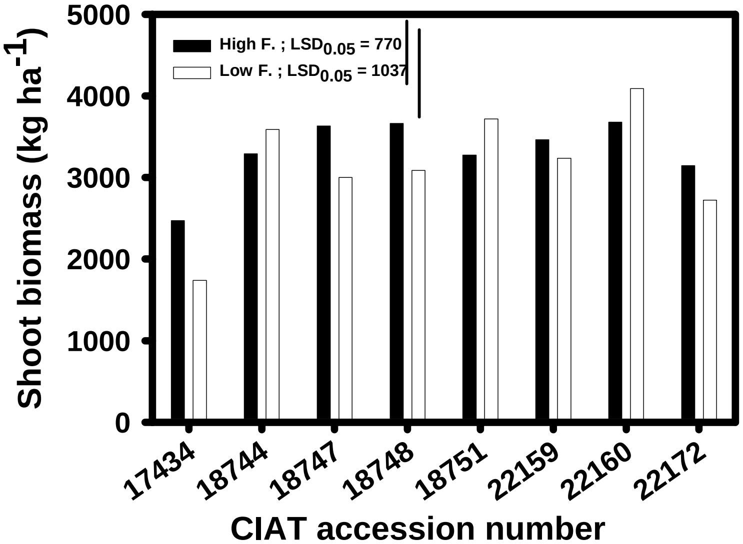


Figure 66. Genotypic variation in forage yield of 8 accessions of *Arachis pintoi* at 12 months after establishment (June 5, 2002) in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

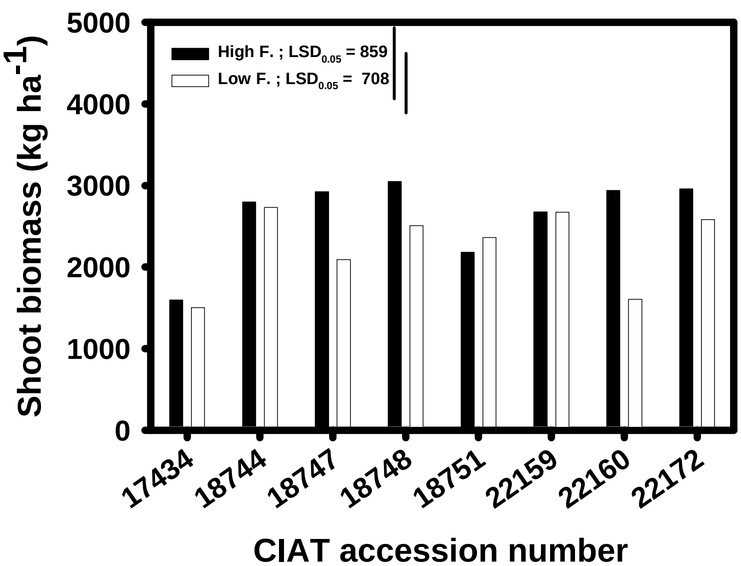


Figure 67. Genotypic variation in regrowth potential (forage yield) of 8 accessions of *Arachis pintoi* in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont. Measurements were made at 79 days after standardization of plots (August 22, 2002). Standardization was done at 12 months after establishment.

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored.

This field study indicated that the *Arachis pintoi* accession CIAT 22159 is superior to the commercial cultivar (CIAT 17434) in terms of rapid establishment as well as regrowth potential.

Activity 3.2 Identify genotypes of grasses and legumes with dry season tolerance

Highlights

- Showed that the superior dry season performance of *B. Brizantha* cv. Toledo (CIAT 26110) which maintained high proportion of green leaves during moderate dry season in the Llanos of Colombia was associated with greater acquisition of nutrients from drying soil.

- Selected accession of sorghum developed by ICRISAT that feed more total forage in a subhumid environment than local checks used for silage production.

3.2.1 Drought tolerance of *Brachiaria*

Determination of the genotypic variation in dry season tolerance in *Brachiaria* accessions and genetic recombinants in the Llanos of Colombia

Contributors: I. M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. Garcia (CIAT)

Rationale

A major limitation to livestock productivity in subhumid regions of tropical America is quantity and quality of dry season feed. A field study is in progress at Matazul Farm in the Llanos of Colombia. The main objective was to evaluate genotypic differences in dry season (4 months of moderate drought stress) tolerance of most promising genetic recombinants of *Brachiaria*. Results from this field study for the past 2 years indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the Llanos of Colombia, was associated with lower levels of K and N content in green leaves. This year, we continued our efforts to monitor the dry season performance into third year after establishment.

Materials and Methods

A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. Among the germplasm accessions, CIAT 26110 was identified from previous work in Atenas, Costa Rica as an outstanding genotype for tolerance to long dry season (up to 6 months). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20 P, 20 K, 33 Ca, 14 Mg, 10 S; and high: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, and shoot nutrient uptake were measured at the end of the dry season (33 months after establishment; April 2002). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet season of 2001 (April 2001).

Results and Discussion

Because of the application of maintenance fertilizer, forage yields with high fertilizer treatment were greater than those with low fertilizer treatment (Table 39). At 33 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 0 to 1834 kg/ha and the highest value of forage yield was observed with a germplasm accession CIAT 26110. This accession was released in Costa Rica as cultivar Toledo and is known for its dry season tolerance. Among the 4 parents, B. decumbens cv. Basilisk (CIAT 606) was outstanding in live forage and dead biomass production with high fertilizer application. A spittlebug resistant *Brachiaria* genetic recombinant, FM9503-S046-024 was superior to other hybrids in terms of greater live shoot biomass and total shoot biomass, particularly with high fertilizer application. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants as it produced almost no live forage after dry season. The leaf to stem ratio values of two genetic recombinanats (BR97NO-0082 and FM9301-1371) were markedly superior to other genotypes.

Table 39. Genotypic variation as influenced by fertilizer application in live shoot biomass, dead shoot biomass and total forage yield of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Leaf to stem ratio		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	745	771	4.82	6.14	1506	1654
BR97NO-0383	710	972	2.88	2.84	1382	1938
BR97NO-0405	765	858	1.92	1.58	1711	1847
FM9201-1873	516	1142	2.77	2.53	1407	2256
FM9301-1371	701	848	6.79	4.01	1433	2718
FM9503-S046-024	705	1348	4.42	2.00	1762	4092
Parents:						
CIAT 606	622	2444	1.37	1.53	2086	8264
CIAT 6294	776	1077	3.24	2.51	2022	2603
BRUZ/44-02	0	0	0	0	0	0
CIAT 26646	931	1126	2.51	1.81	2173	2460
Accessions:						
CIAT 26110	1834	2069	2.17	2.22	2778	3445
CIAT 26318	974	1278	2.09	1.98	2323	2683
Mean	**773**	**1161**	**2.92**	**2.43**	**1715**	**2830**
LSD *(P=0.05)*	361	1300			843	4721

The superior performance of *B. brizantha* cv. Toledo (CIAT 26110) with low fertilizer application was mainly attributed to its ability to produce green leaf biomass during dry season (Figure 68). Results on leaf and stem N content indicated that differences among genetic recombinants, parents and accessions were not significant with either low or high fertilizer application (Table 40). But shoot N uptake with low fertilizer application was markedly greater for CIAT 26110 (Table 40; Figure 69). With high fertilizer application, CIAT 606 was outstanding in shoot N uptake. Shoot uptake of P, K, Ca and Mg were also greater with CIAT 26110 (Tables 41 and 42; Figure 69). Among the parents, CIAT 606 was superior in P, K, Ca and Mg acquisition from high fertilizer application.

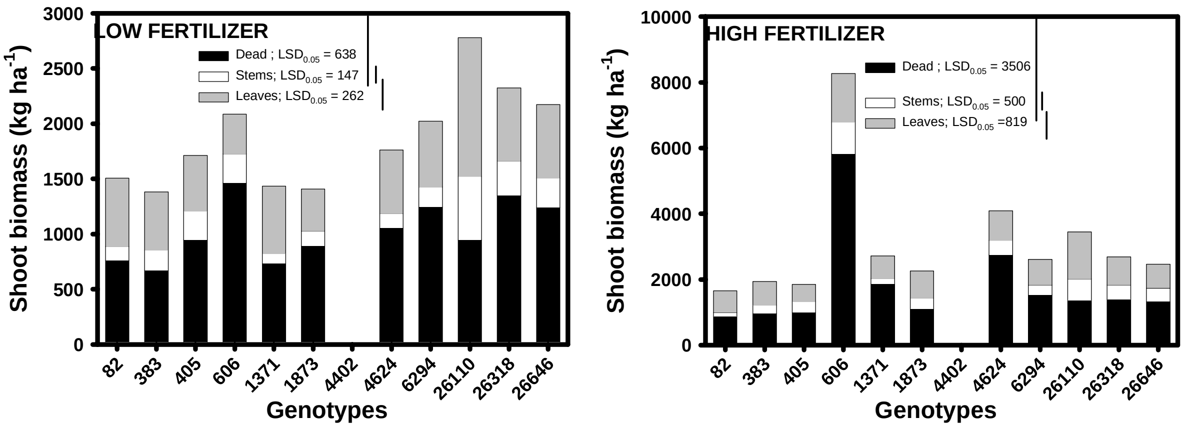


Figure 68. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

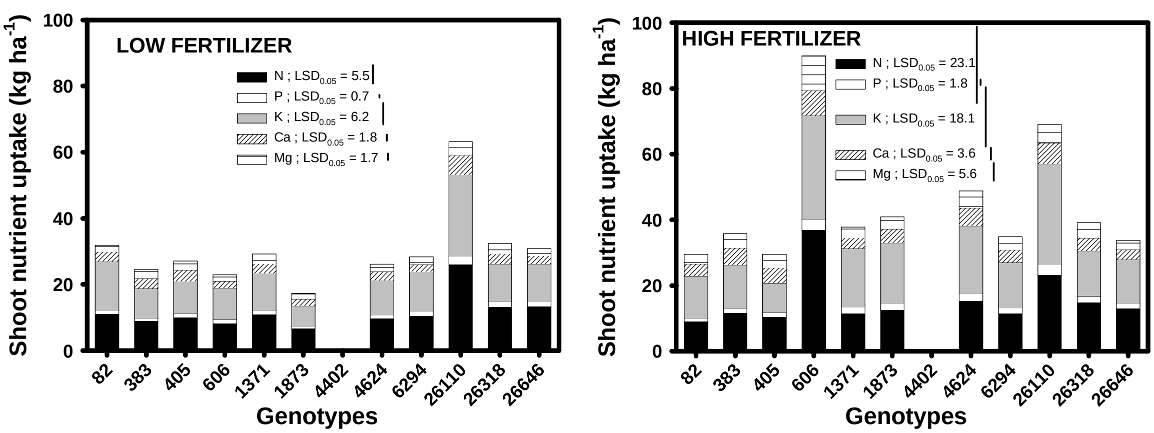


Figure 69. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low and high fertilizer application was associated with greater stem biomass indicating the importance of stem reserves for production of green leaf biomass (Table 43). No significant association was observed between green leaf biomass and level of nutrients in green leaves.

Results from this field study indicated that the superior performance of the germplasm accession CIAT 26110 which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

Table 40. Genotypic variation as influenced by fertilizer application in leaf N content, stem N content and shoot N uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season - April 2002). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		---------- (%) -----------		---------- (kg/ha) -----	
Recombinants:						
BR97NO-0082	1.51	1.26	1.34	0.88	11.07	9.02
BR97NO-0383	1.47	1.34	1.05	0.79	8.98	11.68
BR97NO-0405	1.51	1.53	1.04	0.79	1014	10.47
FM9201-1873	1.52	1.33	1.06	1.08	6.68	12.58
FM9301-1371	1.63	1.47	1.33	1.01	10.92	11.56
FM9503-S046-024	1.45	1.29	1.11	0.82	9.77	15.26
Parents:						
CIAT 606	1.72	1.41	1.07	1.01	8.25	36.88
CIAT 6294	1.47	1.12	0.92	0.89	10.48	11.47
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	1.64	1.31	0.89	0.9	13.38	13.0
Accessions:						
CIAT 26110	1.58	1.28	1.13	0.79	26.01	23.28
CIAT 26318	1.65	1.31	0.91	0.82	13.29	14.78
Mean	**1.56**	**1.33**	**1.08**	**0.89**	**11.73**	**15.45**
LSD *(P=0.05)*	NS	NS	0.26	NS	5.46	23.1

ND = not determined due to small size of the sample; NS = not significant.

Table 41. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season - April 2002). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.16	0.16	0.136	0.124	1.12	1.15
BR97NO-0383	0.12	0.16	0.097	0.119	0.86	1.45
BR97NO-0405	0.15	0.17	0.096	0.098	1.07	1.30
FM9201-1873	0.13	0.17	0.103	0.135	0.65	2.12
FM9301-1371	0.18	0.23	0.153	0.188	1.34	1.83
FM9503-S046-024	0.16	0.20	0.132	0.112	1.06	2.31
Parents:						
CIAT 606	0.21	0.17	0.139	0.116	1.12	3.27
CIAT 6294	0.19	0.17	0.155	0.132	1.39	1.71
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	0.17	0.17	0.098	0.103	1.41	1.69
Accessions:						
CIAT 26110	0.14	0.17	0.138	0.172	2.55	3.31
CIAT 26318	0.18	0.17	0.128	0.112	1.73	1.95
Mean	**0.16**	**0.18**	**0.126**	**0.128**	**1.30**	**2.01**
LSD *(P=0.05)*	0.042	NS	0.046	0.058	0.68	1.79

ND = not determined due to small size of the sample; NS = not significant.

Table 42. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season - April 2002). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	14.83	12.69	2.91	3.68	1.94	2.95
BR97NO-0383	8.88	13.20	3.08	5.14	2.76	4.39
BR97NO-0405	9.76	8.92	3.40	4.71	2.71	4.14
FM9201-1873	6.23	18.29	2.01	4.26	1.72	3.64
FM9301-1371	11.16	17.85	2.91	3.27	2.88	3.30
FM9503-S046-024	10.49	20.65	2.54	5.43	2.24	5.11
Parents:						
CIAT 606	9.61	31.63	1.99	7.64	1.95	10.50
CIAT 6294	12.05	13.75	2.13	3.89	2.32	4.05
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	11.48	13.26	2.51	2.99	2.12	2.76
Accessions:						
CIAT 26110	26.47	30.46	5.84	6.40	4.10	5.59
CIAT 26318	11.22	13.89	3.12	3.85	3.07	4.66
Mean	**11.85**	**17.69**	**2.95**	**4.66**	**2.53**	**4.64**
LSD *(P=0.05)*	6.180	18.09	1.819	3.614	1.732	5.592

ND = not determined due to small size of the sample.

Table 43. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.97***	0.99***
Total forage yield (live + dead) (t/ha)	0.79***	0.89***
Dead biomass (t/ha)	0.41**	0.82***
Stem biomass (t/ha)	0.77***	0.93***
Leaf N content (%)	-0.26	0.27
Leaf P content (%)	0.14	-0.10
Leaf K content (%)	0.02	-0.09
Leaf Ca content (%)	-0.13	-0.32*

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Dry season tolerance of most promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Previous research indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with lower levels of K and N content in green leaves. The main objective of this field study was to evaluate dry season tolerance of the more recent hybrids of *Brachiaria* in comparison with their parents when grown with low nutrient supply in soil at Matazul farm of the altillanura.

Materials and Methods

A field trial was established at Matazul farm on 31 May of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with 3 replications. The plot size was 5 x 2 m.

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at the end of dry season (April 2002; 4 months of moderate drought stress), i.e., at 11 months after establishment of the trial. The trial was managed with strong and frequent mob grazing at 2 months interval.

Results

At 11 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 900 to 1400 kg/ha and the highest value of forage yield was observed with the hybrid 4624 (Figure 70). Differences in dry matter distribution among the hybrids and parents indicated that the hybrid 4624 was superior to other hybrids in terms of green leaf production with both low and high initial fertilizer application.

This is consistent with our previous results obtained for the past 3 years from another trial at the same location. It is important to note that one of the parents, CIAT 606, which is very well-known for its adaptation to low fertility soils, produced greater dead biomass under both low and high initial fertilizer application. This could be an important attribute for recycling of nutrients that contribute to superior persistence under low fertility conditions but may not be a desirable attribute for improved animal performance. The dry matter distribution pattern of the hybrid 4015 was in contrast with that of the parent CIAT 606. This hybrid produced very little dead biomass and a greater proportion of aboveground biomass was in green leaves. Another hybrid, 4132 had markedly lower stem biomass compared with green leaf biomass.

The hybrid 4624 was also outstanding in its ability to acquire nutrients, particularly N and Ca with both low and high application of fertilizer (Figure 71).

Results on nutrient uptake also showed that CIAT 6294, which is known for its moderate level of adaptation to infertile acid soils, was inferior to the 4 hybrids tested in terms of its ability to acquire nutrients, particularly N.

The results obtained on green leaf and stem N and P contents also indicate the superior nutritional quality of the hybrid 1251 under both high and low intial fertilizer application (Figures 72 and 73).

The dry season performance of the 4 hybrids will be monitored for the next 2 years in comparison with the two parents in terms of forage yield and nutrient acquisition.

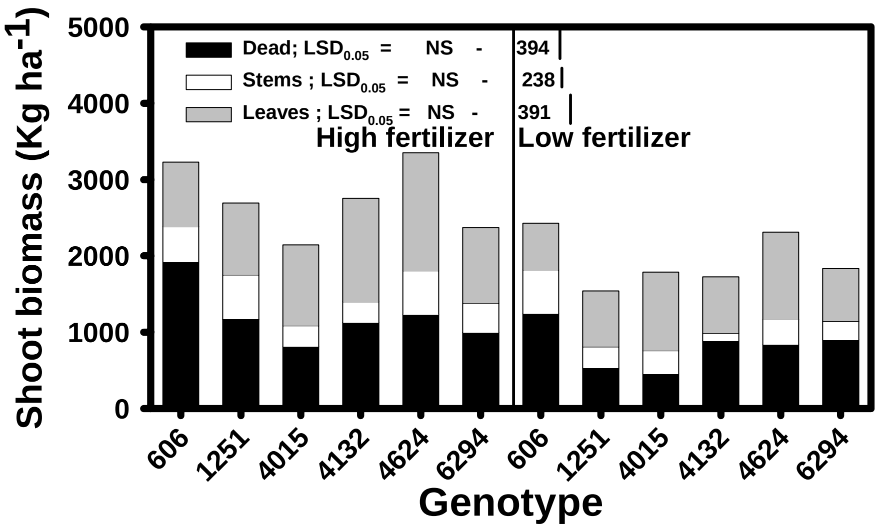


Figure 70. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

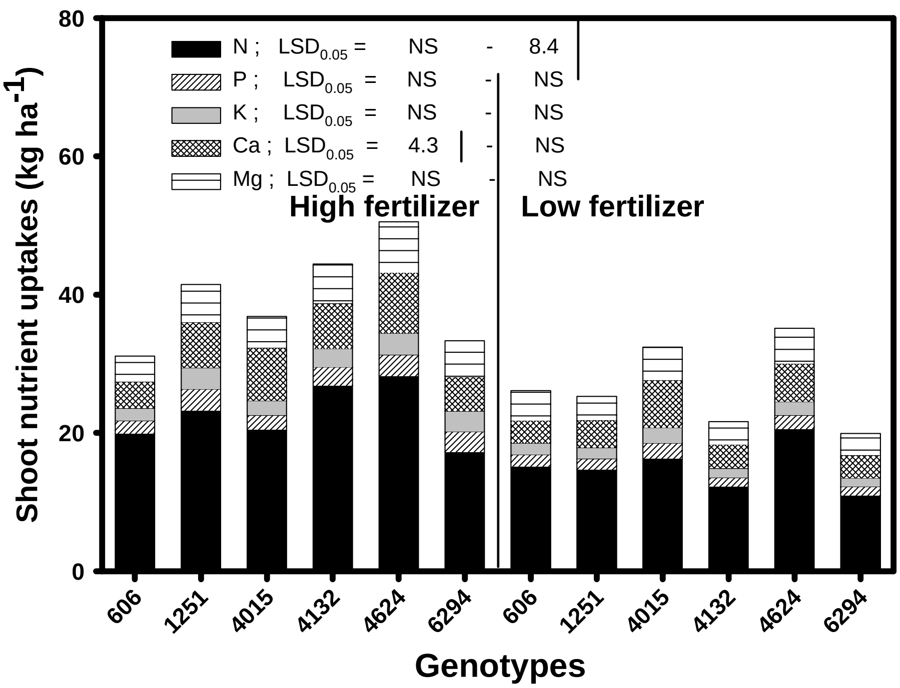


Figure 71. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level. NS = not significant.

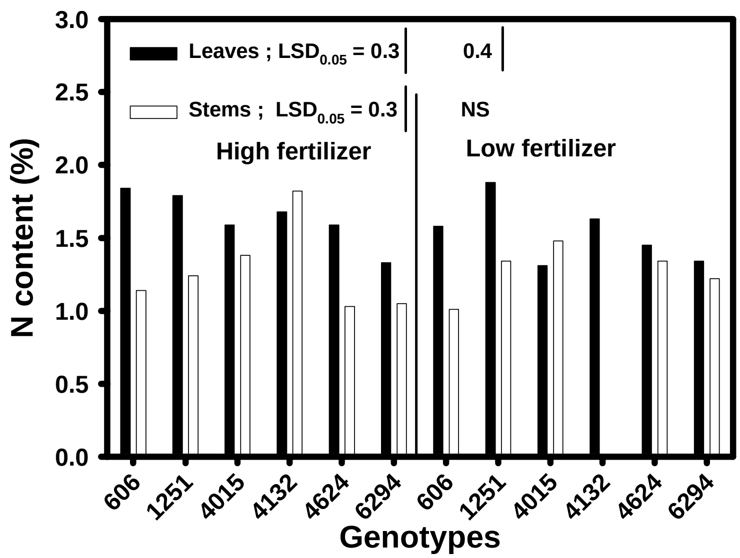


Figure 72. Genotypic variation as influenced by fertilizer application in nitrogen (N) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

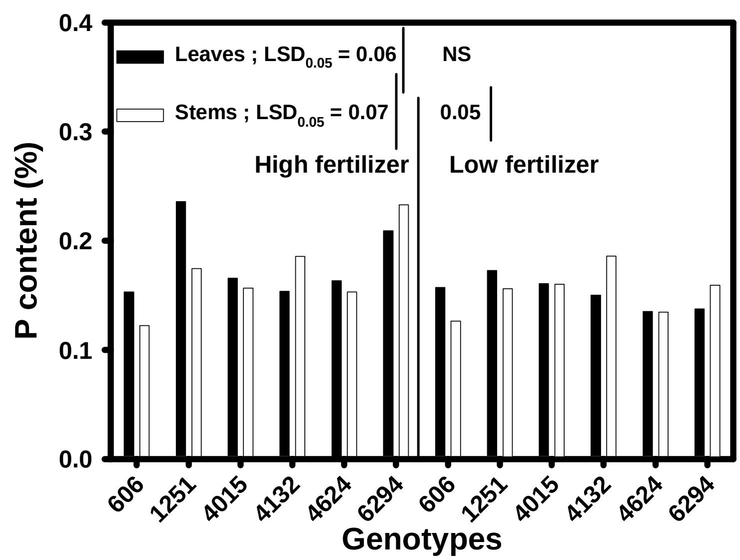


Figure 73. Genotypic variation as influenced by fertilizer application in phosphorus (P) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

3.2.2 Field studies to determine DM yields and quality of forage sorghum lines and pearl millet in a subhumid environment of Costa Rica.

Contributors: Carlos Boschini (UCR), Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

Sorghum is widely used either as forage or as grain for both animal and human consumption. However, the genus is highly variable in terms of plant morphology, flowering time, tolerance to pest and diseases, drought tolerance, grain and forage yields. Commercial lines of forage sorghum are presently under use, particularly in the subhumid tropics, for making silage, for direct grazing or as cut and carry material. New lines are also available from breeding programs carried out elsewhere, thus it is worth to investigate the adaptation and potential yield of these germplasm as compared with more traditional lines.

Materials and Methods

Thirteen lines of forage sorghum (*Sorghum almum*) and 9 lines of pearl millet (*Pennisetum thyphoides*) originated from ICRISAT, were planted for evaluation in a randomized block design with three replicates at Atenas, Costa Rica. Planting distance were respectively 0.50 m between rows and 0.25 m between plants for a plant density of 160,000 plants per ha. The sorghum established normally but the pearl millet failed to establish due to poor quality of the seed. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, and low both in P and Al content. The experiment was planted in September 2001 and harvested 98 days later when the panicles were at milking stage. Panicle and tiller number as well as DM yields of leaves, stems and panicles were recorded. The samples were dried in an air forced oven set at 60 ºC for 72 hours, and then grounded to less than 5-mm particles. Crude protein content (CP) by the Micro-Kjeldahl method, and acid detergent fibre (ADF) by Goering and van Soest method, were determined.

Results and Discussion

All lines of sorghum established well and seedling emergence had a mean of 73% (range 92 to 49) a week after planting. One month later, plant height reached a mean of 33 cm (range 27 to 38 cm), and two months later a mean of 175 cm (range 156 to 194 cm). At this date the more vigorous lines were respectively the numbers 496, 591, 635, 879 and 649-1. Panicle emergence was not uniform and extended from 22 November (lines 979 and 897) through 13 December.

No significant pests or diseases were recorded during the evaluation period, apart of a low incidence of leaf spots caused by *Helminthosporium* spp., particularly in the local check (called Sorgo negro).

Dry Matter yields. There were significant differences (P<0.05) between sorghum lines in DM yields for the components of leaves, stems and panicle as shown in Table 44. This affected total DM yield and a group of at least nine accessions had a total yield superior to 300 g/plant (approximately 48 ton per ha of DM), although the accessions 635 and 634-1 had an estimated yield over 61 ton of DM/ha. These latter accessions had either intermediate or small size panicles as compare to the accession 591 and 897.

Following harvest in December plants were subject to a severe dry period of about 5 months. With the initiation of rains there were significant differences among the accessions in re-growth, which is a highly valuable agronomic characteristic given that sorghum is planted in many subhumid areas of the region with prolonged dry seasons.

In general, all accessions suffered some degree of plant mortality; however, the accessions 897 and 879 produced more shoots than the others – respectively 27 and 23 new shoots out of 108 plants initially planted. It is interesting to note that these accessions had the lowest DM yield, but for an unknown reason they tolerated better the dry season than the other accessions in this particular site. The accession 635 (which was a high yielding accession) produced 16 new shoots following the rains, while 634-1 produced only 6 plants. The local check (Sorgo negro) was also poor to re-growth; this variety had low yield, particularly referred to stems and panicles, as compared to other accessions.

Table 44. Dry matter yields of leaves, stems and panicles 98 days after planting of ICRISAT sorghum lines established for evaluation in Atenas, Costa Rica.

Accession	DM yield (g/plant)			
ICIRISAT No.	Leaves	Stems	Panicles	Total
635	39.2 ab*	320.3 a	29.1 bc	388.6
634-1	44.1 a	324.2 a	13.5 d	381.8
591	38.1 ab	283.9 ab	49.2 a	371.2
613	43.2 ab	306.1 a	21.7 cd	371.1
496	36.8 ab	290.1 ab	26.1 bcd	353.0
639	37.6 ab	283.1 ab	28.9 bc	349.6
638	34.0 bc	273.1 ab	29.7 bc	336.8
623	42.2 ab	287.4 ab	16.8 cd	346.3
645	26.3 cd	256.0 abc	17.6 cd	299.9
649-1	26.4 cd	225.5 bcd	25.3 bcd	277.3
610	42.4 ab	197.6 cde	12.4 d	252.5
Local check	34.7 abc	182.6 de	15.4 cd	232.8
879	24.0 de	150.2 e	23.3 cd	197.5
897	16.7 e	80.9 f	38.7 ab	136.2

*Within each column means followed by the same letter are not statistically different (P<0.05)

Forage quality. The crude protein (CP) and fiber (ADF) in sorghum lines evaluated, are shown in Table 45. As expected, leaves had more concentration of CP than stems and panicles but CP percentages did not vary greatly among leaves, stems and panicle components of different accession, as observed by the low standard deviation. However, there was a clear tendency, particularly in leaves, of more CP concentration in low yielding lines such as the Check and accessions 879 and 897 (Table 44). It is worth indicating again that these accessions produced the larger number of re-growths following the dry period. On the other hand, within the top yielding accessions the accession 634-1 had one of the highest CP contents in the leaves, which makes this an interesting line for future evaluation.

The ADF fraction, which is composed of a hemicelulose, is highly correlated with forage digestibility. The ADF mean was slightly higher in stems (46.4%) than in leaves (41.1%) and panicles (31.2), but are within the values observed for this type of forage. Ten of the best lines are presently under seed multiplication increases for further evaluation. In general, our results indicate that there is great scope for seeding high yielding sorghum was developed by ICRISAT for silage production in the subhumid environment of Central America.

Table 45. Quality component of forage sorghum lines established for evaluation in Atenas, Costa Rica (samples taken 98 days after planting).

Accession	CP (%)			ADF (%)		
ICRISAT No.	Leaves	Stems	Panicles	Leaves	Stems	Panicles
635	11.5	2.4	10.6	41.6	42.7	31.3
634-1	13.6	3.0	9.7	39.3	40.7	37.7
591	11.8	2.3	3.4	44.1	42.9	31.6
613	11.3	4.1	10.1	45.5	51.6	30.6
496	12.1	4.6	10.1	40.4	47.4	29.7
639	12.7	2.4	10.5	40.4	43.4	29.2
638	11.2	3.1	10.3	40.0	47.1	31.4
623	11.0	3.5	9.1	46.5	49.8	42.4
645	10.4	4.0	9.1	41.2	44.6	22.0
649-1	12.6	2.8	10.2	41.8	43.0	27.8
610	10.8	3.6	9.0	42.7	45.2	36.5
Check	15.0	3.6	9.2	38.0	48.6	36.2
879	16.8	4.4	10.1	36.8	48.9	28.6
897	15.2	4.1	10.0	37.7	53.4	22.6
Mean	12.6	3.4	9.4	41.1	46.4	31.2
Sd	1.9	0.8	1.8	2.9	3.8	5.6

3.2.3 Participatory evaluation of multipurpose legumes for enhanced tolerance to drought (cover, feed, green manure)

Contributors: Axel Schmidt (IP-5, PE-2, PE-3), Steve Hughes (SARDI, Australia), Michael Peters (IP-5), Edmundo Barrios (PE-2), Luis Alfredo Hernández (SN-3), Clark Davies (IP-5), Pedro Pablo Orozco (PE-2)

Rationale

Farmers' adaptive capacity to cope with increasing drought incidents in Central America depends to large extent on the availability of drought tolerant crops and forages. Limited through 6-7 months of dry season (November to May/June), smallholders in the hillsides of Nicaragua (750-1100 mm/a) are looking for crop varieties and forage species which safeguard their harvests and permit to feed animals during the dry season. Multipurpose legumes adapted to these dry conditions can play a significant role in enhancing livestock feed during the dry season and, if integrated in cropping systems, they may also improve soil fertility through nitrogen fixation and suppress weeds when used as cover crops (mulch).

Species requiring smaller amounts of water for considerable biomass production (feed, cover, green manure) might even offer the possibility to extent the growing season beyond the two traditional growing periods "*primera*" from May/June to September and "*postrera*" from September to November. To match farmers' demand for such plant materials, it is necessary to screen available germplasm for adaptation to local conditions and for plant characteristics which allow for the integrations of these species into the traditional maize-beans cropping system. In order to improve adoption such germplasm evaluation and selection should be based on farmers' criteria and in collaboration with them.

Material and methods

In collaboration with the South Australian Research and Development Institute (SARDI) and its Australian Medicago Genetic Resource Centre, a set of 30 annual legume species were selected for an adaptation experiment at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The site is known by local farmers as the driest place in San Dionisio and is shared by other CIAT projects and national collaborators for the screening of different crops for drought tolerance (e.g. beans, grain/forage sorghum). The legume germplasm includes a variety of *Trifolium*, *Medicago*, and *Lotus* species mostly from the Mediterranean region (Table 46). The selection criteria were: low water requirement, fast growth and soil cover, annual growth cycle, ease of establishment, late flowering and no seed shattering.

Table 46. Data passport of germplasm from ICARDA

SARDI Acc. No.	Genus	Species	Alt.	Lat.	Long.	Precip. (mm/a)	***Origin***
367	*Trifolium*	*purpureum*	60	33° 54’ N	35° 28’ E	890	Lebanon
687	*Trifolium*	*hirtum*	300	32° 33’ S	118° 13’ E		Australia
1011	*Trifolium*	*vesiculosum*					Italy
3400	*Medicago*	*arabica*					USA
4715	*Trifolium*	*clypeatum*		36° N	27° E		Greece
5036	*Tetragonolobus*	*purpureus*		45° 30’ N	73° 36’ W		Canada
5045	*Trigonella*	*balansae*					Sweden
6125	*Trifolium*	*apertum*	50	45° 02’ N	39° 00’ E		Russia
8687	*Lotus*	*peregrinus*	140	32° 42’ N	35° 12’ E		Israel
12701	*Lotus*	*edulis*	700	36° 30’ N	03° 45’ E	650	Algeria
12953	*Lotus*	*maroccanus*	91	34° 03’ N	06° 33’ W	425	Morocco
14935	*Trifolium*	*spumosum*	400	37° N	30° E		Turkey
17357	*Trifolium*	*diffusum*					USA
22575	*Hymenocarpos*	*circinnatus*	150	39° 41’ N	19° 45’ E	1100	Greece
24228	*Hedysarum*	*carnosum*	200	35° 50’ N	09° 12’ E	300	Tunisia
24578	*Trifolium*	*isthmocarpum*	250	34° 05’ N	05° 00’ W	750-1000	Morocco
32202	*Trigonella*	Calliceras					Canada
32233	*Medicago*	*scutellata*					Australia
32506	*Hedysarum*	*coronarium*					Morocco
32511	*Tetragonolobus*	*palaestinus*					Spain
33621	*Trifolium*	*alexandrinum*					Australia
33816	*Vicia*	*sativa*					Australia
33842	*Lotus*	*ornithopodioides*					Czechoslovakia
33884	*Trifolium*	*incarnatum*					Unknown
34647	*Medicago*	*polymorpha*					Turkey
35361	*Hedysarum*	*flexuosum*		35° 48’ N	05° 45’ W	750-1000	Morocco
35644	*Trifolium*	*salmoneum*	120	35° 07’ N	35° 38’ E	400	Israel
36323	*Medicago*	*polymorpha*					Australia
36400	*Trifolium*	*squarrosum*					Unknown
36441	*Trifolium*	*michelianum*					Bulgaria

The experiment was reestablished in 2002 since the first establishment failed due to the unusually early beginning of the dry season in October 2001. The experiment was designed as randomized blocks with 3 replicates. Seeds were pregerminated and seedlings transplanted in the 4.5 m x 1.5 m plots at the end of September with an overall planting distance of 0.5 m between plants.

Periodical evaluations (every two weeks) take into account: field emergence, plant height, ground cover, drought tolerance, flowering patterns, biomass production, seed production, incidents of pests and diseases, and for farmer selected species forage quality parameters. Evaluations will be carried out until the onset of the next wet season in 2003. At the beginning, in the middle and at the end of the dry season a selected group of experienced farmers will be invited to evaluate the materials in order to access farmers' selection criteria and determine a set of accessions for further evaluation in larger plots in 2003.

Expected results

It is anticipated that farmers' select a number of approximately 10 accessions for further evaluation. Their selection criteria will be documented. In connection with agronomic data, we expect to develop with farmer participation a plant prototype for legume species tolerant to drought conditions and suitable for integration into traditional cropping systems. Such plant type could have a higher probability for adoption since they meet farmers' criteria. The agronomic data will facilitate the search for this new germplasm at genetic resource units like the Australian Medicago Genetic Resource Centre at SARDI or ICARDA.

Activity 3.3 Shrub legumes with adaptation to drought and cool temperatures

Highlights

- Accessions of *Cratylia argentea* with superior productivity and higher digestibility relative to *C. argentea* cv. Veraniega were identified.

- Identified genetic variability plant attributes and for forage quality parameters in *Flemingia macrophylla.*

- Described phenology of *Flemingia macrophylla* and *Cratylia argentea.*

3.3.1 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*

Contributors: M. Andersson (University of Hohenheim), M. Peters (CIAT), J. Tohme (CIAT), R. Schultze-Kraft (University of Hohenheim), L.H. Franco (CIAT), P. Ávila (CIAT), G. Gallego (CIAT), statistician G. Ramirez (CIAT)

Rationale

The work of CIAT on shrub legumes emphasizes the development of materials to be utilized as feed supplement during extended dry seasons. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. *Flemingia macrophylla* and *Cratylia argentea* have shown promising results in such environments and hence work on these genera is part of the overall germplasm strategy of the CIAT Forages team.
C. argentea is increasingly adopted and utilized, particularly in the seasonally dry hillsides of Central America, and more recently in the Llanos of Colombia. However, most research and development is

based on only few accessions and hence activities to acquire and test novel germplasm of *C. argentea* are of high priority. *F. macrophylla* also is a highly promising shrub legume with excellent adaptation to infertile soils. In contrast to *C. argentea*, whose adaptation is limited to an altitude below 1200 masl, *F. macrophylla* can successfully be grown up to altitudes of 2000 masl. However, the potential utilization of *F. macrophylla* is so far limited by the poor quality and acceptability of the few evaluated accessions.

The project aims to investigate the genetic diversity within collections of *F. macrophylla* and *C. argentea* with three main objectives. Firstly, to identify new, superior forage genotypes based on conventional germplasm characterization/evaluation procedures (morphological and agronomic traits, forage quality parameters, including IVDMD and tannin contents). Secondly, to optimize the use and management, including conservation, of the collections. For this, different approaches to identify core collections for each species will be tested and compared based on, respectively: (a) genetic diversity assessment by agronomic characterization/evaluation; (b) germplasm origin information; and (c) molecular markers (AFLPs). Thirdly, to create a planning basis for future germplasm collections with respect to methodology, geographical focus and genetic erosion hazards.

Material and Methods

Agronomic characterization and evaluation: Space-planted, single-row plots in RCB design with three replications were established in Quilichao in March 1999 (*Cratylia argentea*, 39 accessions) and March 2000 (*Flemingia macrophylla*, 73 accessions). Additionally two replications were sown for seed production and morphological observations. The following parameters were measured in the trials: vigor, height and diameter, regrowth, incidence of diseases, pests and mineral deficiencies, and dry matter yield during wet and dry seasons. For the analysis of nutritive value, crude protein content and *in vitro* dry matter digestibility (IVDMD) of the entire collections were analyzed. For the morphological evaluation, qualitative and quantitative parameters were measured, such as days to first flower, days to first seed, flower color, flowers per inflorescence, flowering intensity, pod pubescence, seeds per pod, seed color, leaf area, peduncle length, etc.

For *F. macrophylla*, a more detailed analysis of nutritive value (crude protein and in vitro digestibility) was conducted with a representative subset (25 accessions) which included high, intermediate and low nutritive value accessions. The analysis comprised fiber (NDF, ADF, N-ADF), and condensed and hydrolysable tannin contents. Additionally, another subset of 10 accessions (9 high-quality accessions:18437, 18438, 21083, 21090, 21092, 21241, 21580, 22082, 22327, and 17403) was sampled 2, 4, and 8 weeks after cutting, to investigate the effect of age on digestibility as well as on protein, fiber and tannin content.

Based on variation on morphological, agronomic and forage quality parameters of all accessions, a core collection will be created using multivariate statistic tools (Principal Component Analysis and Cluster Analysis).

Analysis of origin information*:* Based on ecogeographical information of accession origins, a core collection will be created, hypothesizing that geographic distance and environmental differences are related to genetic diversity. The analysis will be conducted with FloraMap™, a GIS tool developed by CIAT, which allows the production of climate probability models using Principal Component Analysis (PCA) and Cluster Analysis.

Genetic analysis by molecular markers (AFLPs): The genetic analysis of the collection of *C.* argentea and *F. macrophylla* is conducted using the AFLP molecular marker technique. Based on the results a core collection will be created, using multivariate statistic tools (PCA and Cluster Analysis).

Data analysis and synthesis*:* Individual and combined data analyses of all generated information will be performed, including the use of GIS tools and multivariate statistics. In the analysis of each of the different approaches (agronomic characterization, origin information, molecular marker analysis), PCA and Cluster Analysis are utilized to create core collections. Eventual correlation between the different approaches and clusters obtained is evaluated. The resulting concept is expected to help deciding which of the three methods or which combination is most appropriate (time and cost efficient) to create a core collection, depending on availability of time and financial resources (e.g., if an agronomic evaluation of the entire collection is not feasible because of time constraints, a core collection may be created using origin information and/or molecular marker analysis).

Based on molecular marker similarities and GIS analysis, we hope to make suggestions on future collections in areas with particularly high diversity, and on sampling strategies (i.e. frequency). Duplicates accessions in the world collections will also be identified.

Results and Discussion

Agronomic characterization and evaluation*:* Three and two evaluations per season were carried out for *Cratylia argentea* and *Flemingia macrophylla*, respectively. Results indicated considerable phenotypic and agronomic variation in the collections. Data for *C. argentea* and *F. macrophylla* are presented in Tables 47 and 48, respectively.

For *C. argentea,* the in vitro digestibility (IVDMD) varied between 61 and 67% and crude protein (CP) content between 19 and 23%. Mean dry matter production was 219 and 202 g/plant in the wet and dry season, respectively. The higher dry season yields indicate good adaptation of *C. argentea* to dry conditions and its sensitivity to moisture excess. Results also showed that CIAT accessions 18674, 22406, 22408, 22409, 22375 and 18957 had the highest dry matter yields ranging from 247 to 319 g/plant. Productivity of these accessions was higher than yields of the material advanced to cultivar in Costa Rica (and soon in Colombia) - an accession mix of CIAT 18516/18668. In addition to the higher yield, accessions 22408 and 22409 also had higher digestibility values (67% and 66%) in comparison to CIAT 18516/18668 (63%).

For *F. macrophylla,* IVDMD varied from 33 to 53% and CP content from 15 to 24%. Mean dry matter production was 208 g/plant in the wet and 118 g/plant in the dry season. In comparison to the results obtained for *C. argentea* the lower yield and lower productivity in the dry season may indicate a lower drought tolerance of *F. macrophylla.* The most productive accessions were CPI 104890, CIAT 7184, 21090, 21241, 21248, 21249, 21519, 21529 and 21580 with a total dry matter production >300 g/plant. Among the materials with dry matter yields higher than 200 g/plant, CIAT accessions 18437, 20622, 20631, 20744, 21083, 21090, 21241 had high digestibility values (>45%). These accessions are superior compared to CIAT 17403, which yielded 208 g dry matter/plant and has a digestibility of 40%.

Based on forage quality results (IVDMD and CP) a representative subset of *F. macrophylla* (CIAT 17403, 17407, 18437, 18438, 19457, 20065, 20616, 20621, 20622, 20744, 20975, 20976, 21083, 21087, 21090, 21092, 21249, 21529, 21580, 21982, 21990, 21992, 22082, J001 --total of 25 accessions of which 10 are erect, 11 semi erect, and 4 prostrate) were chosen for subsequent analysis of NDF, ADF, condensed and hydrolysable tannin content.

Results on fiber analysis in *F. macrophylla* showed that the NDF, ADF and N-ADF content is higher in the dry season than in the wet season. The N-ADF fraction (N not available) was nearly identical for all accessions, as was NDF in the dry season (Table 49).

Table 47. Agronomic evaluation of a collection of *Cratylia argentea* in Quilichao. Data of six evaluation cuts (three in the dry season and three in the wet season). Grey underlaid: Accessions with dry matter yields higher than accession mix CIAT 18516/18668 (material advanced for cultivar release in Costa Rica and Colombia).

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Mean dry matter yields (g/pl) Wet	Dry	Mean	IVDMD (%)	Crude protein (%)
18516	118	103	20	247	238	243	63.35	22.16
18667	119	99	18	218	208	213	63.73	21.34
18668	108	107	18	203	217	210	63.25	21.90
18671	120	102	19	239	190	214	63.56	20.99
18672	102	85	14	185	150	167	60.50	21.10
18674	122	114	23	329	310	319	63.72	21.87
18675	117	95	16	226	208	217	62.68	20.55
18676	115	93	16	231	193	212	61.00	20.42
18957	118	102	18	251	244	247	62.49	21.06
22373	117	91	17	203	207	205	62.82	20.71
22374	124	104	18	247	234	241	64.89	20.96
22375	130	98	18	255	255	255	65.04	22.41
22376	105	78	12	155	146	150	62.51	19.78
22378	109	83	13	155	144	149	61.32	20.65
22379	118	92	16	231	205	218	63.92	20.64
22380	117	93	13	201	166	184	62.23	21.24
22381	111	88	13	178	149	163	63.04	20.59
22382	118	91	14	203	219	211	63.61	21.14
22383	109	95	14	197	163	180	62.00	20.47
22384	120	89	11	210	154	182	64.02	20.45
22386	121	86	13	195	177	186	65.28	20.03
22387	118	91	13	193	185	189	62.22	19.99
22390	104	94	14	174	158	166	64.30	20.07
22391	116	99	16	218	195	207	64.29	20.59
22392	121	88	15	176	150	163	63.96	22.37
22393	117	95	19	203	201	202	63.39	21.96
22394	117	88	15	181	165	173	64.36	22.35
22396	109	80	11	159	149	154	63.89	23.00
22399	109	89	14	160	147	153	66.19	20.93
22400	126	99	18	230	228	229	62.48	21.72
22404	120	97	15	212	226	219	65.68	21.97
22405	120	97	17	235	223	229	64.41	21.52
22406	119	112	22	297	271	284	63.25	21.07
22407	122	99	18	233	220	226	65.62	21.21
22408	129	105	18	278	277	278	67.25	21.54
22409	116	111	18	260	270	265	65.98	22.34
22410	125	96	18	237	214	225	63.07	20.75
22411	109	90	15	193	186	190	64.40	20.31
22412	121	93	13	234	231	233	63.58	19.06
Mean	116	95	16	219	202	211	62.9	21.1
Range	102-130	78-114	11-23	155-329	144-310	149-319	61-67	19-23

Table 48. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Growth habit: e = erect, s = semi erect, p = prostrate. Grey underlaid: Accessions with digestibility >45% and dry matter yield >200 g/plant.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude protein (%)
				Wet	Dry	Mean		
801 (e)	122	91	28	344	180	262	41.81	24.20
7184 (e)	116	98	34	357	266	312	40.01	22.72
17400 (s)	60	93	32	236	108	172	38.26	22.95
17403 (s)	68	98	34	262	153	208	40.29	22.05
17404 (s)	59	85	33	188	113	151	38.58	21.97
17405 (s)	59	79	30	188	131	160	40.63	21.50
17407 (s)	78	100	37	298	181	240	38.15	20.26
17409 (s)	57	104	36	295	157	226	38.22	20.85
17411 (s)	58	89	34	217	173	195	39.51	21.27
17412 (s)	76	98	41	267	167	217	40.37	20.84
17413 (s)	62	95	35	212	127	169	38.82	20.94
18048 (s)	32	48	23	55	26	41	45.05	20.42
18437 (s)	57	99	39	239	159	200	50.04	22.48
18438 (s)	66	79	40	204	84	144	53.41	21.65
18440 (s)	59	85	32	222	112	167	33.16	21.13
19453 (e)	95	71	18	162	93	128	42.44	21.60
19454 (e)	105	85	22	243	134	188	43.29	20.66
19457 (e)	112	86	25	215	175	195	39.10	21.56
19797 (s)	56	84	22	183	105	144	41.40	21.12
19798 (s)	61	93	27	228	153	190	43.27	21.39
19799 (s)	63	84	26	191	122	156	42.01	21.25
19800 (s)	70	92	35	208	150	179	37.21	21.33
19801 (s)	79	92	40	251	151	201	37.63	22.09
19824 (e)	64	96	36	237	164	200	42.10	22.21
20065 (p)	18	19	5	5	2	3	42.19	21.11
20616 (s)	70	104	37	292	149	221	38.79	21.94
20617 (s)	74	90	29	210	123	166	33.92	21.03
20618 (s)	72	91	31	209	136	172	35.62	21.74
20621 (e)	70	78	30	142	118	130	35.97	21.99
20622 (e)	142	90	29	323	223	273	45.36	22.35
20624 (s)	65	100	35	269	177	223	34.71	20.72
20625 (e)	122	87	25	305	185	245	44.28	23.30
20626 (e)	113	91	29	269	214	241	43.68	22.82
20631 (e)	110	89	24	321	204	262	45.22	21.84
20744 (e)	116	89	27	296	180	238	45.42	22.93
20972 (p)	26	54	32	62	36	49	41.45	21.14
20973 (p)	28	44	18	66	26	46	35.64	19.93
20975 (s)	48	77	41	134	71	103	46.32	20.09
20976 (s)	44	54	25	66	59	63	42.87	19.88
20977 (s)	30	32	9	18	12	15	43.21	19.13
20978 (s)	47	50	21	64	29	46	44.53	20.55
20979 (s)	48	77	41	142	78	110	39.89	20.60
20980 (s)	44	55	29	94	46	70	43.68	20.29
20982 (s)	50	62	28	89	57	73	43.33	19.64

Continues…………..….

Table 48. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Growth habit: e = erect, s = semi erect, p = prostrate. Grey underlaid: Accessions with digestibility >45% and dry matter yield >200 g/plant.

Continuation…..

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Mean dry matter yields			IVDMD (%)	Crude protein (%)
				Wet	Dry	Mean		
21079 (e)	44	76	41	168	63	115	40.52	20.01
21080 (s)	38	58	13	94	32	63	39.49	17.20
21083 (e)	102	92	42	317	145	231	50.10	19.97
21086 (s)	26	44	9	43	30	36	37.13	15.11
21087 (s)	59	61	45	144	73	109	45.92	21.29
21090 (s)	93	112	59	520	201	361	49.81	19.73
21092 (s)	74	82	24	214	136	175	51.53	18.89
21241 (e)	131	95	26	362	195	278	45.30	22.20
21248 (e)	126	101	32	406	248	327	39.38	21.79
21249 (e)	127	106	34	508	257	382	41.42	21.71
21519 (e)	124	99	28	359	193	276	42.93	22.26
21529 (e)	128	98	31	420	200	310	43.31	22.24
21580 (e)	131	104	36	586	272	429	42.76	21.03
21982 (p)	21	58	35	98	30	64	41.74	21.52
21990 (p)	37	67	50	117	51	84	37.92	18.65
21991 (p)	31	53	25	64	29	46	39.35	21.00
21992 (p)	31	54	27	66	33	49	46.22	18.85
21993 (s)	40	71	40	117	59	88	43.17	20.02
21994 (p)	26	49	13	42	25	33	37.10	16.90
21995 (p)	32	56	32	77	27	52	38.12	19.80
21996 (p)	22	41	15	25	14	20	42.28	20.00
22058 (e)	81	59	12	135	86	111	38.15	18.69
22082 (s)	77	91	63	274	116	195	43.56	19.81
22087 (p)	24	46	16	44	14	29	42.33	18.12
22090 (s)	41	43	11	36	10	23	37.05	17.72
22285 (s)	46	73	46	144	62	103	38.20	19.72
22327 (s)	36	58	29	76	44	60	46.31	18.67
C10489 (e)	99	95	35	376	196	286	39.82	22.94
I15146 (e)	105	82	26	343	179	261	42.98	23.77
J001 (e)	120	88	31	315	175	245	41.64	23.52
Mean	69	78	30	208	118	163	41.06	20.91
Range	18-142	19-112	5-63	5-586	2-272	3-429	33-53	15-24

Table 49. Fiber content of a representative subset (25 accessions) of *Flemingia macrophylla*. Data of three evaluation cuts (two in the wet season and one in the dry season). IVDMD = *in vitro* dry matter digestibility, NDF = neutral detergent fiber, ADF = acid detergent fiber, N-ADF = nitrogen bound to acid detergent fiber.

Forage quality	IVDMD (%)		NDF (%)		ADF (%)		N-ADF (%)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
High	51.9	49.1	36.3	41.8	26.6	27.4	1.2	1.5
Medium	45.6	42.3	37.8	41.6	29.4	29.9	1.2	1.6
Low	40.7	36.9	39.2	41.6	29.6	30.5	1.3	1.7
Mean	45.1	41.7	38.0	41.6	28.9	29.6	1.2	1.6

A subset of 10 high-quality accessions of *F. macrophylla* was analyzed for forage quality (IVDMD, CP, fiber and tannin content) after 2, 4, 6 and 8 weeks of regrowth in the wet season. The IVDMD, CP and fiber content varied with time. Fiber content (ADF and NDF) seemed to be related with IVDMD and generally had its highest values after six weeks of regrowth. The crude protein content exhibited little variation (Figure 74).

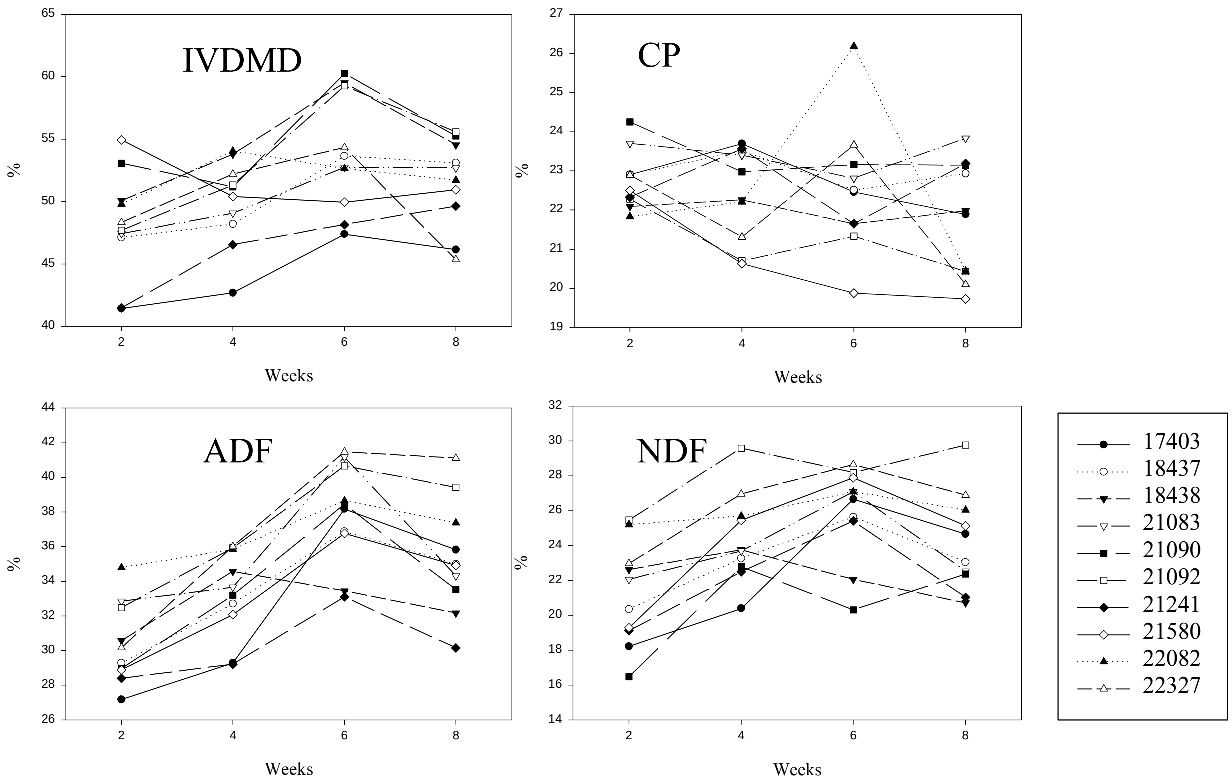


Figure 74. Variability in IVDMD, crude protein and fiber content of 10 high-quality accessions of *Flemingia macrophylla* after 2, 4, 6 and 8 weeks of regrowth. IVDMD = in vitro dry matter digestibility, CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber

Morphological characterization and evaluation*:* The phenology study is not yet completed, but available data allows some preliminary assessment using principal component analysis (PCA).

For *Cratylia argentea*, the parameters included so far are: days to first flower, days to 50% flowering, volubility, flowering intensity, flowers per node, length of inflorescence and inflorescence peduncle, pods per plant, seeds per pod, pod pubescence, length and width of pod, g/100 seeds, leaflet area, and data of a visual evaluation after 8 weeks of regrowth (height, diameter, vigor, plagues, diseases). Principal component analysis revealed correlations between days to first flower and 50% flowering (r= 0.96); between flowering intensity and flowers per node (r =0.60); between seeds per pod and pod length and width (r =0.57); between pod width and g/100 seeds (r= 0.60); and between plant height and leaf area (r= 0.52).

Cluster analysis (UPGMA) resulted in nine groups of *C. argentea*, but groups were quite non - homogeneous and there were no conspicuous characteristics to clearly distinguish and describe them. Six groups contained only one accession, among them CIAT 18516 of the accession mix released as cultivar Veraniega in Costa Rica. The first group contained 11 accessions, which were precocious (time to first ripe seed after establishment: 368 days), had low volubility and low pod pubescence. The second group of 19 accessions took on average three weeks longer to first seed production, had higher volubility and higher pod pubescence. Group three included two accessions with very low flowering intensity and on average only one flower per node. Accession 18672 as group four had an especially low leaflet area (148 cm^2), while accession 22387 as group five had the highest leaflet area of the whole collection (394 cm^2) and a great plant height as well (168 cm). Accession 18671 in group six had the longest inflorescences (53 cm), the longest pods (16 cm), highest number of seeds per pod (7) and of pods per plant (387). Accession 22400 as group seven had very light seeds (22 g/100 seeds), while 18516 as group eight had the highest seed weight (38 g/100 seeds). The last group was made up by accession 22393, which was taller than most other accessions (158 cm).

For *Flemingia macrophylla*, the parameters included were: days to first flower, days to 50% flowering, length of inflorescence peduncle, flower color, length of bracts, seed color, inflorescence branching (terminal/axillar), flowering intensity, leaf(let) area, leaf peduncle length and –pubescence, and data of a visual evaluation after 8 weeks of regrowth (height, diameter, vigor, plagues, diseases). PCA performed with the agronomic data of 71 accessions of *F. macrophylla* revealed high correlations between seed color, length of the inflorescence peduncle, inflorescence branching and height, as well as between days to first flower and days to 50% flowering (>70%).

Cluster analysis (UPGMA) resulted in eight groups of *F. macrophylla*. The first group contained 18 of the 19 erect growing accessions, which were characterized by their tall height (160 to 290 cm), and by a terminal, pedunculate and branched, raceme-like inflorescence with light rose-colored flowers (Photo 13) and black seeds. The average leaflet area was 40 cm^2 with an average peduncle length of 5 cm. The remaining erect-growing accession (No. 21249), with the same growth and inflorescence characteristics, was in a group of its own, distinguished by lower vigor and high disease attack. Group 3 (20 accessions) was composed nearly exclusively of semi-erect accessions with an average height of 134 cm and diameter of 205 cm. This group also included two prostrate accessions (19797, 20624) because of their extensive height and diameter.

Photo 13. Inflorescence of the erect growth type of *F. macrophylla*.

The inflorescences of these accessions were axillary and sessile, with dark pink flowers in dense, cylindrical racemes (Photo 14) with brown and mottled seeds. Accession No. 18437 with the same characteristics fell into a separate, fourth group of its own because of higher disease attack, as well as No. 21090, due to very late flowering (355 days till 50% flowering). The remaining 26 semi erect and prostrate accessions, with the same inflorescence characteristics as mentioned before but with a lower average height (86 cm) and diameter (137 cm), fell into a sixth, large group. Groups seven and eight were different to all other accessions with respect to the bracts and bracteoles. Generally in *F. macrophylla* the bracts and bracteoles are shorter than the inflorescence.

Photo 14. Inflorescence of the semi erect and prostrate growth type of *F. macrophylla*.

In some accessions the bracts and bracteoles were longer than the inflorescence (Photo 15), and as a result these accessions fell into two distinct groups: Group seven with accessions 21080, 21994, 22058 and group eight with accession 21086. These accessions also had conspicuously larger leaves (average leaflet area was 68 cm^2, while the average of the whole collection was 34 cm^2) which resemble tobacco leaves. This growing type therefore was named “tobacco”, in contrast to the three other growth types mentioned above: “erect”, “semi erect” and “prostrate”.

Photo 15. Inflorescence of the “tobacco” growth type of *F. macrophylla*.

Genetic analysis by molecular markers *(AFLPs):* Efforts made in genetic analysis showed as a preliminary result that common manual DNA extraction methods do not work well with *F. macrophylla* and *C. argentea*. The modified protocol, which was used to extract DNA, showed promising results at the beginning. But later frequent degradation, contamination and partial digestion occurred, due to secondary plant compounds, probably polyphenols. In preliminary trials with a commercial extraction kit instead, the DNA purity was higher and so far problems occurred neither in digestion nor in amplification.

3.3.2 Agronomic characterization of a collection of *Rhynchosia schomburgkii*

Contributors: M. Peters, P. Avila, L.H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

From the evaluation of a range shrub legume with tolerance to cool temperatures *Rhynchosia schomburgkii* emerged as one of the most promising species for higher altitude hillsides. Thus, we were interested in characterizing its agronomic performance and potential feed value and palatability of this species to see it potential for crop and livestock uses (Photo 16).

Photo 16. *Rhynchosia schomburgkii at* Quilichao.

Materials and Methods

A total of 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, were planted at Quilichao. Plants were transplanted into single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Results and discussion

In 2002 the experiment was finalized completing analysis of palatability of *R. schomburgkii* by cattle. Earlier observations had indicated some acceptance of the fodder by cattle. However, results from the cafeteria trial this year indicated that R. *schomburgkii* was rejected by cattle. Hence this plant species may be an option for green manure and fallow improvement, but not as cattle feed. Table 50 shows data on forage quality, updated with digestibility and crude protein data for the 2nd dry season, for reference.

Table 50. Fodder quality of accessions of a collection of *Rhynchosia schomburgkii* under minimum and maximum precipitation, Quilichao

Accession	Season					
	Minimum		Maximum			
	IVDMD	CP	IVDMD	CP	Tannins	
					Soluble	Bound
8582	44	21	41	21	4.81	0.78
19235	42	21	40	21	3.68	0.40
20800	42	21	38	21	2.49	0.84
17918	44	19	45	19	3.06	0.73
20456	44	19	46	21	3.31	0.68
22134	41	19	41	20	3.69	4.55
7389	41	20	42	21	2.95	0.67
7810	41	20	41	20	5.23	0.72
18490	40	20	49	21	3.44	0.92
918	36	19	36	21	5.85	0.72
LSD($P<0.05$)	NS	NS	5.5	NS		

Activity 3.4 Selection of legumes for multipurpose use in different agro-ecosystems and production systems

Highlights

- Accessions of *Vigna unguiculata* (cowpea) with good performance across sites in Central America Haiti, and Colombia identified.

- *Lablab purpureus* (lablab) accessions with adaptation to acid and alkaline soils identified.

- Forage quality of *Lablab purpureus* was high but influenced by soil fertility of the location.

3.4.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua and Honduras

Contributors: M. Peters, Luis H. Franco, A. Schmidt, H. Cruz Flores, P. Avila, B. Hincapié, G. Ramírez, (CIAT), and B.B. Singh (IITA, Nigeria)

Rationale

Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed for livestock, but the utilization of cowpea in Latin America is so far limited. We visualize that, cowpea could be an alternative crop for the second planting season in the central hillsides region of Nicaragua and Honduras where the legume could provide not only higher grain yields as compared to common beans, but could also allow for a third crop in November/December in order to provide hay as animal feed in the dry season or contribute to soil fertility enhancement for the following maize crop. Cowpea could also be used for hay, silage and feed meal, production, which in turn could be an option for income generation by smallholder livestock and non-livestock owners.

Adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for a successful development of a cowpea option for the traditional maize-bean cropping systems in Central America. It remains to be seen if cultural traditions allow for the inclusion of cowpeas in the daily menu of people in Central America.

A) Evaluation of cowpea in Quilichao and Palmira, Colombia

In 2001/2002, a selected set of cowpea accessions from IITA were sown in the same season in Quilichao and Palmira to compare the effect of climate and soil on performance and to identify accessions with broad adaptation, which is key for Central American Hillsides with highly variable soil and climatic conditions (Photo 17).

Photo 17. Cowpea *Vigna unguiculata at* Quilichao.

Materials and Methods

Of the original collection of 15 cowpea accessions obtained from Dr. B.B. Singh, cowpea breeder of IITA and complemented with two local accessions from Colombia (cultivar Sinu) and Brazil (cultivar Verde Brasil), accessions IT86D-715, IT86D-716, IT89KD 288, IT6D-733, IT86D-719, IT95K-1088-2 and IT95K-1088-4 were selected on the basis of dry matter yield and adaptation.

Accessions were evaluated for forage yield and their value as green manure with yield of maize after incorporation of the green manure. Materials sown in 2002 were incorporated at different physiological stages:

1. Incorporation before initiation of flowering 7 to 8 weeks after planting;
2. Same as 1, but removing the aerial part (simulating use as feed or for sale) and only incorporating root biomass;
3. Removing green pods (for human nutrition) and incorporating the residue;
4. Removing grains (for human nutrition) and incorporating the residue.

In Palmira another stage similar to 4 was included, but removing the whole aerial part (grains for human nutrition and standing hay as feed) and only incorporating roots (data forthcoming). Maize was sown to observe the effect of green manure on grain yield as a result of incorporation of the cowpea at different physiological stages.

Results and Discussion

As reported in the 2001, establishment of the cowpea accessions was slower in Palmira than in Quilichao, due to higher incidence of insects and weeds. The higher pressure of weeds and insects negatively influenced the DM yields obtained in Palmira. In Table 51, we show DM yields of the cowpea accessions in Quilichao before incorporating in the soil. No significant differences were observed between accessions, but differences between treatments were significant (P<0.05) as expected. The highest cowpea DM yields (2724 kg/ha) was measured for treatment 1 (green manure incorporated before flowering) while the lowest DM yields were recorded when harvesting of grains was allowed and the plant had dried up (treatment 4). Due to prolonged drought it was not possible to harvest the grain.

Table 51. Dry matter yield (kg/ha) of cowpea green manure herbage before soil incorporation in Quilichao, 2002

Accessions	Cowpea herbage (kg/ha)				
	Treatment				Mean
	1	2	3	4	
IT95K-1088/2	3265	1953	2567	1907	2423
IT86D-715	2890	1560	2367	2173	2248
IT89KD-288	2412	1993	2747	1687	2210
IT6D-733	2676	2060	2607	1307	2162
IT95K-1088/4	2377	1800	2313	1967	2114
Mean	2724	1873	2520	1808	

LSD (P<0.05) between treatments (accessions combined) = 655.3 kg/ha; LSD (P<0.05) between accessions (treatments combined) = 507.9 kg/ha

In contrast to Quilichao, differences in DM yields between accessions (P<0.01) and harvest treatment (P<0.05) were measured in Palmira (Table 52), but no interaction between accessions and treatments was observed. Accession IT89KD-288 had the highest DM yield and yield was lowest in treatment 4, as plants had dried up by then. In contrast to Quilichao, there was a tendency for accessions to regrow after senescence, indicating a weak indeterminate growth habit if environmental conditions are favorable.

Table 52. Dry matter yield (kg/ha) of cowpea green manure herbage before soil incorporation in Palmira, 2002

Accessions	Cowpea Herbage				
	Treatment				Mean
	1	2	3	4	
IT89KD-288	2767	2513	2487	2133	2475
IT86D-716	1533	1473	1633	927	1392
IT86D-719	2547	2353	2133	2027	2265
IT95K-1088/2	2147	1900	1773	1707	1882
IT95K-1088/4	1947	2233	1907	1307	1848
Mean	2188	2095	1987	1620	

LSD (P<0.05) between treatments (accessions combined) = 655.3 kg/ha; LSD (P<0.05) between accessions (treatments combined) = 507.9 kg/ha

B) Evaluation of cowpea in Nicaragua

Materials and Methods

In Nicaragua and Honduras a core collection of 19 accessions of *Vigna unguiculata* (Table 53) was established. In Nicaragua, this collection was located at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The accessions were replicated three times in a randomized block design. Plots measured 5 x 2.5 m and seeds were sown at a distance of 0.25 m within a row and 0.5 m between rows. Measurements include seed emergence, ground cover, plant height, plant vigour, biomass/grain production flowering patterns, and incidence of pest and disease. Local farmers are being invited to participate in the evaluation of the core collections and soil fertility enhancement effects will be measured through the planting of a maize crop at the onset of the next wet season and comparing maize yields with N-fertilized plots.

Table 53. Accessions of *Vigna unguiculata* sown in San Dionisio/Nicaragua and Yorito/Honduras and Nicaragua as green manures for maize-based systems; *Lablab purpureus* DICTA was sown in Honduras only as a local check.

Accessions	Accessions
Vigna unguiculata IT86D-277/2	*Vigna unguiculata* IT86D-715
Vigna unguiculata IT90K-284/2	*Vigna unguiculata* IT96D-740
Vigna unguiculata IT89KD-391	*Vigna unguiculata* IT95K-1088/4
Vigna unguiculata IT86D-716	*Vigna unguiculata* IT89KD-288
Vigna unguiculata IT93K-503/1	*Vigna unguiculata* IT93K-573/5
Vigna unguiculata IT93K-637/1	*Vigna unguiculata* CIDDICO1
Vigna unguiculata IT86D-719	*Vigna unguiculata* CIDDICO2
Vigna unguiculata IT95K-1088/2	*Vigna unguiculata* CIDDICO3
Vigna unguiculata IT6D-733	*Lablab purpureus* DICTA

Results and Discussion

No significant differences (P>0.05) were observed between cowpea accessions in terms of DM yield and grain yield. Mean DM yield was 1790 kg/ha and mean grain yield 170 kg/ha. Accessions with highest DM yields were 637/1, 284/2, 719 and local varieties Café and Rojo. Highest grain yields were obtained for accessions 716, Rojo, 288, 391 and 719 with more than 200 Kg/ha; Rojo (a local check) was one of the best accessions both in terms of DM yield and grain yield (Table 54).

Table 54. Dry matter yields and grain (kg/ha) of cowpea before soil incorporation in Nicaragua.

Accessions	Biomass Cowpea DM kg/ha	Grain yield cowpea kg/ha	Accessions	Biomass Cowpea DM kg/ha	Grain yield cowpea kg/ha
637/1	3156	133	1088/4	1677	153
284/2	2315	182	1088/2	1645	135
719	2237	200	573/5	1481	153
Café	2117	167	733	1478	173
Rojo	2021	212	716	1477	234
Control	1965	196	715	1397	165
740	1953	125	288	1376	202
Blanco	1872	128	277/2	1256	164
Negro	1825	161	503/1	1073	144
391	1683	200	1088/4	1677	153
LSD (Biomass Cowpea) = 1102;			LSD (Grain yield cowpea) = 105.1		

C) Evaluation of cowpea in Honduras

In 2001, an experiment was established in the SOL Yorito site to evaluate a core collection of cowpea (IP-5 Annual Report 2001). In this case the experiment was complemented with the inclusion of *Lablab purpureus* DICTA as a control. In Yorito, the focus is again on selecting cowpeas as green manures in maize-based systems for soils with neutral to alkaline pH. The maize crop sown after the green manure in 2001 was destroyed by a storm and hence the trial was replanted in 2002. Dry matter yields for the evaluation in 2002 in comparison to 2001 are presented in Table 55. Yields of DM in 2002 were substantially higher than in the previous year with a mean of 7545 kg/ha. Accessions with highest DM yields were IT90K-284/2, 9611, CIDDICO$_2$, 391 and 1088/4 with more than 9 t/ha DM, harvested 9 to 10 weeks after planting.

Table 55. Dry matter yields of *Vigna unguiculata* (cowpea) genotypes before soil incorporation before a maize crop in Yorito, Honduras. 2001 – 2002

Accessions	Soil cover (%) 2001	Soil cover (%) 2002	DM yield kg/ha 2001	DM yield kg/ha 2002	Accessions	Soil cover (%) 2001	Soil cover (%) 2002	DM yield kg/ha 2001	DM yield kg/ha 2002
CIDICCO3	93	85	6212	8718	IT6D-733	50	58	3827	5349
IT90K-284/2	90	97	6123	12132	IT93K-503/1	60	67	3672	4484
9611	-	88	-	11440	CIDICCO2	78	95	3521	10724
CIDICCO1	83	68	5282	5010	IT93K-573/5	50	68	2926	6866
Lablab purpureus DICTA	85	78	5230	7368	IT89KD-391	43	90	2867	10158
IT96D-740	72	48	5112	3229	IT89KD-288	53	80	2734	8085
IT93K-637/1	72	88	5101	8397	IT86D-719	38	65	2381	7422
IT86D-716	67	58	4944	8138	IT86D-715	37	43	2175	5110
IT95K-1088/2	72	72	4042	7992	IT90K-277/2	33	60	1754	6370
IT95K-1088/4	68	70	3923	9208					
LSD (P<0.05)								2014	4243

3.4.2 Evaluation of a core collection of Cowpea (*Vigna unguiculata*) for multipurpose use in Atenas, Costa Rica

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

Some Cowpea lines are used as source of food in Caribbean and Latin American countries, but this plant has also the potential to improve the soil as green manure and as feed for livestock, particularly in countries like Haiti that have a predominance of subsistence farmers in hillside landscapes.
The development of Cowpea lines adapted to soil and climatic conditions, particularly to water stress, are prerequisites to select and offer farmers new options of this legume to fit into their production systems; and thus options are available given the wide variability found within the species.

Methods

Fifteen accessions of Cowpea originated from IITA were planted for evaluation in a randomized complete block design replicated three times at Atenas, Costa Rica. Plot size was 2.5 m wide x 4.0 m long, and half of the plot was left to measure grain yield, while in the other half, seedling emergence, plant vigor, plant cover, flowering initiation, DM yields and the incidence of pest and diseases were measured. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, and low P and low aluminum content. The experiment was planted at the end of July and harvested for DM yields 62 days after; grain was harvested 80 days after planting.

Results and Discussion

All accessions established well in Atenas. Seedling emergence was good and we had a mean of 9 plants per m^2 (range 6 to 10) two weeks after planting. Plant vigor was also high and one month later plant height had a mean of 22.3 cm (range 19 to 31). One of the most vigorous accessions was IT95K-1088/2, followed by IT89KD-391, IT90K-284/2 and IT96D-740. Flower initiation was not uniform between accessions; the earliest accession to flower was IT93K-637/1 while the accession IT86D-716 was still vegetative 80 days after planting. There was a low incidence of leaf eater insects and fungal foliage diseases.

There were significant differences ($P<0.05$) between accessions for both grain and DM foliage yields (Table 56). The accession IT96D-740 produced the highest grain and herbage yields, followed by the accession IT93K-503/1. Other outstanding accession was IT90K-277/2 particularly for the high foliage yield, while the accessions IT89KD-288, IT96D-759 and IT95K-1088/4 were among the poorest in both grain and herbage yields. Seed color varied between accessions and ranged form reddish (IT95K-1088/4), to cream (IT89KD-391) and white (IT96D-740). The latter line also produce smaller seeds.

The highest yielding line (IT96D-740) produced a type of grain similar to the Cowpea traditional lines available in the Caribbean and some Latin American countries, namely white grain with a hilum black spot, and may be of interest to investigate further to compare with traditional lines. This line, as well as the more promising accessions, are under seed multiplication to support future testing elsewhere.

Table 56. Grain and dry matter yields of Cowpea lines established in Atenas, Costa Rica

Accession	Yields (kg/ha)	
	Grain	DM herbage
IT96D-740	3698.7 a*	4728.9 a
IT93K-503/1	2580.7 ab	2644.5 abcd
IT90K-277/2	2216.0 bc	4080.4 ab
IT89KD-391	2182.7 bc	3522.0 abc
IT86D-716	2168.0 bc	2885.0 abcd
IT86D-715	1830.0 bcd	2275.7 bcd
IT90K-284/2	1681.3 bcd	2322.3 bcd
IT93K-637/1	1556.0 bcd	1420.4 cd
IT93K-573/5	1500.0 bcd	940.9 bcd
IT95K-1088/2	1429.3 bcd	1947.9 bcd
IT6D-733	1258.0 bcd	1555.5 cd
IT86D-719	1235.3 bcd	2872.7 abcd
IT89KD-288	948.0 cd	950.9 d
IT96D-759	842.7 cd	1592.5 cd
IT95K-1088/4	756.0 d	1071.3 d

*Within columns means followed by the same letter are not statistically different (P<0.05)

3.4.3 Evaluation of a core collection of *Lablab purpureus* for multipurpose uses (Quilichao and Palmira)

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Lablab purpureus is a free seeding, fast growing or short-term perennial legume, with widespread use through the tropics as a fodder plant. In Africa the use of Lablab for human consumption is also common. The origin of the Lablab germplasm currently utilized is mainly Eastern/Southern Africa and Asia. In addition, it is well documented that *Lablab purpureus* is best adapted to areas with rainfall regimes of 750–2000 mm/year. This species grows in a variety of soils, but the ideal pH for growing Lablab is reported to be between 5.0 and 7.5.

In order to evaluate the potential of Lablab in tropical America, we obtained a collection available at ILRI/CSIRO. Our main objective with the collection is to select accessions with broad adaptation to different soils and climate conditions in tropical America. However, of immediate interest is to evaluate the Lablab collection in acid and neutral soils to define niches of Lablab for green manure and fodder (especially for hay and silage or deferred feed), with emphasis on Central America where soils are highly variable in pH.

Materials and Methods

A total of 44 accessions of *Lablab purpureus* were initially sown on an acid soil (pH 4.0) in the Quilichao Research Station for seed multiplication. In 2001, 42 and 25 accessions were planted for agronomic evaluation in neutral (Palmira) (Photo 18), and acid soil (Quilichao), respectively. From this collection accessions (including the cultivars Rongai, Highworth, Endurance and Koala) were selected based on vigor, cover and DM yield for 2nd phase (2002) evaluations in Palmira and Quilichao, respectively.

Photo 18. *Lablab purpureus at* Palmira.

Results and Discussion

In Quilichao (acid soils) the *Lablab purpureus* accessions I-11630, CPI 106471, I-14441 y I-14442 had the most rapid establishment, with more than 90% soil cover and vigor ratings of 4 to 5 twelve weeks after planting (Table 57). Most accessions had soil covers superior to 60%.

Highest DM yields were obtained for accessions I-6533, CPI 67639, CPI 34777 and CPI 106471, while lowest yields were recorded for accessions 76996, CQ-2975 and 106494. In comparison to 2001, yields in 2002 were slightly reduced, due to early initiation of the dry season.

As in 2001, in Palmira (alkaline soils) vigor and yields of *Lablab* were higher than in Quilichao. Significant differences ($P<0.05$) in DM yields between accessions were also observed. Most accessions rapidly covered the soil, with 83% of accessions having soil covers above 90% twelve weeks after planting; this indicates a high ability of *Lablab* to compete with weeds.

Accessions of *L. purpureus* best adapted to soils in Palmira were CPI 34777, CPI 106471, CIAT 21603, CPI 52535, Highworth, I-11635, I-11635, L-987, I-14437, I-11630, CPI 81626, CQ-2975, 29398, 29398, Rongai, I-14441 and I-6533, with DM yields superior to the average of the collection (3918 kg/ha) (Table 58). Among the cultivars, Highworth and Rongai performed best while Koala and Endurance had very low DM yields.

Table 57. DM yield (kg/ha), vigour and soil cover (%) of *Lablab purpureus* accessions in Quilichao, 2002

Accessions	Vigour	Soil cover (%)	DM yield (kg/ha)
	1 a 5	12 Weeks	
I-6533	3	75	2300
CPI 67639	3	77	2013
CPI 34777	3	77	1973
CPI 106471	5	95	1953
CPI 36903	3	67	1933
I-11632	3	80	1873
CPI 52535	3	73	1873
I-14441	4	92	1853
Koala	2	67	1853
I-14437	4	88	1807
I-11630	5	98	1780
I-14442	5	92	1753
Rongai	4	85	1733
CIAT 21603	4	77	1667
I-14411	4	85	1653
Highworth	4	83	1640
CPI 76998	4	77	1633
Endurance	3	75	1527
CPI 106548	3	48	1353
CPI 81626	3	62	1293
CPI 99985	3	75	1287
CPI 106494	3	72	1067
CQ-2975	3	65	1013
CPI 76996	3	65	953
LSD (P<0.05)			1100

Table 58. Dry matter yield (kg/ha), vigour and soil cover (%) of *Lablab purpureus* accessions in Palmira, 2002.

Accessions	Vigour	Soil cover (%)	DM yield (kg/ha)
	1 a 5	12 Weeks	
CPI 34777	5	100	5293
CPI 106471	5	100	5053
CIAT 21603	5	100	4853
CPI 52535	5	100	4733
Highworth	5	97	4620
I-11635	3	87	4480
L-987	5	100	4227
I-14437	4	100	4413
I-11630	5	100	4353
CPI 81626	3	93	4213
CQ-2975	5	100	4187
CPI 29398	4	97	4167
Rongai	4	100	4113
I-14441	5	96	4040
I-6533	4	97	4033
CPI 52544	4	97	3840
CPI 35894	3	90	3713
CPI 67369	4	93	3673
CPI 76998	4	100	3613
CPI 100602	4	97	3513
I-14411	5	100	3460
CPI 99985	3	93	3387
I-11613	4	90	3220
CPI 106548	4	87	3200
CPI 96924	3	63	3160
CPI 36903	4	97	3093
Koala	3	80	3067
I-14442	4	100	3067
Endurance	3	80	2653
LSD P<0.05)			1505

Accessions of *Lablab* harvested in Palmira had higher quality than accessions harvested in Quilichao. While in Palmira digestibility's (IVDMD) and crude protein (CP) content varied between 81-86% and 22-28%, respectively, for Quilichao corresponding values were 73-83% and 18-25%, respectively (Tble 59). Overall, the forage quality of *Lablab purpureus* was very high.

Table 59. Forage quality of a collection of *Lablab purpureus* evaluated in Quilichao and Palmira, 2002

Accession	IVDMD		CP		NDF	
	%					
	Quilichao	Palmira	Quilichao	Palmira	Quilichao	Palmira
I-6533	79.1	85.8	25.0	25.6	19.4	20.4
I-14411	76.5	82.1	25.2	27.0	19.5	19.0
I-14437	76.0	84.3	22.6	26.9	21.8	22.4
I-14441	80.1	81.9	22.5	26.7	21.2	19.7
I14442	78.2	82.0	23.8	27.9	19.3	17.9
CPI 18626	79.6	86.3	20.0	23.0	20.5	18.6
CIAT 21603	79.2	83.3	23.0	26.3	21.2	20.3

Continues………….

Table 59. Forage quality of a collection of *Lablab purpureus* evaluated in Quilichao and Palmira, 2002.
Continuation…………..

	IVDM		CP		NDF	
			%			
Accession	Quilichao	Palmira	Quilichao	Palmira	Quilichao	Palmira
CQ-2975	81.6	84.3	18.3	26.5	25.3	19.9
CPI 34777	79.3	81.6	19.7	*	18.9	20.7
CPI 36903	81.5	82.7	18.4	25.8	19.5	18.3
CPI 52535	81.9	82.7	18.9	25.0	23.3	21.3
CPI 67639	83.0	84.1	20.2	26.4	20.4	20.5
CPI 76996	80.0		22.4		21.1	
CPI 79998	82.1	82.6	21.7	27.0	21.2	20.6
CPI 99985	81.7	83.5	25.5	26.3.	25.3	19.6
CPI 106471	80.5	84.5	25.4	24.2	15.8	20.7
CPI 106494	77.3	.	24.3		19.5	
CPI 106548	80.7	82.8	23.1	26.4	22.1	20.8
I-11630	80.6	83.1	21.9	26.8	23.5	20.5
I-11632	80.8		22.7		15.8	
Koala	72.9	84.3	21.6	23.2	19.7	21.3
Rongai	80.7	83.8	22.3	25.3	21.8	21.2
Endurance	80.4	83.1	20.1	24.8	28.3	26.2
Highworth	81.6	82.1	22.1	25.4	18.2	18.7
I-11613		85.0		26.4		19.5
I-11615		83.9		23.6		17.8
CPI 29398		84.3		26.1		20.5
CPI 35894		85.4		22.1		21.3
CPI 52544		85.3		26.8		21.7
CPI 96924		86.1		21.7		19.7
CPI 100602		84.0		27.8		20.5
L-987		84.0		24.3		21.5

*Data lost will be repeated. Plank spaces correspond to accessions not common across sites

3.4.4 Genotype by Environment trial with Mucuna, with focus on L-Dopa content

Contributors: L. H. Franco, M. Peters, B. Hincapié, G. Ramírez, CIEPCA/IITA

Rationale

Further progress on developing food and feed uses for Mucuna is restricted by the current confusion in Mucuna taxonomy, by the lack of characterization of different Mucuna accessions, and by our poor understanding of the relative impact of genotype and environment on various characteristics of Mucuna. Of particular interest is the impact of genotype and environment on the content of L-dopa, as Mucuna's high content of L-dopa is perhaps the greatest bottleneck to its increased utilization as a food and feed.

To determine with certainty the comparative importance of genotype and environment, the same genotypes need to be grown in various environments.

The project reported here is undertaken as a part of the larger project that aims to increase Mucuna's potential as a food and feed crop, which is coordinated by CIEPCA/IITA (Center for Cover Crops

Information and Seed Exchange in Africa/International Institute of Tropical Agriculture). The study includes measurement of L-dopa levels in eight (8) Mucuna accessions grown in nine (9) locations, in latitude from 18° S to 38° N, during the year 2001-2002. This experiment is a continuation of a smaller pilot study conducted during the year 2000-2001 when four (4) accessions were grown in six (6) locations. Therefore the specific objectives of this study are: 1) to estimate L-dopa concentration in Mucuna's seed, leaf, stem, and root parts, and 2) to assess variability in these plant parts of Mucuna grown at different latitudes.

Materials and Methods

Eight accessions of *Mucuna sp*., obtained from CIEPCA, are being evaluated in a randomized complete block design with two replicates. Plots consisted of a single plant, typically supported by a 2.5 m high metal (or wooden) frame, 1 m^2 at the base. A corridor of 1.5 m between trellises was left, as Mucuna tends to spread very aggressively. The trial is being conducted in 9 locations that vary in latitude between 18°S and 38°N (Table 60 and Photo 19).

Table 60. Location, institutions, collaborators, and latitudes of the G X E trial.

Country	Latitude	Collaborating institution
Zimbabwe	18° S	University of Zimbabwe
Colombia	3° N	CIAT
Benin	6° N	CIEPCA/IITA
Honduras	14° N	CIDICCO
Mexico (Chiapas)	16° N	Universidad Autonoma de Chiapas
Mexico (Yucatan)	20° N	Universidad Autonoma de Yucatan
USA (Florida)	26° N	University of Florida
USA (Alabama)	33° N	Auburn University
USA (California)	38° N	University of California, Davis

Photo 19. *Mucuna* sp. *at* Quilichao.

Results and Discussion

Samples for L-Dopa analysis, separated for different plant parts were collected and sent for analysis to a central laboratory and results are pending. In Table 61 data on DM yield of different plant part and vigor are presented. Based on the agronomic data the most interesting accessions are *Mucuna pruriens* cv. Utilis, cv. Jaspeada and cv. IRZ.

Table 61. Dry matter yields and vigour of a collection of Mucuna accessions, (Quilichao 2001/2002).

Genus	Species	Cultivar	Total	Dry Weight Leaf	Dry Weight Petiole	Dry Weight Stem	Roots	Vigour
			g/plant					
Mucuna	*sp.*	Ghana	180.0	75.5	28.5	76.0	8.5	3
Mucuna	*Pruriens*	Utilis	951.5	278.0	98.0	575.5	29.0	4
Mucuna	*sp.*	Deeringiana	563.0	185.0	36.0	342.0	18.0	3
Mucuna	*Pruriens*	IRZ	572.0	170.0	67.5	334.5	26.5	4
Mucuna	*Pruriens*	Cochinchinensis	957.0	276.0	94.5	586.5	33.5	2
Mucuna	*sp.*	Preta	1153.5	424.5	159.0	570.0	31.5	3
Mucuna	*Pruriens*	Jaspeada	887.5	396.0	82.0	409.5	27.5	4
Mucuna	*sp.*	Rajada	480.0	199.5	48.0	232.5	15.5	1

Results on reproductive parameters (Table 62) indicate large variation in days to flowering and seed yield among cultivars.

Table 62. Reproductive parameters of a collection of Mucuna accessions, Quilichao 2001/2002

Cultivar	Dry seeds	Dry Weight Total Seeds	First Open Flower	First Pod Mature
	No.	g	Days	
Ghana	129	170	37	85
Utilis	267	227	61	125
Deeringiana	66	77	61	121
IRZ	406	459	61	120
Cochinchinensis	207	185	77	129
Preta	199	207	75	125
Jaspeada	245	361	54	123
Rajada	376	337	95	145

3.4.5 Effect of cutting height a frequency on DM yield and quality of *Chamaecrista rotundifolia* and *Desmodium velutinum* in Atenas, Costa Rica.

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

Shrub legumes tolerant to prolonged dry seasons are valuable sources of forage in sub-humid environments, since they may offer good quality feed either as browse plants or in cut and carry systems. A number of little known shrub legumes have been collected during the last years and are presently available for characterization, including plant adaptation and seasonal yield; therefore it is important to carry out agronomic work to identify promising lines of multipurpose use.

Material and Methods

The shruby legumes *C. rotundifolia* var. *grandiflora* CIAT 18252 and *D. velutinum* CIAT 13953, were established for evaluation in Atenas, Costa Rica. The experiment consisted in a factorial of 2 cutting heights (15 and 30 cm) and 3 cutting frequencies (6, 8 and 10 weeks of re-growth), arranged in a randomized complete block design replicated three times. Plot size was 2 m x 2 m, and the species were planted at 0.50 m x 0.50 m between rows and plants. At each plot, 9 central plants were harvested and the forage oven dried at 60 ºC for 72 hours to determine DM yields. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, and low P and low aluminum content. The experiment has been evaluated for one year and preliminary results are presented.

Results and Discussion

Both species have very small seeds, and although seed viability was high, seedling emergence was poor due to heavy rains following planting. The experiment needed re-planting several times to obtain the adequate number of plants. The experiment is still underway, however *Ch. rotundifolia* did not stand the cutting imposed independent of cutting height or frequency and we recorded high plant mortality (a mean of 4 dead plants out of 9 experimental plants) at the middle of the first dry season following planting, for this reason this legume was removed from the esperiment. During the dry period there was only one evaluation cut for the frequencies of 6 and 8 weeks, but plants did not respond at 12 weeks re-growth. It seems that *Ch. rotundifolia* is very sensible to cutting in sites with prolonged dry seasons such as Atenas. On the other hand, *D. velutinum* has not experienced so far plant mortality under cutting, although the DM yields during the dry period are obviously lower compared to the wet season (Table 63). As expected, both legumes tend to increase DM yield as the cutting frequency increases from 6 to 12 weeks, and it seems that cutting height has little effect on total DM yield. The experiment will continue under evaluation for another year.

Table 63. Dry matter yields of *Chamaecrista rotundifolia* and *Desmodium velutinum* evaluated under cutting at Atenas, Costa Rica.

	Cutting frequency (weeks)						
	6		8		12		
Species/Cutting	Wet	Dry	Wet	Dry	Wet	Dry	Mean
Height (cm)	(g/plant)						
Ch. rotundifolia							
15	21.7	10.6	39.5	7.9	77.2	0	26.2
30	26.2	7.8	40.1	3.2	69.7	0	24.5
Mean	16.6		24.7		36.7		
D. velutinum							
15	14.7	3.6	26.7	11.2	36.2	17.4	18.3
30	17.6	5.5	22.6	10.2	43.9	14.8	19.1
Mean	10.3		17.4		28.1		

3.4.6 Participatory evaluation of improved forages into smallholder dairy systems in Matagalpa, Nicaragua

Contributors: Axel Schmidt (IP-5), Michael Peters (IP-5), Luis Horacio Franco (IP-5), Pedro Argel (Consultant IP-5), Luis Alfredo Hernandez (SN-3)

Rationale

Scaling of research results is considered as the most important issue to be addressed in current R&D activities due to increasing pressure from donors and civil society that money spent in R&D must result in a lasting impact on the lives of the rural poor. Furthermore, it is recognized that many relevant technologies and approaches are not achieving their full potential impact due to low levels of adoption, which has led to more emphasis on the effectiveness of research to produce adoptable technological options. There is a need not only to increase impact, but also so show good quality research results which attract different stakeholders in the R&D environment (e.g. NGOs, farmer associations) in order to achieve a high degree of adoption in combination with a faster dissemination process.

The BMZ funded forages project 'Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America' achieved the development of a technology package made up of different components such as:

1. Participatory diagnosis and stakeholder analysis,
2. Nursery plots for farmer participatory evaluation and selection and agronomic G x E data,
3. On-farm animal production trials including economic analysis,
4. Complementary training modules (participatory evaluation and selection, forage utilization/management, participatory monitoring and evaluation), and
5. Seed systems (informal and formal, public and private).

This package attracted among others the attention of the ASDI-funded bilateral development project FONDEAGRO (Fondo de Desarrollo Agropecuario) based in Matagalpa with the objective to enhance milk production in the projects' target region Matiguás and Rio Blanco in Nicaragua. The target group consists of approximately 1000 livestock holders in three zones. After several visits to the reference site in San Dionisio, where the technology package was applied in the last three years, FONDEAGRO asked CIAT to implement the package within their project.

Materials and Methods

CIAT was contracted 1) to select and implement three forage germplasm nursery sites offering a wide basket of germplasm options to farmers, 2) implement on-farm animal production experiments with the objective to demonstrate pasture management and increased milk production based on improved grasses and grass-legume associations, 3) implementation of dry season feed opportunities based on the shrub legume *Cratylia argentea* in combination with cut&carry grasses, 4) conduction of training modules on forage agronomy and pasture management, participatory methods for evaluation and extension, seed production and participatory monitoring and evaluation, and 5) development of seed production system within the project in order to secure long term sustainability and adoption of the selected forage options.

The project started in late August with the selection of the sites and their preparation. Plots and pastures were sown at the beginning of October. The first training module was held late September on forage agronomy for all technicians of the project. A visit to the reference site San Dionisio was included (Photo 20) and demands for detailed training were identified. An additional visit to CIAT seed producers in Honduras was arranged for early next year.

Photo 20. Training on forage species and pasture management of FONDEAGRO technicians at reference site San Dionisio

All plots will be established, training modules implemented, technicians and project staff prepared and a seed production system will be in place until the end of 2003 when the initial contract with FONDEAGRO for the first phase of the project will end. The second phase of the project (2004-2007) will put emphasis on dissemination within the project area.

The implementation of CIAT's forage based technology package within the FONDEAGRO project indicates one suitable way to speed the dissemination and adoption process of research results. However, in order to attract the attention of such development projects, clear, easy understandable and flexible technology packages have to be offered. Information materials on the packages for non-professionals should be available.

The alliance with development projects such as FONDEAGRO allows CIAT to access additional funding that is currently difficult to obtain. This kind of funding, however, is decidedly development oriented and, as such, impact driven. These funds can be used effectively for 'going to scale' with technologies that are well advanced or with clear practical applications at the field level but are less useful for basic research. Ideally an on-going alliance with development organizations should allow CIAT to leverage research funds more effectively by presenting clear links to demand – development organization as proxies for final clients – as well as viable dissemination networks through which research results (product packages) will contribute to improved rural livelihoods.

Activity 3.5 Genotypic variation in inhibition of nitrification and nitrous oxide emission in *Brachiaria*

Highlights

- For the first time confirmed the phenomenon of nitrification inhibition by *Brachiaria humidicola* using refined bioassay methodology and root exudates from plants grown in the hydroponics and in the field.

3.5.1 The biological phenomenon of nitrification inhibition in *Brachiaria humidicola*

Contributors: G.V. Subbarao, T. Ishikawa, K. Okada and O. Ito (JIRCAS) and I.M. Rao (CIAT)

Rationale

Ammonium-N is transformed into nitrite-N and nitrate-N by soil microorganisms, a process known as nitrification. By controlling nitrification in soils, it will be possible in future to reduce nitrogen fertilizer inputs into agricultural production systems and also minimize nitrate pollution in aquatic systems and ground water. We have demonstrated earlier that a tropical grass, *Brachiaria humidicola* that is widely adapted to lowland agro-ecosystems (savannas) of the humid and sub-humid tropics, particularly in South America, has the ability to suppress nitrification in soil and emission of nitrous oxide to the atmosphere (Ishikawa et al., 2001; 2002 a, b, c). This year, we tested the hypothesis that root exudates of this tropical grass have the ability to suppress nitrification.

Materials and Methods

Bio-assay methodology - calibration and refinement: The basic methodology was adopted from Izumi et al. (1998), which is developed to detect nitrification inhibitors in the municipal-waste water treatment plants. This methodology was improved and calibrated to detect nitrification inhibition in the root exudates of *Brachiaria humidicola*. Different luminometer settings involving integral time of the measurement, the time to initiate the measurement after injection of the sample, the incubation time of the bacterial culture with the root exudates were tested during this phase to standardize the methodology to get reliable and repeatable data.

Root exudates collection, concentration and characterization for inhibitory activity: Using non-destructive root exudates collection techniques, root exudates were collected at weekly intervals from hydroponically grown plants. For collecting root exudates, the styroform block containing plants from each hydroponic tank is placed in 5L of distilled water for 24 h. Intact plant roots were washed initially with tap water and then with deionised water before using them for collecting root exudates. After 24 h of root exudates production in deionized water, plants were removed and returned to the hydroponic tanks, and the collected root exudates were stored at $4^{o}C$ until further processing for the bioassay. This experiment was continued for about 5 months.

Protocol for bioassay using luminometer for detecting nitrification inhibition: The *Nitrosomonas* bacterial cells were concentrated by centrifuging at 10,000 g. Usually 600 ml *Nitrosomonas* broth culture of 10 d old is concentrated to 10 ml, and then suspended in fresh P buffer before the bioassay. For the bioassay, 250 µL of the concentrated *Nitrosmonas* culture is transferred to a test tube, and 20 µL of the test compound (either root exudates processed sample or rhizosphhere soil aqueous extract or phenolic test compound) is added, and incubated at the room temperature for 30 min. After incubation, then 100 μL of this *Nitrosomonas* with test compound mixture is injected into luminometer after adding 2.5 μL dicylaldehyde into luminometer to determine the bioluminescence. Every measurement is repeated four times and the average of these four measurements is used for presenting the data for each sample.

Results and Discussion

Bioassay methodology: The bioluminescence of *Nitrosomonas* showed a distinct two peak pattern during the 30 seconds time after the injection of the sample; the first peak was substantially larger than the second one and lasted about 15 seconds, thus 15 second integral time gave relatively consistent results compared with other integral times tested. Also, 2.2 seconds delay time for the initiation of the measurement after injection of the sample was found out to be the best condition. For the incubation time, 30 minutes was ideal to get consistent and stable data. With these settings in place for luminometer and incubation time, the bioassay method is found to be stable and measurements have <5% coefficient of variation. Also, the bioluminescence, which is an indicator of the metabolic status of the *Nitrosomonas* bacteria, was closely correlated with nitrite production ($r^2 = 0.94$) (Figure 75).

This suggests that by measuring bioluminescence, we can quickly assess the metabolic status of the bacteria and evaluate whether the test compound (i.e. root exudates or tissue extract or synthetic chemical) has inhibitory effect on the functioning of *Nitrosomonas*, which derives its metabolic energy from the conversion of NH_3 into nitrite.

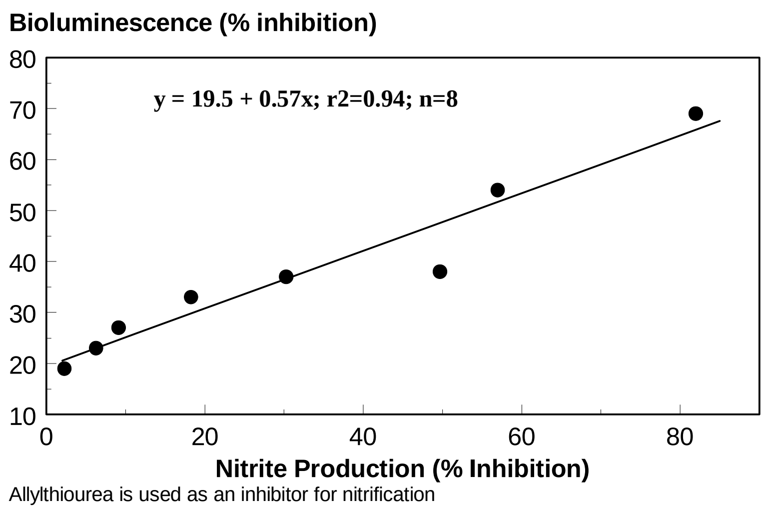


Figure 75. Relationship between *Nitrosomonas* activity and nitrite production in the incubation medium

Root exudates activity at various growth stages of *B. humidicola*: The nitrification inhibition activity increased with plant growth and reached close to maximum (i.e. >95% inhibition) at the time of heading stage of the plant growth, remained stable for the rest of the growth period (Figure 76).

The specific inhibitory activity (i.e. inhibitory activity g^{-1} root dwt) decreased with plant age. The total activity per plant was maintained because of higher root mass with time. Also, root exudates collected from field grown (EMBRAPA research station experimental fields in Campo Grande, Brazil) plants of *B. humidicola*, and rhizosphere soil extracts showed inhibitory activity on nitrification (data not presented). Thus root exudates from hydroponically grown- and field -grown plants showed nitrification inhibition, thus confirming this phenomenon for the first time.

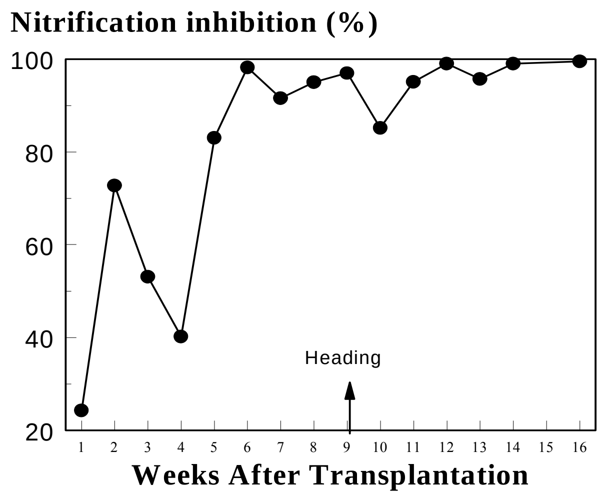


Figure 76. Total nitrification inhibition activity of the root exudates at various growth stages of *B. humidicola*

Our study is perhaps the first comprehensive study to demonstrate the biological phenomenon of nitrification inhibition using both hydroponically grown and field grown *Brachiaria humidicola.* Several studies in the past have hypothesized such a possibility based on empirical observations, but was never demonstrated through experimental evidence. Also, our research has produced a reliable technique to detect and also quantitatively determine nitrification inhibition activity in root exudates, plant tissue extracts or rhizosphere soil water extracts.

One of the most important challenges for nitrification inhibition research is the absence of a reliable technique that can detect nitrification inhibition activity rapidly and reliably. When published, our methodology to detect nitrification inhibition would become a standard procedure for researchers who wish to explore/exploit this research area in future. Also, this technique will become a powerful tool in exploiting the research potential of the biological nitrification inhibition phenomenon from a genetic improvement perspective.

These technical tools are necessary and perhaps essential to develop more efficient agro-pastoral systems based on grasses such as *B. humidicola* for tropical savannas of Latin America. It appears that *B. humidicola* not only is well-adapted to infertile and poorly drained acid soils but also conserves and utilizes nitrogen more effectively because of its ability to inhibit nitrification in soils.

The nitrification inhibitory activity in the root exudates of *B. humidicola* increased with plant growth and appeared to be following in a similar fashion to normal growth curve of root or shoot. Thus, root mass in *B. humidicola* can to some extent determine the degree of nitrification inhibition activity. Nevertheless, the specific nitrification inhibitory activity (i.e. NI activity g^{-1} root dwt) decreased with the plant age (data not shown).

Further studies are underway to isolate the active compound from the root exudates and determine its structure and possible metabolic pathways of synthesis.

3.5.2 Tropical forage grasses and their influence on inhibition of nitrification and emission of nitrous oxide from acid soil

Contributors: M. Rondon, I. M. Rao, C. Lascano, E. Barrios (CIAT); T. Ishikawa, G. V. Subbarao, K. Okada and O. Ito (JIRCAS, Japan)

Rationale

Tropical grasses, both native and improved, account for most of pastureland in the tropics. Among the tropical grasses, *Brachiaria* species are widely adopted, particularly in Latin America where they are planted on about 70 million hectares. Their prime merits of adaptation to infertile acid soils, high yield, and ease of propagation have allowed a major economic transformation of vast areas of unproductive tropical savannas, not only by directly sustaining ruminant livestock production, but also as base of viable production systems that include annual crops. One of the most important cultivars of *Brachiaria* grasses is *B. humidicola* CIAT 679 cv. *Humidicola (koroniviagrass)*.

The Soil Microbiology section of the Tropical Pastures Program (TPP) of CIAT reported inhibition of nitrification in the soil under *Brachiaria humidicola* CIAT 679 (CIAT Annual Reports of TPP – 1983, p. 211; 1985, p. 224; 1986, p. 194). Based on this research work, a paper was published showing that under pure established grasses very little nitrification occurs in Carimagua (acid soil of the Llanos of Colombia) soil where as under bare soil and under legumes high rates of nitrification are observed (Sylvester-Bradley et al., Soil Science 39: 407-416, 1988). Further research showed that: (i) the inhibitory effects on nitrate accumulation observed are a direct effect of the grass roots on nitrification and not an indirect effect of recycling of litter; (ii) rates of N mineralization under N fertilized *B. humidicola* CIAT 679 and *B. dictyoneura* CIAT 6133 were lower than those under N fertilized *B. decumbens* CIAT 606; and *B. humidicola* CIAT 679 reduces net total mineralization and specifically inhibits the conversion of ammonium-N to nitrate-N (CIAT Annual Report of TPP 1989, p. 10-23). A study conducted by soils/plant nutrition section of the TPP showed that *B. humidicola* CIAT 679 takes up nitrogen as the ammonium ion rather than as nitrate as many plants do (CIAT Annual Report of TPP 1984, p. 190). The ability of *B. humidicola* CIAT 679 to take up ammonium for plant growth and inhibit nitrification in soil could contribute to its superior adaptation to very infertile acid soils of the tropics. Recent work by JIRCAS colleagues has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere (IP-5 2001 Annual Report). The release of nitrous oxide from the soil comes about in the process of nitrification as plant residues decompose. A corollary of *B. humidicola*'s capacity to inhibit nitrification is to reduce nitrous oxide emissions compared with other tropical grass species.

In general, pastures of *B. humidicola* remain remarkably free of weeds as long as the pasture is reasonably vigorous. It is at least plausible that this occurs because the grass inhibits nitrification and in doing so denies nitrate to invaders. Weeding is a major cost in most agricultural systems, for cultivation and/or herbicide application in mechanized systems, and labour inputs in subsistence systems. If nitrification inhibition is the reason why *B. humidicola* CIAT 679 pastures remain weed free and if the precise mechanism can be identified, it might be possible to incorporate the characteristic into other species and make them essentially weed resistant. If the gene can be identified, it may be found or introduced in other plants so that it might be possible to 'switch on' the enabling mechanism by genomics techniques. The possibilities are mind-boggling, and potentially could affect most agricultural production with huge economic impact. This would be in addition to any potential mitigation by reducing the evolution of oxides of nitrogen, which are powerful greenhouse gases, during the nitrification process

Given these findings with one genotype of koroniviagrass, there is a need to determine the extent of genetic variation among tropical grasses in their ability to inhibit nitrification and reduce emissions of N_2O. This information will be extremely useful to develop screening methods to select genetic recombinants of *Brachiaria* grasses that not only are resistant to major biotic and abiotic constraints but also can protect the environment. Given the vast areas under *B. humidicola*, reductions in net emissions of N_2O could have important environmental implications. The main objective was to quantify differences among several tropical grasses to inhibit nitrification and associated reductions in N_2O emission under greenhouse conditions using infertile acid soil. Also we intend to correlate nitrification inhibition with root biomass and length, and to monitor nitrate and ammonium levels in the soil after addition of ammonium –N as fertilizer.

Materials and Methods

A sandy loam oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot). Nutrients were applied before planting (kg/ha): 40 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. A total of 6 different tropical grasses were used as test plants at wo levels of ammonium sulfate application (0 and 100 kg/ha). Six tropical grasses included: *B. humidicola cv. Humidicola*; *B. decumbens cv. Basilisk*; *B. dictyoneura cv. Llanero*; *B. hybrid cv. Mulato*; *B. brizantha* cv *Marandu*; *P. maximum cv. Common*. Two control treatments were included: soil without plants that had no application of ammonium sulfate or had application of ammonium sulfate. The experiment was arranged as a completely randomized block desin with 4 replications. Plants were allowed to grow for 6 weeks and were cut to 10 cm to stimulate regrowth for 2 weeks and were cut again at 10 cm height to allow regrowth and to simulate grazing effects under field conditions. After another 10 days of regrowth ammonium sulfate was applied in solution to monitor the processes of nitrification and emission of greenhouse gases. To monitor flux measurements of gases (CH_4, CO_2 and N_2O), a closed chamber was adapted to tightly fit the pots during the 30 minute period of measurement. Gas samples were collected using glass syringes. Samples were analyzed within 36 hours of collection. Analysis of CH_4, CO_2 and N_2O were made in the same sample by combined FID-ECD gas chromatography. (Shimadzu, CG14A, Valco 14 port two-position valve). Just before application of the ammonium sulfate, soil samples were collected using a core sampler (2 cm diameter) across the soil depth. Soil was homogenized and analyzed for NO_3 and NH_4 contents. Similar procedure was used for all experimental pots at 2 weeks after application of the N fertilizer. At the end of the experiment, plants were harvested and separated into shoot and roots. Leaf area was determined using a leaf area meter. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and roots was determined.

Results and Discussion

The samples are being analyzed and the results will be reported next year.

Output 4: In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research

Activity 4.1: Develop of partnerships with NARS, NGO's IARC's, ARIS and the private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use

Highlights

- Identified *P. maximum* cv. Mombasa, *B. brizantha* cv. Toledo and one accession of cowpea as promising lines for multipurpose use in Haiti
- Confirmed the high seedling vigor, tillering and plant development of *Brachiaria* hybrid cv. Mulato in on-farm plantings in 5 countries of the Latin American tropics
- Release by Corpoica of *B. brizantha* (CIAT 26110) cv. Toledo, *C. argentea* (CIAT 18516 and 18668) cv. Veranera and *D. heterocarpon* subsp. *ovalifolium* (CIAT 13651) cv. Maquenque for the Llanos of Colombia.
- Rapid adoption of *Cratylia argentea* by small dairy farmers in the Llanos of Colombia, which is replacing concentrates and reducing cost of milk production.
- The most profitable livestock production system in the lowlands are the dual-purpose system whereas in the highlands the specialized and large farms produced milk at lower costs and had higher net incomes than medium and small farms.

4.1.1 On-farm evaluation of new grasses and legumes options for livestock systems in the llanos of Colombia

Contributors: C. Plazas, J. Miles and C. Lascano

We continued the evaluation of new grass and legume options for livestock systems in the llanos of Colombia with funding from the Ministry of Agriculture (MADR) of Colombia.

Introduction of legumes to recuperate degraded pastures

Piedmont: In 1999 four farms were selected in the piedmont (2) and altillanura (2) to recuperate degraded Brachiaria pastures with *Arachis pintoi* and *Desmodium ovalifolium*, respectively.

The introduction of Arachis to recuperate degraded pastures in the piedmont has been very successful, which was not the case in the altillanura (well drained savanna) due to lower soil fertility. After 4 years of grazing the proportion of legume in the pasture has varied between 10 and 40% in the association with *B. decumbens* and between 16 and 28% in the association with *B. humidicola* (Figure 77).

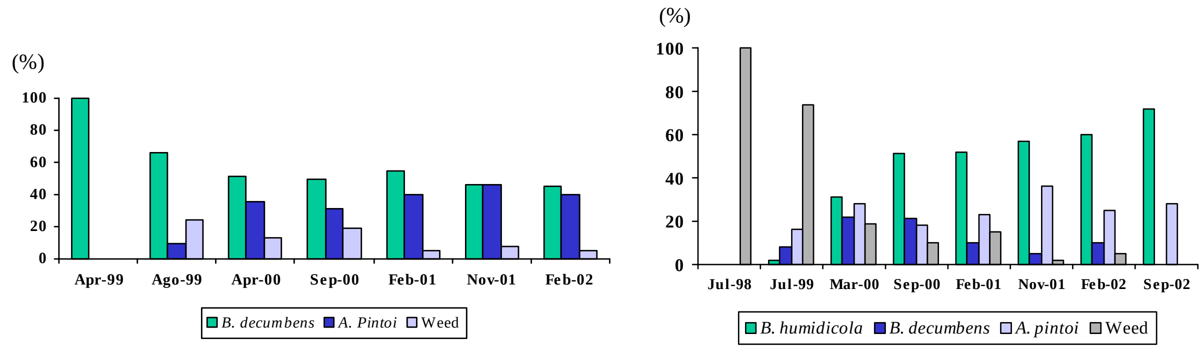


Figure 77. Legume content in pastures of *Brachairia decumbens* (left) and *Brachiaria humidicola* (right) being evaluated in the piedmont (Llanos of Colombia)

As expected, crude protein (CP) content has been consistently higher in the grass/legume pastures than in the grass alone pasture as illustrated in Figure 78. In the association of *B. decumbens*/*A. pintoi* and grass alone pasture the CP level varied between 9 and 10% and 4 and 9%, respectively.

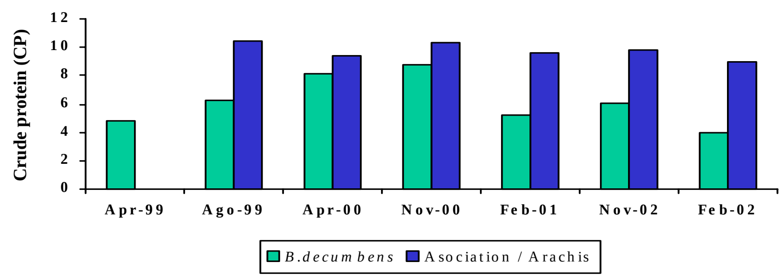


Figure 78. Crude protein of the forage on offer in pastures of *Brachiaria decumbens* alone and in association with *Arachis pintoi*

The low level of CP in *B. humidicola* when growing in low fertility soils is recognized as a limiting factor of this grass and as result animal performance is low. As expected, the introduction of *A. pintoi* in pastures of *B. humidicola* resulted in high levels of CP in the wet season (10%) and dry season (6-7%) (Figure 79).

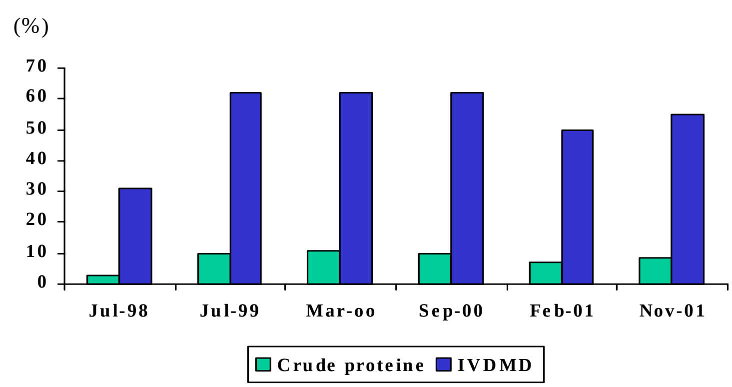


Figure 79. Crude protein and in digestibility of the forage on offer in a pasture of *Brachiaria humidicola* associated with *Arachis pintoi*

One major advantage of the Arachis- based pastures in the piedmont has been there high carrying capacity. For example, the B. *humidicola* /*A. pintoi* pastures has been stocked at the rate of 2.0 animals/ha, which is considerably higher than the average stocking rate (1.0 animals/ha) used in the region. In the *B. decumbens*/*A. pintoi* pasture the stocking rate used by the farmer has also been very high (3.1 steers/ ha).

Well-drained savanna: After failing with the introduction of *Arachis pintoi* to recuperate degraded pastures in well-drained low fertility soils in the savanna (altillanura), we concentrated our attention on *Desmodium ovalifolium* CIAT 13651 given its better adaptation to acid-low fertility soils.

Since May 2000, we have been monitoring the botanical composition of a *B. decumbens* /*D. ovalifolium* pasture in a farm in the llanos. The legume content in the pasture had increased up to 25% in the rainy season of last year, but due to excessive grazing (5 animals/ha), the legume content dropped to 5% at the end of the dry season of this year (Figure 80). Observations made recently indicate that the legume is increasing again as a result of the rains and more lenient grazing.

This year CORPOICA released *D. ovalifolium* CIAT 13651 as cv Maquenque for use as cover in plantations and to recuperate degraded pastures in the llanos. The challenge ahead is to ensure a high and rate of adoption by farmers of the technology. However, for this to happen we need to ensure a continuous supply of seed. The options that need to be examined are: a) to have oil palm growers harvest seed and commercialize seed and b) develop seed multiplication-purchasing arrangements between commercial seed companies and farmers.

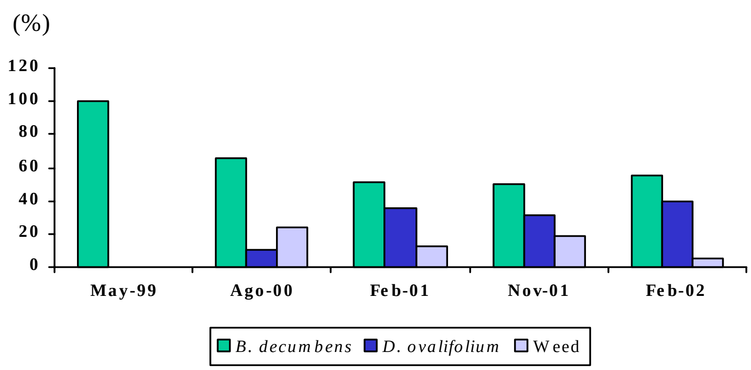


Figure 80. Legume content in a *B. decumbens* pasture associated with *D. ovalifolium* in a farm in a well-drained site in the llanos

Evaluation of new grass options for the llanos

Piedmont: In 2000 we established *B. brizantha* CIAT 26110 in a farm in the piedmont that fattens steers. Results so far have been extraordinary in terms of stocking rate (3.4 animals /ha) and liveweight gain (600 g/steer/d). Forage on offer has varied between 2,600 and 4800 k DM/ha and CP content in the forage on offer varied between 8 and 11%.

The excellent performance of *B. brizantha* CIAT 26110 in the piedmont was used by CORPOICA as justification to release this genotype as cv Toledo. Given that this cultivar is commercial in Brazil we expect that abundant seed will soon reach the Colombian market.

Well-drained savannas: In 2000 we established two accessions of *B. brizantha* (CIAT 26110 and 26318) in a farm in the llanos. The following year two new *B. brizantha* accessions (CIAT26990 and 26124) were also established in the same farm.

Results on forage yield in the wet season indicate that accessions 26990 and 26110 have been superior to the other two accessions. In the dry season forage on offer has also been greater with the accession 26110 (Figure 81)

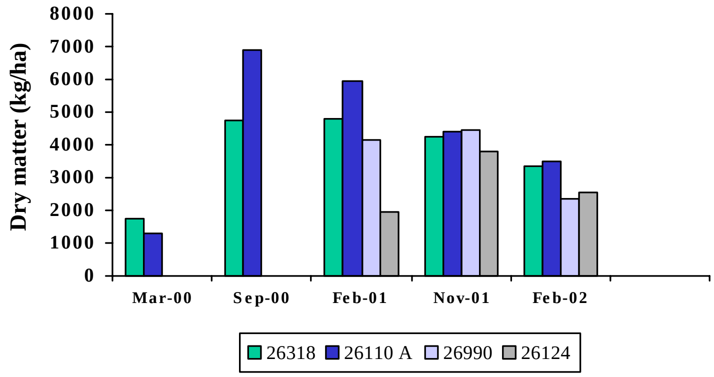


Figure 81. Forage on offer in pastures with different accessions of *Brachiaria brizantha* in well-drained site in the llanos of Colombia

Results from this year indicate that CP levels of the *Brachiaria* accessions being evaluated varied between 4.5 and 6.5% and that the highest level was found in CIAT 26990.

Based on a performance of the Brachiaria accessions and on preference by farmers, we pre-selected three accessions (CIAT 26318, 26124 and 26990) for seed increase. We expect that by early 2003 will we have sufficient quantity of seed of the pre-selected accessions so that CORPOICA could increase the number of farmers testing the new materials.

4.1.2 Evaluation of legumes as covers for plantations in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, L.H. Franco, B. Hincapie (CIAT) and Oil Palm and Rubber Growers of the Colombian Llanos

Rationale

In plantations of the Llanos of Colombia there is a need to find sustainable ways to reduce weed infestation, to maintain and improve soil fertility, to control erosion and increase soil fauna biomass. There is currently a trend to promote plantation systems in the Llanos. In the rubber plantation the target group for this promotion are small to medium size farmers who want to diversify there farming operations. In the oil palm plantations plots of up to 5 ha are rented out to landless farmers to manage the oil palms for the oil palm industry.

In 1999 a range of legume accessions of the species *Arachis pintoi*, *Desmodium heterocarpon* subsp. *ovalifolium* and *Pueraria phaseoloides* were sown under shade and no-shade conditions in the Meta department of Colombia. Based on initial results, this work was expanded to include the evaluation of different establishment procedures for the most promising cover (*Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651) in comparison with the most commonly used cover *Pueraria phaseoloides*.

Materials and Methods

In plots of 80 m^2 we established legumes covers in commercial rubber (young and old) and oil palm plantations in the savannas and Piedemont areas of the Llanos. The following legumes were sown in a Randomized Block Design with three replications: *Arachis pintoi*: 17434, 18744, 18748, 22159, 22160 (seed rate 10 kg/ha); *Desmodium heterocarpon* subsp. *ovalifolium* (*D. ovalifolium)*: 350, 13105, 13110, 13651, 23762 (0.5 kg/ha); *Pueraria phaseoloides*: 8042, 9900 (3 kg/ha). Additionally a mixture of *Arachis pintoi* CIAT 18744 and *Desmodium ovalifolium* CIAT 13651 was sown. Measurements include % cover, DM yield and weeds.

Results and discussion

Monitoring of soil covers to assess persistence under established (productive) and recently planted rubber, continued in 2002. In rubber plantations in production (i.e. closed canopy), the soil cover of *Arachis pintoi* and *Pueraria phaseoloides* was strongly depressed in 2002 in comparison to earlier years, with covers below 15%. In contrast, *D. heterocarpon* continued to cover the soil well, notably accession CIAT 13651 with soil covers increasing in 2002 to 83%.

In the more recently established rubber plantations, characterized by more light penetration, in 2002 soil cover of *Arachis pintoi* increased to 67 - 78%, while soil cover of *D. heterocarpon* and *Pueraria phaseoloides* decreased in comparison to 2001. However soil cover of *D. heterocarpon* CIAT 13651, with 68% was similar to *A. pintoi* (Table 64).

Table 64. Soil cover of different forage legumes under rubber two and three years after sowing in two sites in the Llanos of Colombia.

Treatments	Cover sown under productive rubber plants: Average 2001	Cover sown under productive rubber plants: Average 2002	Cover sown under recently established rubber plants: Average 2001	Cover sown under recently established rubber plants: Average 2002
	Cover (%)			
A. pintoi CIAT 17434	33	5	35	67
A. pintoi CIAT 18744	64	13	63	78
A. pintoi CIAT 18748	50	7	47	68
A. pintoi CIAT 22159	43	13	62	70
A. pintoi CIAT 22160	44	7	67	67
D. heterocarpon CIAT 350	74	60	68	40
D. heterocarpon CIAT 13105	68	70	68	23
D. heterocarpon CIAT 13110	64	67	77	40
D. heterocarpon CIAT 13651	79	83	87	68
D. heterocarpon CIAT 23762	73	65	53	38
P. phaseoloides CIAT 8042	30	5	37	22
P. phaseoloides CIAT 9900	42	5	37	18
Assoc. *A. pintoi/D. heterocarpon*	81	50	87	77

Similar results were obtained in oil palm plantations. While in the wet season of 2002 soil cover of *A. pintoi* and *P. phaseoloides* decreased to 55% and 37%, respectively, with *D. heterocarpon* average soil covers was 71% and 85% with the association of *A. pintoi* with *D. heterocarpon*. In general our results indicate that, *D. heterocarpon* subsp. *ovalifolium* CIAT 13651, due to low establishment cost, rapid establishment and high persistence is the best soil cover option for rubber and oil palm plantations in the Llanos Orientales de Colombia, while *A. pintoi* and *P. phaseoloides* lacked persistence under competition for light.

4.1.3 Evaluation of green manures in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, L.H. Franco and B. Hincapie

Rationale

One of the aims of the Forage Project is to develop alternative green manures for maize and rice based systems in the Llanos of Colombia. It is expected that suitable legumes will reduce the need for external inputs (herbicides, fertilizer) and thus make the crops more competitive.

Materials and Methods

Two types of potential green manures were selected for different temporal niches. 1) Rapidly establishing legumes to be used as green manures incorporated 80-90 days after planting and 2) Slower establishing species to be utilized for fallow improvement. The following species and accessions were compared:

Green manures
Vigna unguiculata (IT86D-716)
Vigna unguiculata (IT6D-733)
Vigna unguiculata (IT89KD-288)
Vigna unguiculata cv. Cabecita negra.
Mucuna pruriens CIAT 9349
Stylosanthes guianensis CIAT 11844
Stylosanthes guianensis CIAT 11833
Stylosanthes guianensis CIAT 184
Stylosanthes guianensis population 3

Fallow improvement
Cajanus cajan CIAT 913
Centrosema macrocarpum CIAT 5713
Centrosema pubescens CIAT 15160
Canavalia brasiliensis CIAT 17009
Pueraria phaseoloides CIAT 9900
Stylosanthes guianensis CIAT 11844
Stylosanthes guianensis CIAT 11833
Stylosanthes guianensis CIAT 184

Results and discussion

Green manures

No significant differences (P>0.05) were measured between legume accessions in terms of ability to cover the soil, vigor and DM yields. Highest soil covers were obtained with the *Vigna unguiculata* (cowpea) and *Mucuna pruriens* accessions (Table 65).

Table 65. Soil cover and vigor of green manures 80 days after planting, La Libertad, Piedemonte

Treatment	Soil cover (%)	Vigor (1–5)
V. unguiculata IT86D-716	100	4
V. unguiculata IT6D-733	100	4
V. unguiculata IT89KD-288	100	4
Vigna unguiculata local	99	4
M. pruriens CIAT 9349	100	4
S. guianensis CIAT 11844	78	4
S. guianensis CIAT 11833	73	4
S. guianensis CIAT 184	53	3.8
S. guianensis CIAT P3	74	3.8
Natural fallow	95	3.7

The highest DM yields were recorded for the *V. unguiculata* accessions with 4 to 5 t/ha DM and *M. pruriens* CIAT 9349 with con 2.4 t /ha DM (Figure 82).

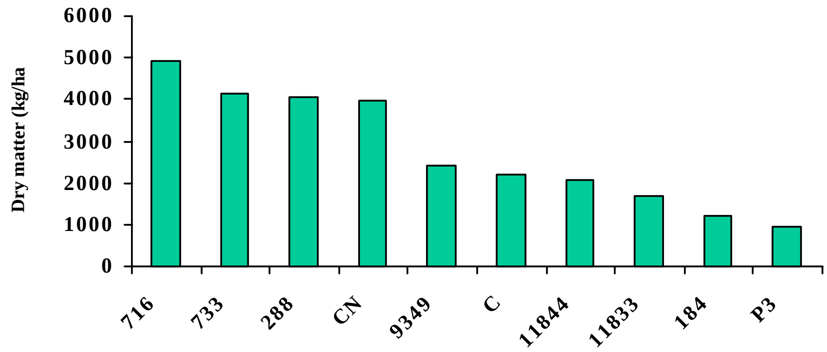


Figure 82. DM yield before soil incorporation of different green manure eight weeks after planting, La Libertad, Piedemonte

In both 2001 and 2002 no significant differences (P>0.05) were encountered for rice yields following green manure alone or green manure plus 40 kg/ha nitrogen fertilizer. In 2001, highest rice yields were obtained after *Mucuna pruriens* CIAT 9343, followed by *V. unguiculata* IT6D-733. Yields of 3.5 t/ha rice after green manures, were comparable to rice yields obtained with 40 to 80 kg N/ha. On the other hand, the residual effects of the green manures were very limited as indicated by the rice yields obtained in 2002, though they were higher than with no nitrogen application (Table 66).

Table 66. Rice yield (kg/ha) after green manure incorporation, C.I. La Libertad, Piedemonte

Treatment	Rice yield 2001*	Rice yield 2002**
	kg/ha	
V. unguiculata IT86D-716	3065	1254
V. unguiculata IT6D-733	3565	1139
V. unguiculata IT89KD-288	3286	1480
Vigna unguiculata local	3089	1257
M. pruriens CIAT 9349	3769	1062
S. guianensis CIAT 11844	2941	1294
S. guianensis CIAT 11833	3036	1296
S. guianensis CIAT 184	2956	1253
S. guianensis CIAT P3	3305	1179
Natural fallow	3391	1267
NO	3247	980
N40	3296	2956
N80	3685	2782
N120	3641	3398
N160	2928	3948
N240	3631	3754

*after legume incorporation; ** residual effect of green manures incorporated in 2001

Fallow improvement. Highest soil covers and highest ability to compete with weeds were recorded for *Cajanus cajan* CIAT 913, *Canavalia brasiliensis* CIAT 17009, *S. guianensis* CIAT 184, *Centrosema macrocarpum* 5713, *S. guianensis* CIAT 11844 and *Centrosema pubescens* CIAT 15160 with soil covers of 80% or higher. Highest DM yields (41-58 t/ha) were for *Cajanus cajan* CIAT 913. Yields for *S. guianensis* CIAT 11844 and *S. guianensis* CIAT 184 were between 7-9 t/ha DM.

Highest rice yields in plots without extra fertilization were harvested after *Centrosema pubescens* CIAT 15160 (2.8 t /ha rice), followed by *C. macrocarpum* CIAT 5713 (2.2 t /ha) and *Pueraria phaseoloides* CIAT 9900 (2.1 t /ha). Yields after *Centrosema pubescens* CIAT 15160 were higher than obtained with N-fertilization of 40 or 80 kg/ha; however maximum rice yields were obtained with a fertilization of 160 kg N/ha with 5.7 t/ha (Figure 83).

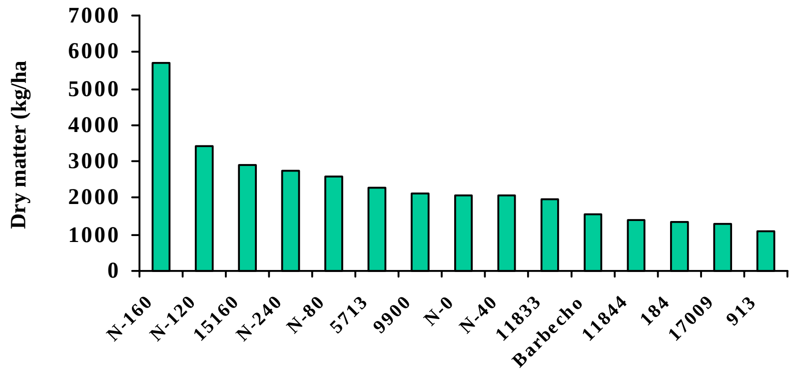


Figure 83. Rice yield after legume fallow, without any further N-application, compared to N fertilization up to 160Kg/ha, La Libertad, Piedemonte

Applying 40 kg/ha nitrogen to rice, in addition to fallow, led to increased rice yields after *Stylosanthes guianensis* CIAT 11833 with 3.7 t /ha and *Centrosema macrocarpum* CIAT 5713 with 3.4 t /ha (Figure 84).

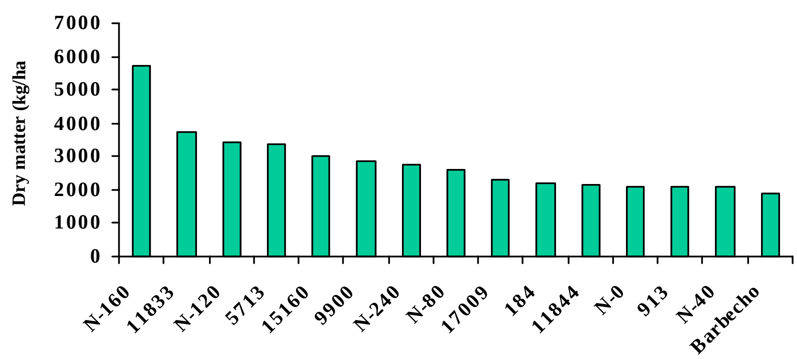


Figure 84. Rice yield after legume fallow, with application of 40 kg N/ha, compared to N fertilization up to 160Kg/ha, La Libertad, Piedemonte.

In conclusion, short-term green manures may provide an option to increase or maintain rice yields without further N application.

4.1.4 Utility of Cratylia in small dairy farms of the llanos

Contributors: C. Plazas and C. Lascano

During 2002 we continued to implement in the piedmont region of the llanos a project funded by PRONATTA to validate the utility of *Cratylia argentea* (*Cratylia*) as source of fodder for livestock in small dairy farms.

A total of 14 farms were selected for the establishment of fodder banks for cut and carry (11), for sowing in association with grasses (2) and for seed production (9) (Table 67). A total of 6 ha of Cratylia were sown to supplement dairy cows in the dry season. In addition, 3,5 ha of sown Cratylia will be used to produce seed that will be sold by the farmers.

Table 67. Areas sown with *Cratylia argentea* for different uses in dairy farms in the llanos of Colombia.

Farms	Cut and Carry (m^2)	Seed Production (m^2)	Strips for grazing (m^2)
Los Pinos		4360	
Bruselas	8400		
La Gloria		1800	
Morichal	1160		
La Isla	8640	3000	
Chaguani	8000	3000	30000
La Pata Rosa		10000	
Poco a Poco	5320		
Fecena	5700	2300	
Las Nieves	3000		
Paraíso	2150	2150	
Rancho Alegre	10000	1700	
Madrigal	3180		
El Hachón	4400	7000	
Total	59950	35310	30000

Average yield of Cratylia for the second cut (70 days regrowth) following a uniformity cut varied among farms and averaged 2 T of DM /ha with an average height of 1.2 m.

Farmers participating in the project have supplemented *Cratylia* either alone or in combination with other forages, including maize silages. Some farmers have also made *Cratylia* silage from the surplus forage that is produced and not fed in the wet season. The average CP content of *Cratylia* forage has been 25%, which is similar to the CP found in other shrub legumes (i.e. *G. sepium*) used by a limited number of farmers in the region.

On-farm documentation of the effect of supplementing *Cratylia* to dairy cows has not been possible, simply because farmers have refused to allow a control (cows not receiving *Cratylia*). However, farmers have consistently indicated that because they now have *Cratylia* in there farms they can milk during the dry season or that they can replace expensive supplements with *Cratylia* with no effect on milk yield. Other farmers have used *Cratylia* to supplement cows in the times where their pastures have very high water tables in the rainy season, which makes it difficult for cows to graze. Other comments from farmers are related to better body condition of cows and of cows coming in heat sooner with *Cratylia* supplementation.

One key aspect of this project is multiplication of *Cratylia* seed in a scale large enough to meet the increasing demand of seed by farmers in the region and outside the region. One strategy that we have followed has been to motivate dairy farmers participating in the project to produce seed, but this has not been easy given the lack of tradition they have on this activity. The other strategy has been to sign contracts with Rural Agriculture Schools (8) so that they could produce seed of *Cratylia* for there own use (50% of the seed) and the rest for sale.

Finally, as part of the strategy to promote the use of *Cratylia* a number events including field days (2) and seminars (5) were carried out this year in the piedmont region. As a result of these events the demand for seed of *Cratylia* has grown considerably and it is fair to say the process of adoption of this legume by small dairy farmers of the llanos of Colombia is underway.

4.1.5 Ex-ante economic evaluation of *Cratylia argentea* in dual purpose cattle production systems in the Llanos of Colombia

Contributors: Federico Holmann (Consorcio Tropileche), Carlos Lascano and Camilo Plazas

Rationale

Dual-purpose livestock production accounts for 78% of the livestock inventory and 41% of the milk produced in tropical Latin America. This type of exploitation is mainly found on small farms that depend, to a large extent, on forage resources that present nutritional limitations and therefore restrict animal productivity.

Forage legumes are an alternative to improve animal nutrition in dual-purpose systems. This is the case of the shrub legume *Cratylia argentea*,, characterized by its adaptation to a broad range of soils, mainly well-drained, low-fertility acid soils, and its capacity for regrowth during the dry season. However, the use of *Cratylia* in cut-and-carry systems in some regions of the tropics is not viable because of high labor costs involved. This study aims to evaluate the impact of supplementing *Cratylia* to milking cows in both cut-and-carry system and under direct grazing associated with *Brachiaria decumbens* pastures.

The objective was to analyze the potential role of *Cratylia argentea* in dual purpose animal production systems in the Llanos in order to reduce feed costs (a) offered alone under cut-and-carry systems at milking time; (b) mixed with grass or molasses in cut-and-carry systems at milking; and (c) under direct grazing associated with *Brachiaria decumbens* with two densities of seeding, 2500 shrubs/ha and 5000 shrubs/ha and three frequencies of replacement: 3, 4, or 5 years.

Materials and Methods

Data were obtained through direct interviews with 32 livestock owners located in the municipality of Villavicencio in order to understand their production systems, use of resources, prices of inputs and products, and management practices. A linear programming model developed in an electronic sheet was utilized as a tool to evaluate ex-ante the costs and profits of current and potential use of *Cratylia* under different scenarios.

Results and Discussion

The current situation shows that cost of milk production was about US$ 0.18/kg vs. a milk price received of about US$0.20/kg for a net margin of 11%. Beef production cost was US$ 0.64/kg live weight vs. a beef price received US$ 0.74/kg for a net margin of 16%. However, due to the low volume of milk and meat sold annually by farms representative of the region, this net margin represented an average income of about US$ 952/farm/yr without considering the opportunity cost of family labor. As a result, the current dual-purpose production systems were low profitable.

Cut and carry. The adoption of *Cratylia* under cut and carry systems could reduce the production costs of milk and beef by 7% when supplemented with molasses, by 11% if *Cratylia* were mixed with grass such as Napier, and by 13% if offered alone at milking time (Table 68). This suggests that the nutritive content found in *Cratylia* was enough to maintain the current productivity of milking cows without the need to provide additional inputs (ie., molasses or Napier grass) to the basal diet (i.e., *Brachiaria decumbens)*.

Table 68. Cost of producing a kilogram of milk under different alternatives with the shrub legume *Cratylia argentea* in the Llanos.

Alternative	Cost of milk production (US$/kg)
Cratylia under cut-and-carry system	
- Alone	0.162
- Mixed with Napier grass	0.167
- Mixed with molasses	0.174
Cratylia under direct grazing with a planting density of 2,500 plants/ha	
- Replaced every 3 years	0.151
- Replaced every 4 years	0.162
- Replaced every 5 years	0.183
Cratylia under direct grazing with a planting density of 5,000 plants/ha	
- Replaced every 3 years	0.155
- Replaced every 4 years	0.167
- Replaced every 5 years	0.192

Direct grazing. Lower production costs was found when *Cratylia* was established with a density of 2,500 shrubs/ha has and not with 5,000 shrubs/ha. Similarly, lower production costs were obtained when *Cratylia* was replaced every 5 years vs 4 or 3 years because the shorter the replacement period, the greater the production cost. As a result, the establishment of *Cratylia* under direct grazing with a planting density of 2,500 shrubs/ha reduced the production cost of milk and beef by 19% when this option was replaced every 5 years, by 13% when *Cratylia* was replaced every 4 years, and by 2% when it was replaced every 3 years. The adoption of *Cratylia* under direct grazing associated with *B. decumbens* not only made it possible to replace the use of supplements such as concentrates, but also to release between 18% of the current area allocated to pastures if *Cratylia* were replaced every 3 years up to 25% of the area if the frequency of replacement was every 5 years (Figure 85). However, little information is currently available about the agronomic management of *Cratylia* under direct grazing. Thus, it is necessary to carry out on-farm research to determine the optimal replacement period of *Cratylia* under rotational grazing systems.

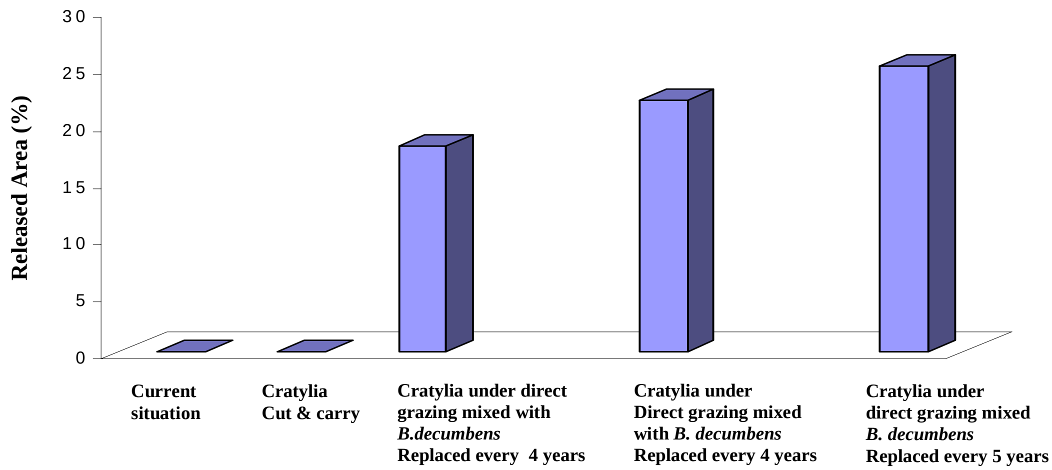


Figure 85. Proportion of farm area that could be released as a result of the adoption of forage alternatives based on *Cratylia argentea* in Villavicencio.

4.1.6 Release of multipurpose forage for the Llanos

Contributors: C. Plazas, J. W. Miles and C. Lascano (CIAT)

One very important outcome of the work carried out by CIAT and CORPOICA in the llanos with funding from the Ministry of Agriculture of Colombia (MADR) was the release this year by CORPOICA of two legumes and one grass. The species released were:

1. *Desmodium heterocarpon subsp ovalifolium* (CIAT 13651) cv Maquenque
2. *Cratylia argentea* (CIAT 18516 and 18668) cv Veranera
3. *Brachiaria brizantha* (CIAT 26110) cv Toledo

Technical Bulletins (Photo 21) were produced and distributed among researchers, extension people and interested farmers. Each Bulletin included a description of the species, methods for propagation and alternative uses and management.

Photo 21. Technical Bulletins of forage cultivars released by Corpoica and CIATfor the Llanos of Colombia

The main attributes of each species for the different production systems that prevail in the llanos of Colombia are summarized as follows:

D. ovalifolium **cv Maquenque:** Herbaceous legume, which can be used for pasture reclamation or for cover in plantations (oil palm and rubber) in well drained low fertility savannas or in the more fertile soils of the piedmont. It is a species well adapted to acid –low fertility soils, that produces abundant seed and it is very persistent under grazing. The cost of establishment of cv Maquenque are very low given that sowing rates range from 0.5 to 1 kg / ha, for pastures and covers, respectively.

C. argentea **cv Veranera:** Shrub legume, which can be used in cut/carry systems, for direct grazing or to make silage in well drained savannas with low fertility soils or in the piedmont with more fertile soils. Small dairy producers in the llanos piedmont are rapidly adopting this legume that is extremely tolerant to the dry conditions that prevail in this region for 3 months.

B. brizantha **cv Toledo:** Grass characterized by fast establishment and fast growth rate following defoliation. As a result pastures of cv Toledo have very high carrying capacities, which is a key attribute in the piedmont region of the llanos where land prices are high. This cultivar is drought tolerant but requires medium soil fertility. Consequently it is recommended for intensive dairy and fattening systems in the llanos piedmont.

To support the release by CORPOICA of the new forage alternatives for the llanos, we provided basic seed (20 to 30 kg) for multiplication by commercial seed producers. In addition, we are promoting the multiplication of *D. ovalifolium* cv Maquenque in plantations where the legume is being used as a cover and of *C. argentea* cv Veranera by farmers using the legume to feed livestock in the dry season.

Finally, in collaboration with CIAT's Impact Assessment Group a study was completed in order to determine the economic ex- ante benefits of the new forage alternatives released to farmers in the llanos and of forage alternative still in the pipeline. The results of the study were included in a publication (see picture) for distribution among policy makers in the Ministry of Agriculture in Colombia researchers, extension people and farmers. An additional publication with relevant results of new forage alternatives being evaluated in farms of the llanos was produced and distributed (Photo 22).

Photo 22. Bulletins on attributes of new forage options under evaluation in farms of the llanos (left) and on the ex-ante impact assessment of new forage options for the llanos (right).

4.1.7 Use of forages for the recuperation and conservation of degraded areas in hillsides of Colombia

Contributors: FIDAR (NGO lead partner), UMATA, Comite Cafeteros, REVERDECER, CVC, Alcaldia de Restrepo, Universidad de Valle, Comite de Cafeteros

Rationale

A high natural diversity and richness of natural resources characterize the study area located in the north of the 'Valle de Cauca'. However inappropriate land use has led to degradation of the natural resource base, threatening social, economic and environmental sustainability of the region.

The deterioration of natural resources is leading to loss of fauna and floral biodiversity, lack of vegetative cover and resulting high erosion, and reduced crop yields. Communities at the lower end of the watershed face an increased risk of natural disasters. Companies utilizing water for electricity and for human consumption at the downstream of the water lines have increased costs in maintenance of plants due to increased sediments as a result of erosion, increasing electricity costs and posing at risk the availability of water of high quality. Hence there are multiple effects on well being and environmental quality as a result of the environmental degradation.

In the past the recuperation of such fragile areas was addressed by isolated activities carried out by public and private institutions, often without incorporating the communities themselves and without long-term follow up. Often the costs of the suggested solutions were high, reducing the possibility for wide adoption and maintenance by the community.

The present collaborative project aims to develop a concerted effort with different actors in the region including the community to reverse the degradation problem in the watershed. In the project, the Fundación para la Investigación y el Desarrollo Agrícola (FIDAR), local organizations and CIAT try to offer sustainable alternatives based on multipurpose grasses and legumes (herbaceous, shrubs, trees), and on development of an evaluation system that incorporates the community.

The expected outputs of the project are:

- Development methods for participatory planning, monitoring and evaluation for recuperating and stabilizing fragile soils
- Stabilization of degraded zones through vegetative covers an mechanical barriers
- Economic and agronomic evaluation of different vegetative covers and mechanical options for recuperation and conservation of degraded lands, with focus on cost-effectiveness
- Farmer groups with the means and tools to continue recuperation and conservation of soils for wider application
- Develop and validate DST for the recuperation and conservation of soils, with focus on the adaptation of existing tools

Results and Discussion

In 2001 and 2002, a total of 50 ha of degraded land were revegetalized and stabilized using biomechanical barriers and covers with herbaceous, shrub and tree materials planted in areas of moderate and severe erosion. With communities, economically attractive proposals for utilizing stabilized zones were developed; such as suggesting a silvopastoral arrangement with improved grasses and sparsely separated trees. One family is developing a degraded zone by selectively applying green manures and irrigation. In the process of interaction with communities organizational and technical capacities of local institutions including schools were strengthened. Though the project concentrates on working with small and medium sized producers, as a spillover effect larger farmers were also sensitized for environmental protection and now contribute to stabilization of degraded areas. An inventory of the flora on eroded zones was elaborated and a total of 98 species were encountered, among these some species have potential for stabilizing eroded zones, most notably Amago (*Daphnosis* sp.). Utilizing the SWAT (Soil and Water Assessment Tool) priority areas for soil recuperation and conservation were identified.

Specific work with forage-based technologies is described in more detail below. Forage options were selected based on their adaptation to low fertility, degraded soils. Two grasses with aggressive growth, i.e. *B. dictyoneura* CIAT 6133 and the locally utilized *Cynodon nlemfuensis* and five herbaceous legume accessions with high ability to cover soil and improve soil physical conditions were chosen. Legumes include the perennials *Arachis pintoi*, *Centrosema macrocarpum* and *Stylosanthes guianensis* and the annuals *Mucuna pruriens* and *Cavavalia brasiliensis*. While the latter were chosen to provide a rapid cover and green manure, the perennial species were included for a more long-term cover. Moreover, three shrub legumes, (i.e. *Leucaena leucocephala*, *Calliandra calothyrsus* and *Cratylia argentea)* were included as options to provide feed and improve eroded soils. These were complemented by local tree species, (i.e. *Senna spectabilis, Daphnosis* sp. and *Eucaliptus grandis).*

Establishment was constrained by irregular rainfall pattern, and *A. pintoi* and *Leucaena leucocephala* were strongly affected by ants. Grasses established well on completely exposed soils, though establishment was favored by an existing minimum cover. *Cynodon* sp. initially had a superior ability to cover the soil in contrast to *B. dictyoneura* CIAT 6133; 100 days after planting 25% and 10% of soil was covered by *Cynodon* and *B. dictyoneura* CIAT 6133, respectively. Stolons of *Cynodon* sp. extended 3.5 m, in contrast to 1.0 m for *B. dictyoneura*. In respect to number of stolons 50 days after planting 30 and 20 stolons per plant were recorded for *Cynodon* sp. and *B. dictyoneura*, respectively. However, over time number of stolons decreased for *Cynodon* and increased for *B. dictyoneura*, to 20 and 25 stolons per plant 100 days after planting, respectively (Figure 86).

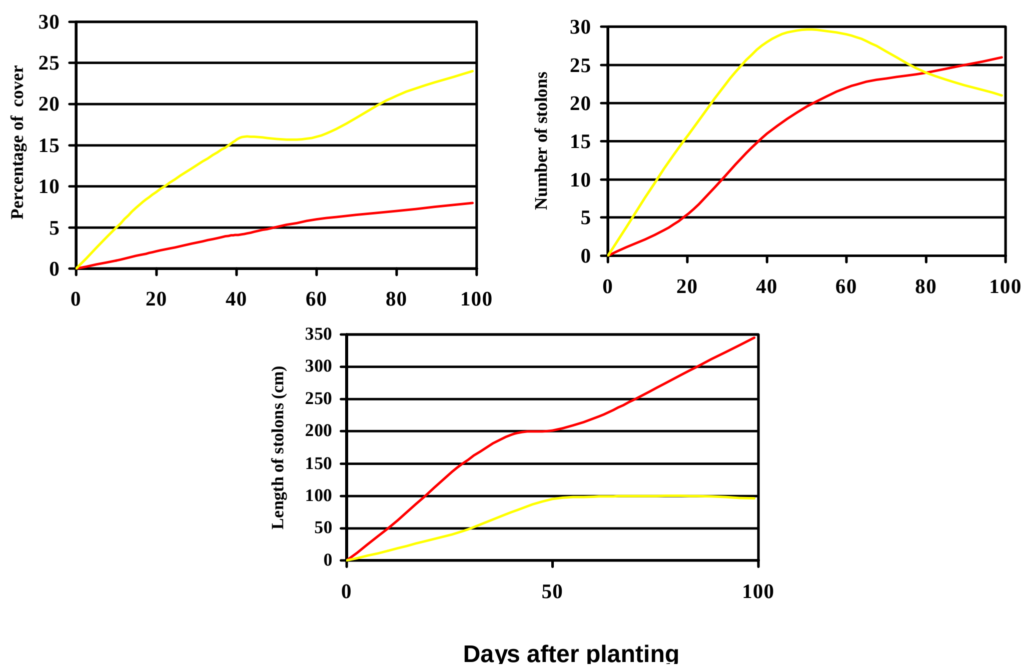


Figure 86. Soil cover, stolon number and stolon extension of *Brachiaria dictyoneura* CIAT 6133 and *Cynodon* sp. on degraded soils, Municipio de Restrepo, Valle del Cauca, Colombia

The herbaceous legumes adapted well to the degraded soils, with *C. macrocarpum* and *M. pruriens* having the highest soil cover and biomass contribution. However, *S. guianensis* was particularly adapted to strongly eroded soils with minimum soil covers.

For the shrub legumes, establishment was very slow, possibly as a result of the relative high altitude and soil conditions (Figure 87). Soil physical conditions with a very hard top soil also appear to have adversely affected development of species, with exception of the very vigorous Amago (*Daphnosis* sp.), a species endemic in the area. Amago has an apparent ability of its roots to break the hard topsoil. Among the introduced species, *Leucaena* and *Calliandra* had the best growth while *C. argentea* has the lowest. Of the local species 'Flor Amarillo', 'Carbonero' and 'Amago' performed best, tolerating extended dry periods and adapting to infertile soils. The utilization other than soil recuperation of these species still needs to be studied, but 'Amago' is highly valued by the community for persistence on degraded soils.

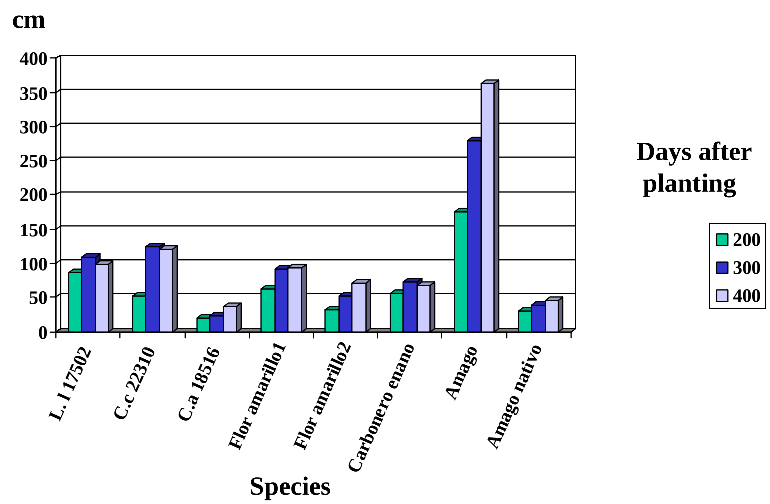


Figure 87. Height of different shrub species after planting

4.1.8 Analysis of intensification of milk production systems in Colombia

Contributors: Federico Holmann, Libardo Rivas, Juan Carulla, Luis A. Giraldo, Silvio Guzman, Manuel Martinez, Bernardo Rivera, Anderson Medina, and Andrew Farrow.

Rationale

Dairying in Colombia has been a dynamic activity during the last 30 years. During the 70's grew at an annual rate of 4.7%, then it had a sustained exceptional growth of 6.5% during the 80's, and in the 90's the production of milk grew at 3.8%/yr, producing in 2000 approximately 5,486 million liters of fluid milk. This growth allowed the population to increase milk consumption from 57 liters per capita in 1970 to 130 liters in 2000, a 128% increase. The high growth in milk production obtained during the 80's was due mainly to the incorporation of thousands of herds into the dual-purpose production system.

With regards to trade, Colombia is basically self-sufficient in milk production. During the 90's the country imported an average of 2% of its annual production. On the other side, Colombia has been a net exporter of beef during the last two decades, but with a clear loss of relative importance since the beginning of the 90's. In 1991 only 5% of the domestic production was exported. From then on, exports have been decreasing and since 1996 the country exports less than 1% of its total beef production.

Colombia has a proven capacity to increase its milk production, and socioeconomic reasons to expand the sector. However, there is internal discussion concerning the most suitable technologies to achieve its development, and if these will be sufficient to make the livestock sector competitive within and outside the region under a scheme of open and unsubsidized economies. Available technologies vary largely with regard to their social, economic, and environmental impact, in the short and long-term. In addition, the information available in different disciplines is scattered and it is not always adequate. It is necessary to systematize it, integrate it, and interpret it so that it facilitates the decision-making process in accordance with the priorities of Colombia.

Objectives of this study were to: (1) identify and quantify the effect of technologies on the increase in milk productivity in dual purpose and specialized dairy systems in different regions of Colombia; and (2) analyze the relationship between productivity, technological level, and profitability.

Materials and Methods

Data came from a survey to 545 farms during the period February to November of 2000 in five regions distributed in the following way: (a) 145 farms in the lowlands of the Savannas piedmont (states of Arauca, Casanare, and Meta), (b) 116 in the lowlands of the Caribbean region (Atlantico, Guajira, Magdalena, César, Bolivar, and Córdoba states), (c) 105 in the midland of the coffee growing area (Quindío, Valle, Caldas, and Risaralda), (d) 97 in the mountain highlands of Antioquia, and (e) 82 farms in the Highlands of the Savanna Cundiboyacense (states of Cundinamarca and Boyacá). These five regions produce more than 80% of milk of the country.

The survey was designed to quantify inputs and products in order to determine costs and prices at the farm level that were then utilized to (1) calculate the variable costs of feeding, labor, health, reproduction, fertilization, and irrigation; (2) calculate the gross income from milk and beef sales, and (3) characterize farms according to levels of productivity and management practices.

The surveys were executed through the coordination of the faculties of animal production of the Universidad de los Llanos in the lowlands of the savannas piedmont, of Fundación San Martín in the Caribbean Region, of the Universidad de Caldas in the midland coffee growing area, and of Universidad Nacional (headquarters Medellín and Bogotá) in the mountain highlands of Antioquia and the savanna highlands of Cundinamarca and Boyacá.

Results and Discussion

Regardless of the production system utilized or the region where farms were located, the increase in competitiveness was in direct relationship with herd size. Therefore, as herd size increased, the production cost per unit of milk and beef decreased, net income per cow increased, and the annual return to capital invested improved. However, when the increase in competitiveness was associated with productivity, this trend was not observed, which suggested those highly productive farms may not necessarily be profitable (Table 69). In addition, these results confirm the fact that economies of scale exists, which has large implications for the livestock sector in Colombia because 70% of dairy farmers produce less than 100 kg of milk/day. Thus, smaller herds producing milk at higher costs have greater disadvantages to stay competitive given the scale size in which they operate.

Table 69. Multivariate analysis containing the production cost of milk, net income, annual return on capital invested, and productivity of milk and beef by production system and region based on herd size.

Multivariate analysis group by production system and region	Number of farms per group	Herd size (# cows)	Milk production cost ($/kg)	Net income ($/cow/yr)	Annual return to capital invested (%)	Milk productivity (kg/ha/yr)	Beef productivity (kg/ha/yr)
Dual Purpose							
1	108	20	0.24	- 66	-0.7	894	140
2	21	35	0.21	58	1.3	2193	247
3	136	83	0.16	106	2.8	734	134
4	17	78	0.20	87	2.6	5472	173
5	13	337	0.13	164	6.0	636	140
6	5	730	0.13	82	6.1	226	78
Specialized dairy							
1	54	17	0.25	- 152	-2.9	9100	360
2	52	24	0.26	- 163	-3.7	2976	128
3	35	37	0.20	180	4.6	15760	262
4	24	62	0.18	227	6.0	7970	130
5	31	105	0.20	57	1.7	3090	79
6	13	159	0.16	413	6.2	14358	245
Llanos							
1	59	19	0.19	12	0.2	1099	178
2	30	23	0.30	-184	-1.9	742	75
3	9	45	0.08	463	8.5	662	392
4	29	56	0.16	61	1.0	728	109
5	5	56	0.28	-182	-1.7	1463	84
6	8	108	0.17	23	0.3	326	109
Caribbean							
1	9	48	0.32	-130	-1.8	377	56
2	27	73	0.19	25	0.4	750	112
3	35	111	0.14	140	4.8	1028	151
4	17	175	0.11	253	8.8	758	152
5	10	528	0.15	84	2.9	410	116
6	1	926	0.10	280	9.0	108	80
Coffee area							
1	13	8	0.30	-341	-3.6	9300	378
2	28	19	0.24	-55	-0.8	1460	186
3	18	28	0.24	-70	-0.8	10100	291
4	13	76	0.15	115	2.8	600	157
5	29	85	0.19	179	2.8	3800	99
6	1	265	0.15	210	3.1	6400	114
Antioquia							
1	14	13	0.29	-361	-9.6	8500	428
2	14	18	0.27	-195	-4.8	2370	105
3	36	26	0.25	48	2.7	20200	385
4	12	34	0.23	21	1.5	6090	153
5	10	113	0.20	90	1.7	2800	80
6	10	117	0.20	255	5.6	14600	197
Cundiboyacense Highland							
1	10	10	0.25	-178	-4.7	4900	197
2	14	21	0.22	-86	-0.6	10600	263
3	18	38	0.19	25	0.5	2100	126
4	25	72	0.16	278	5.4	9400	183
5	7	170	0.15	567	7.9	15800	279
6	1	330	0.15	591	8.1	12,700	263

The most profitable production system in the tropical lowlands (Llanos and Caribbean regions) was the dual-purpose system whereas in the highlands (Coffee, Antique and the Cundiboyacense highland) was the specialized dairy. As a result, Colombia should have different strategies for research and technology transfer in order to exploit more efficiently the comparative advantages of each region (Table 70). With regards to technological change, the adoption of improved pastures generated higher profits (Table 70) as well as higher productivity (Table 71) in all five regions. In addition, the investment in greater number of grazing paddocks for a more efficient use of improved pastures to increase the quality and quantity of biomass generated higher higher productivity (Table 71) in all five regions and higher profits in all regions except the Caribbean (Table 70).

The use of strategic supplementation to the basal (forage) diet had mixed effects. The best economic response to supplementation in the lowlands (ie., Llanos and Caribbean) was by offering small quantities to milking cows (i.e., <0.5 kg MS/cow/day) while in the highland regions (i.e., Coffee, Antioquia and Cundiboyacense highland) it was supplementing milking cows with moderate quantities of concentrate (i.e., between 0.5 and 2 kg MS/cow/day).

Table 70. Observed variability in profitability, expressed as net income per cow per year, as a function of technological change in different regions of Colombia.

Technological Change	Category	Region				
		Llanos	Caribbean	Coffee	Antioquia	Cundiboyacense Highland
Production System	Dual Purpose	135 a	111	11 b	53 b	145 b
	Specialized dairy	-140 b	ND	86 a	158 a	236 a
Amount of supplement offered (kg of dry matter/cow/d)	Less than 0.5	164 a	118 a	117 a	-457 c	238 b
	0.5 to 2.0	32 b	102 a	58 a	300 a	263 a
	Greater than 2.0	-49 c	47 b	-46 b	39 b	163 c
Proportion of cows in milk (%)	Less than 60	118 a	72 c	-39 a	-27 b	212 a
	60 to 80	123 a	126 b	43 b	83 a	177 a
	Greater than 80	178 a	234 a	67 b	86 a	242 a
Proportion of improved pastures established on farms (%)	Less than 33	127 a	27 b	29 a	48 a	-358 a
	33 to 67	118 a	116 a	37 a	62 a	203 b
	Greater than 67	167 a	121 a	49 a	135b	316 c
Milking frequency (# of times/day)	Once	126 a	108 a	17 a	-68 b	173 a
	Twice	157 a	156 a	83 b	80 a	216 a
Fertilize pastures	No	148 a	140 a	39 a	130 a	266 a
	Yes	72 b	65 b	36 a	74 b	197 b
Irrigate pastures	No	131 a	121 a	42 a	119 a	189 a
	Yes	-107 b	79 b	7 b	-85 b	231 a
Reproductive management	Natural service	127 a	128 a	53 a	68 a	177 a
	Both	131 a	51 b	1 b	131 a	213 a
	Artificial insemination	123 a	195 a	16 b	62 a	226 a
Number of grazing paddocks on the farm	Less than 10	126 a	102 a	-75 b	-4 c	158 b
	10 to 20	148 a	139 a	60 a	112 a	180 b
	Greater than 20	194 b	83 a	58 a	102 b	255 a
Experience at producing milk (years)	Less than 5 years	92 a	88 b	-42 b	66 a	144 a
	5 to 15	138 b	73 b	50 a	73 a	244 b
	More than 15 years	162 b	136 a	78 a	91 a	236 b
Frequency of deworming (# times/yr)	Less than 2	151 a	112 b	55 a	302 a	399 a
	2 to 3	111 a	179 a	30 a	59 b	173 b
	More than 3	66 b	-14 c	-27 b	53 b	210 b
Frequency of treatment against external parasites (# times/yr)	Less than 6	249 a	139 a	40 b	140 a	201 a
	6 to 12	87 b	59 b	76 a	23 b	223 a
	More than 12	135 b	98 b	-2 c	59 b	222 a
Herd size (# adult cows/farm)	Less than 30	101 b	48 b	-10 b	-11 b	88 c
	30 to 100	168 a	123 a	57 a	166 a	234 b
	More than 100	227 c	109 a	87 a	217 a	422 a
Commercial value of land (US$/ha)	Less than 3,000	145 b	114 a	37 a	114 a	143 b
	3,000 to 6,000	182 a	126 a	43 a	102 b	246 a
	More than 6,000	26 c	-51 b	25 a	3 c	232 a

The use of the fertilization and irrigation increased productivity but not income, except in the Cundiboyacense highland, which suggested the need for investing resources in research to determine the economic response at various levels of N_2 and irrigation methods based on grass species utilized (Tables 70 and 71). A management practice which increased both productivity and profitability was milking twice a day.

Table 71. Observed variability in productivity, expressed as milk production per cow per day, as a function of technological change in different regions of Colombia.

		Region				
Technological Change	Category	Llanos	Caribbean	Coffee	Antioquia	Cundiboyacense Highland
Production System	Dual Purpose	5.1 b	4.2	8.4 b	12.7 a	11.0 b
	Specialized dairy	9.7 a	ND	9.6 a	14.2 a	14.0 a
Amount of supplement offered (kg of dry matter/cow/d)	Less than 0.5	4.8 c	3.9 b	5.1 c	10.0 b	9.0 c
	0.5 to 2.0	6.1 b	4.8 b	8.0 b	10.7 b	12.1 b
	Greater than 2.0	8.2 a	7.0 a	12.5 a	14.5 a	17.2 a
Proportion of cows in milk (%)	Less than 60	4.9 b	4.3 a	6.9 b	16.0 a	13.1 ab
	60 to 80	5.7 a	4.1 a	9.1 a	14.1 b	12.0 b
	Greater than 80	4.3 b	4.4 a	9.9 a	13.2 c	14.3 a
Proportion of improved pastures established on farms (%)	Less than 33	3.7 b	3.4 b	6.8 b	10.7 b	12.3 a
	33 to 67	5.2 a	4.2 ab	6.6 b	12.8 b	14.8 a
	Greater than 67	5.7 a	4.4 a	9.4 a	15.1 a	19.1 b
Milking frequency (# of times/day)	Once	5.1 b	4.1 b	4.8 b	8.9 b	10.5 b
	Twice	6.8 a	5.2 a	10.6 a	14.0 a	13.5 a
Fertilize pastures	No	5.0 b	4.1 a	5.0 b	8.6 b	10.8 b
	Yes	5.9 a	4.4 a	9.9 a	14.2 a	13.9 a
Irrigate pastures	No	5.2 a	4.2 a	8.3 b	13.2 a	10.5 b
	Yes	5.9 a	4.2 a	11.6 a	16.3 a	15.6 a
Reproductive management	Natural service	4.9 b	4.2 a	7.6 c	11.6 b	9.2 b
	Both	5.7 b	4.3 a	10.0 b	15.6 a	13.1 a
	Artificial insemination	6.9 a	5.1 a	12.7 a	16.0 a	15.0 a
Number of grazing paddocks on the farm	Less than 10	4.7 b	4.2 a	8.0 a	12.6 a	12.2 a
	10 to 20	5.3 b	4.2 a	8.3 a	14.4 a	13.6 a
	Greater than 20	7.4 a	4.4 a	9.3 a	14.3 a	13.8 a
Experience at producing milk (years)	Less than 5 years	4.9 a	3.7 a	10.3 a	16.0 a	13.9 a
	5 to 15	5.4 a	4.5 a	8.2 b	12.9 a	13.2 a
	More than 15 years	5.1 a	4.2 a	8.2 b	13.9 a	13.1 a
Frequency of deworming (# times/yr)	Less than 2	5.1 a	4.2 a	8.6 b	13.7 a	13.6 a
	2 to 3	5.7 a	4.2 a	8.0 b	15.1 a	11.2 b
	More than 3	4.9 a	4.0 a	10.7 a	13.2 a	12.7 ab
Frequency of treatment against external parasites (# times/yr)	Less than 6	4.9 a	4.3 a	8.4 b	14.3 a	13.5 a
	6 to 12	5.2 a	4.0 a	8.3 b	14.0 a	13.1 a
	More than 12	5.3 a	4.2 a	9.6 a	13.2 a	12.5 a
Herd size (# adult cows/farm)	Less than 30	5.2 a	3.6 a	8.9 a	12.4 b	11.9 b
	30 to 100	5.4 a	4.4 a	9.0 a	15.9 a	13.1 b
	More than 100	3.5 a	4.1 a	7.5 b	14.3 a	16.1 a
Commercial value of land (US$/ha)	Less than 3,000	5.0 a	4.2 a	6.9 c	12.7 b	8.9 b
	3,000 to 6,000	5.3 a	4.0 a	8.8 b	14.1 b	13.5a
	More than 6,000	5.5 a	5.8 a	12.4 a	16.2 a	15.1 a

However, it is necessary to have electricity as well as cooling equipment installed to store milk. Farms, which treated against external parasites and dewormed cattle with low frequency, increased net profits but not productivity when compared to farms that practiced these with high frequency. In addition, farms with more years of experience at producing milk had higher incomes but were not more productive (Tables 70 and 71). This indicates that investing in training could have a large impact on farmer's incomes.

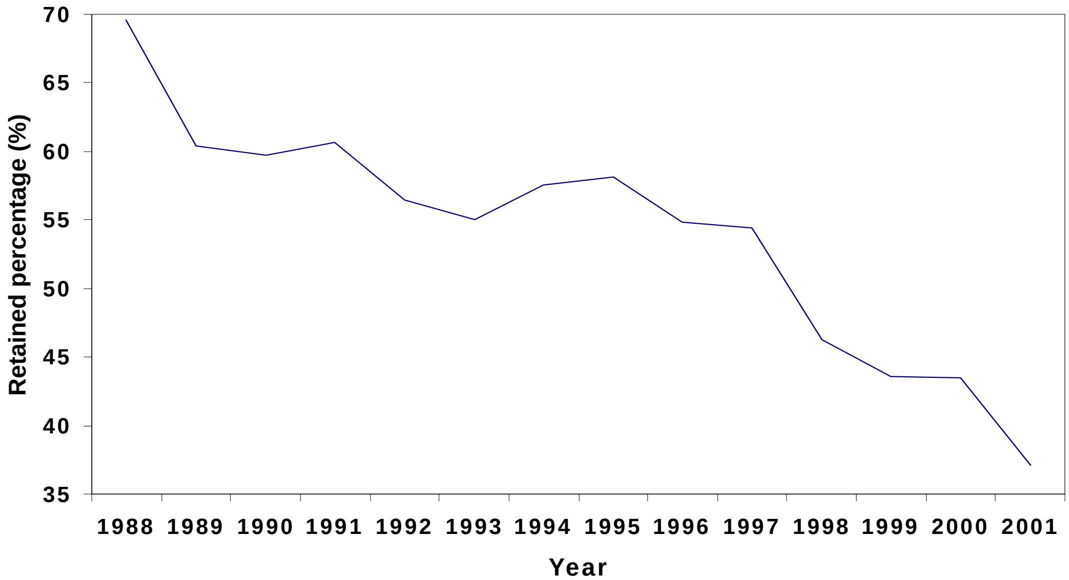
Comparing the evolution of the dairy sector with results from 12 years ago (Table 72), it was observed that milk productivity per hectare increased by 44% in dual purpose systems and by 14% in specialized dairy systems. This increase reduced the cost of milk production by 16% and 10% in dual purpose and specialized dairy systems, respectively, due to an increase in the stocking rate of 15% and 17% in dual purpose and specialized dairy systems, respectively, as well as to the increase in the investment in infrastructure and equipment (ie., adoption of improved pastures, greater number of grazing paddocks, mechanical grass-cutters, irrigation equipment, and other facilities), which increased by 258% in dual purpose systems and by 37% in specialized systems.

Table 72. Evolution of productivity costs of production, investment, profitability, and product prices in dual purpose and specialized dairy systems in Colombia between 1988 and 2000.

Parameter	Milk production System			
	Dual Purpose		Specialized Dairy	
	1988[a]	2000	1988[a]	2000
Productivity				
- Milk production (kg/ha/yr)	453	654	4,132	4,708
- Beef production (kg/ha/yr)	115	107	212	114
- Stocking rate (AU/ha)	1.3	1.5	2.3	2.7
Production cost				
- Milk (US$/kg)	0.19	0.16	0.21	0.19
- Beef (US$/kg)	0.73	0.57	0.98	0.60
- Both (US$/ha)	172	174	1,098	903
Profitability				
- Gross income (US$/ha/yr)	239	223	1,906	1,153
- Net income	67	49	806	250
Investment (US$/ha)				
- Land	1,828	2,479	7,120	5,201
- Livestock	688	461	2,868	1,042
- Facilities & Equipment	117	419	1,126	1,544
- Total	2,632	3,359	11,114	7,786
Annual return on capital investment (%)	4.2	2.7	6.8	2.8
Product prices (US$/kg)				
- Milk	0.27	0.21	0.37	0.22
- Beef	1.02	0.82	1.71	1.24

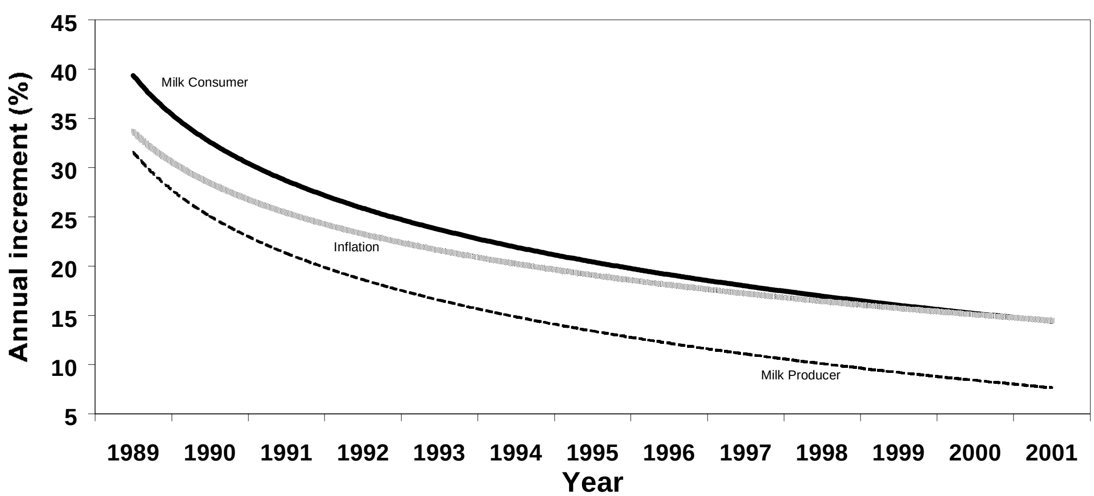
[a]Adapted from Aldana (1990). Currency figures from 1988 were inflated to constant Colombian pesos of 2000 and then expressed in US dollars at the average exchange rate of the year 2000 of 2,084 pesos to the dollar. Productivity figures were estimated from a weighted averaged of both improved and intensive dual purpose and specialized dairy systems.

However, net income per hectare during this period decreased 27% in dual purpose systems and 69% in specialized dairies due to a reduction in the producer price of milk of 22% in dual purpose systems and of 41% in specialized dairies because adjustments in the milk price were always below the inflation rate (Figure 88). On the other hand, the reduction in the price of milk to the producer was never translated in lower consumer prices because the adjustments in the consumer price were above the inflation rate (Figure 88). Thus, if producers were receiving a lower price and the consumers were paying more, who benefited? Figure 89 shows the percentage of the milk price paid by the consumer that milk producers retained. As shown, this percentage went from 70% in 1989 down to 37% in 2001. The largest portion of this difference was retained in the hands of a sector whose growth has been dramatic in the last decade: the supermarkets.



Source: CEGA (2002); DANE (2002)

Figure 88. Trend in inflation rate and annual increments in the consumer and producer price of milk



Source: CEGA (2002); DANE (2002)

Figure 89. Percentage of the milk price paid by the consumer that the Colombia milk producer retains

Through informal interviews with managers of milk processing plants and supermarkets in the city of Cali it was determined that supermarkets request from milk plants that: (a) the first two deliveries of dairy products be free; (b) all expenses in advertising and marketing must be paid by the milk plants whose products are on sale; (c) a permanent discount of 5% compared with the price offered to small (ie., neighborhood or "mom and pop") stores; (d) pay leasing space inside the supermarket at USD 400/lineal meter; (e) pay an annual quota equivalent to 1.8% of estimated annual sales at the supermarket. The strategy of the milk plants has been to translate these marketing costs down to the producer. Likewise, and as a reaction to low profit margins, milk plants begun in the mid-90's to promote the installation of milk cooling tanks in farms to reduce transport and milk collection costs, favoring large and medium producers in detriment of small farms.

Public and private development agencies in Colombia should internalize the fact that policies oriented to markets will increasingly be "oriented towards supermarkets." If one adds that in Colombia three or four chains command up to 50% or more of the supermarket sector the conclusion is that development programs and policies will need to learn how to deal with just a handful of giant companies. This in a huge challenge, and demands an urgent review of ideas and strategies.

We suggest that Livestock Association in Colombia should monitor these price relationships in order to: (a) influence in a proactive manner the milk agroindustrial chain to facilitate negotiations with public and private entities, (b) present to policy makers the appropriate documentation of the impact of these market practices on the livestock sector in Colombia. Otherwise, the new rules of the game could induce a massive exodus of producers in the short term and in a relatively short period of time.

4.1.9 Validation/promotion of *Brachiaria* hybrid cv. Mulato and other forage lines by NARS and farmers

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Rationale

The initial adoption of new forage varieties is almost always limited by low availability of commercial seed, or because the ignorance of both farmers and extension agronomists on the potentiality and requirements of the new varieties. A program that involves the supply of seed and on-farm demonstration on the advantages of the new forages is desirable and will hasten the adoption and diffusion of promising forage lines. However, these activities are usually costly and difficult for government agencies to carry out, thus the support of the private sector is essential for successfully validate and promote new forages.

Methods

A protocol was defined for the validation/promotion of *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *Cratylia argentea* cv. Veraniega (CIAT 18668/516) in cattle farms of small to medium size producers located in Central America, Venezuela, Colombia and Dominican Republic.

It was agree that the experimental seed would come either from CIAT or from Semillas Papalotla, a seed company from Mexico, and that this company will cover the expenses involved in seed shipping, travel to different countries and the costs associated with the establishment and the follow up of the plots.

Collaborators were chosen from INIA-Venezuela, CORPOICA-Colombia, IDIAP-Panamá, MAG-Costa Rica, INTA-Nicaragua, DICTA-Honduras and IDIAF-Dominican Republic. It was not possible to identify a suitable collaborator in Guatemala.

In each country 3 to 5 dual purpose cattle farms were selected and a plan of soil preparation, pasture establishment and management was defined with each collaborator, usually a forage agronomist from the national research institution. Measurements included seedling emergence, plant cover, plant height and DM production at 30, 60 and 90 days after planting. Once the pasture was established a protocol for measurement of milk production will be defined for each country.

Results and Discussion

Five hundred and fifty kg of cv. Mulato seed was supply by Semillas Papalotla and stored for distribution at CIAT Seed Units of Cali (Colombia) and Atenas (Costa Rica); basic seed of cv. Veraniega was supplied either by national institutions or by CIAT.

Six countries of the region were visited between January and July 2002 to make field visits and arrangements for the establishment of the forage species, in particular cv. Mulato. Table 73 shows the countries visited, the region chosen, the number of selected farms, the area to be planted, and the main collaborator responsible for the activity. At least 31 farms were selected and 83.2 ha of cv. Mulato were established during the last planting season; also 1.9 ha of cv. Vearniega were planted in 4 farms near Yorito in Honduras.

The only country of the region not visited this year was Guatemala, given that potential collaborators either from the national or private institutions were not possible to identify; however, the initiative will be taken again during 2003. Contacts were made in Venezuela and a visit paid during April 2002 that covered the savannas of Maturin (Monagas) and El Tigre (Anzoátegui). Farms were chosen for the validation of cv. Mulato with the assistance of agronomists from INIA; however, this institution requested the signing of a letter of understanding before embarking with on-farm activities. This negotiation is still underway and consequently Mulato was not planted this year.

Up to date follow up visits have been made to sites in Honduras, Nicaragua, Costa Rica and Panamá. *C. argentea* cv. Veraniega was planted by direct seeding and established well in Honduras, although some degree of foliage chlorosis was observed two months later probably due to soil nutritional deficiencies, particularly in soils with high calcium content that predominate in the area Yorito-Sulaco.

In Costa Rica cv. Mulato was been planted in sites that ranged from humid to sub-humid tropics, respectively with means of 2500 to 1600 mm of annual rainfall. The soils have pH around 5.0, Al content less than 0.5 meq/100 g soil and contents of OM from 2 to 9%. Cultivar Mulato established vigorously in all farms. Seedling vigor is probably one of the most outstanding characteristics of this cultivar, as well as tiller number, plant development and rapid cover of the soil that allows an early grazing of the paddocks in relation with other grasses.

Table 74 shows results recorded in four farms Costa Rica that indicate that regardless of soil preparation there was an adequate number of seedlings per square meter, and that the first grazing was carried out as early as 2.5 months after planting. Similar observations have been made in Honduras, Nicaragua, Panamá and Colombia.

Table 73. Countries, main region, number of farms, area planted and principal collaborators involved in the validation/promotion of *Brachiaria* hybrid cv. Mulato.

Country	**Region**	**Number of Farms**	**Area planted (ha)**	**Collaborator/ Institution**
Colombia	-Montería	4	13.0	H.Cuadrado/CORPOICA L. Torregrosa/U. de C.
	Villavicencio	2	22.5	C. Plazas/CIAT
Panamá	-Gualaca	1	3.0	B. Pinzón/IDIAP
	Vijagual	2	7.0	H. Hartentains/IDIAP
Costa Rica	-San Gerónimo	1	1.5	M. Lobo/MAG
	Miramar	1	0.7	B. Sandoval/MAG
	San Miguel	1	0.7	J. Florez/MAG
	Puriscal	1	2.5	M. Mesén/MAG
Nicaragua	Matagalpa/Other	5	8.5	A. Schmidt/CIAT M. Mena/INTA
	León/Chinadega	4	5.8	D. Soto/MAG-FOR
Honduras	-Yorito/Sulaco	4	10.7	B. Burgos/DICTA H. Cruz/CIAT
	Olancho	2	7.3	O. García/DICTA
R. Dominicana	-San Juan	1	Na	L. Martínez/SECRETARIA
	La Vega	1	Na	Y. Soto/IDIAF
	Pedro Brand	1	Na	
	Total	**31**	**83.2**	

Table 74. Number of farms, soil preparation, plants per m^2 and date at first grazing of *Brachiaria* hybrid cv. Mulato established in Costa Rica (Information supplied by Marco Lobo and María Mesén, MAG).

Locality	Soil preparation	Area (ha)	Plants/m^2 (2-3 mths later)	First grazing (mths after planting)
San Gerónimo	Conventional	1.5	7.2	3.5
San Miguel	Minimum	0.7	15.0	2.5
Miramar	Zero tillage	0.7	17.0	2.5
Puriscal	Zero tillage	2.5	na	Na

Photo 23 shows the excellent growth condition of cv. Mulato 50 days after planting in a farm in Honduras. It can be noted the high vigor and development of the grass. Similar establishment conditions have been observed in other farms located at the localities of Victoria, Yorito and Olancho.

In the Llanos of Colombia (called locally Altillanura), 15 ha of cv. Mulato were planted jointly with a maize crop, thus the grass is taken advantage of the residual fertilizer applied to the crop as shows

Photo 24. This practice may open a crop-pasture rotation system for an ecosystem like the Colombian savannas characterized by extensive grazing of poor quality forages.

Photo 23. A vigorous plant of *Brachiaria* hybrid cv. Mulato (CIAT 36061) 50 days after planting in a dual-purpose cattle farm in Sulaco (Honduras).

Photo 24. *Brachiaria* hybrid cv. Mulato (CIAT 36061) planted with a maize crop on a farm at the Colombian Altillanura. Note the good growth of the grass between the maize rows. (Photo courtesy of Dr. John Miles)

Plans are presently underway to evaluate cv. Mulato with dual purpose milking cows at the farm level in the different countries established; therefore, both technicians and farmers will experience the quality and productivity of the grass under grazing. During the establishment phase, a low incidence of spitllebug has been recorded in one farm in Costa Rica, as well as a low degree of foliage fungi diseases.

4.1.10 Participatory evaluation of forages for multipurpose use in Haiti

Contributors: Garline Amboise, Myrtho Jerome, Levael Eugene and Jean Osmy Chéry of CIAT/HAP and Luis Horacio Franco (CIAT-Colombia) and P. J. Argel (CIAT-Costa Rica)

Rationale

Haiti is country dominated by hillsides with an extension of 27,500 km^2, and with an estimated population of 8 million people. The soils are either basaltic or of calcareous origin, shallow depth and highly eroded. There is high pressure for cropland with and estimated land tenure of 0.5 to 1.0 ha per family; thus agriculture and livestock practices are fundamentally subsistence activities in the country.

Proteins of animal origin are very important in the population diet, and a significant amount of the government revenues are spent in the importation of milk and beef cattle. However, in the country a high cattle and goat population exists, and practically every family has a variable number of animals, which are highly valued for economic and social reasons. Animals are usually maintained along roadsides grazing poor quality forages or crop residues; therefore animal productivity is very low.

The Forage Project of CIAT has been involved in Haiti since 2000 with the Hurricane George Recovery Program (HGRP) financed by USAID, but lately a new CIAT contract has been signed with the Hillside Agriculture Program (HAP) that involves several crops including forages for multipurpose use. As part of a general work plan of CIAT for Haiti, a number of forage field trials were proposed during 2002 as green manure, for soil erosion control and as sources of grain for humans and feed for livestock. The evaluation strategy contemplates farmer participatory evaluation of forage establishment, production and harvesting of green foliage and grain, as well as dry matter measurements. It is expected that from these trials we can select at least 3 grasses and 3 legumes for seed multiplication and for promotion among farmers.

Methods

The multipurpose forage trials proposed included the evaluation of Cowpea (*Vigna unguiculata*) as grain and green manure, the agronomic evaluation of multipurpose grasses and legumes, and the evaluation of multipurpose forages as barriers for erosion control and feed for livestock in a cut and curry system. Six grasses, 4 herbaceous legumes and 6 shrub legumes were proposed for evaluation. Experimental protocols for each activity were prepared and the experimental seed delivery to the CIAT/HAP team of Haiti.

Evaluation of multipurpose grasses and legumes. The following species were selected for evaluation in a complete block design replicated 4 times:

The grasses *Panicum maximum* cv. Mombasa, *P. maximum* cv.Tanzania (CIAT16031), *Brachiaria decumbens* cv. Basilisk (CIAT 606), *B. brizantha* cv. Toledo (CIAT 26110), *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *Paspalum atratum* cv. Pojuca (CIAT 26986).

The herbaceous legumes *Centrosema macrocarpum* cv. Ucayali (CIAT 25522), *C. pubescens* CIAT 15160, *Arachis pintoi* CIAT 22160 and *Stylosanthes guianensis* cv. Pucallpa (CIAT 184).

The shrub legumes *Cratylia argentea* CIAT 22374, CIAT 22386, CIAT 22379, CIAT 22382 and cv. Veraniega (CIAT 18516/18668), and *Leucaena macrophylla* subsp. *nelsonii* OFI 47/85.

Agronomic adaptation and forage barrier trials are under establishment at the localities of Cap-Rouge, Larevoir and La Vallée in the South-East of the country, and at the Fondation Vincent Salésiens de Don Bosco and Bertin in Cap Haitien in the North. Due to the unreliability of the rains at the beginning of the year, most of the May planting failed, thus the trials were replanted again in mid August. Most of the soils are either basaltic or of calcareous origin with medium fertility, low in P and organic matter, and high in calcium content. At each site the rainfall pattern is bimodal and altitude varies form 0 to 800 masl.

Cowpea evaluation. A local check and the following lines of Cowpea originated from IITA were established at the localities of Cap-Rouge (South-East) and Matador (North): IT86D-716, IT90K-277/2, IT89D-391 and IT95K-1088/4.

A randomized complete block design with 3 replicates was used. Plot size was 5.0 m long by 2.5 m wide; half of the plot was used to measure plant vigor, cover and height, and grain and DM yields of the Cowpea lines. The other half was incorporated at grain filling and subsequently the plot planted with a maize crop one week after the incorporation of the foliage. An additional maize experiment was planted in adjacent plots with 0, 40, 80, 120, 160 and 200 kg/ha of nitrogen to compare maize grain yields between the two experiments and measure the N contribution of the Cowpea trial to the soil. Farmer participatory evaluations were also conducted at each site. The experiment is still under evaluation in Cap-Rouge while at Matador the Cowpea lines were harvested, foliage incorporated and maize planted.

Results and Discussion

Planting of the multipurpose forage trials had problems due to the unreliability of rains, which did not come as expected in April-May, and as a result the first planting was missed. However in the North of the country *B. brizantha* cv. Toledo and *P. maximum* cv. Mombasa have had acceptable growth in the live barrier trial established at Bertin, but plots are not complete and were re-planted again in August. At this site *C. argentea* cv. Veraniega failed to germinate.

The forage agronomic trial planted at Fondation Vincent also had problems of seedling emergence because a long dry period occurred following planting. New plantings have come out well but growth of the plants has been limited by goats that occasionally graze the plots. However, it is possible to see good vigor of the grasses *B. brizantha* cv. Toledo, *B. decumbens* cv. Basilisk, *Brachiaria* hybrid cv. Mulato and *P. maximum* cv. Mombasa. The best forage legumes are *C. pubescens* CIAT 15160, followed by *C. macrocarpum* cv. Ucayali and *Arachis pintoi. C. argentea* cv. Veraniega shows good color but poor plant vigor, which is consistent with results reported elsewhere in calcareous soils for this shrub. In sites with calcareous soils the plant establishes slowly for the first year, but then become very vigorous. This experiment will continue under evaluation and a fence to protect the plots was planned.

At Cap-Rouge and La Vallée the forage agronomic experiments were planted in August – September and plant emergence is good with the exception of *S. guianensis* cv. Pucallpa and *L. macrophylla* subsp. *nelsonii* that had poor germination. At Larevoir experimental plots are not complete but it is possible to see good vigor of the grasses *P. maximum* cv. Tanzania and *B. decumbens* cv. Basilisk. At this site *C. argentea* cv. Veraniega shows growth signs similar to the observed at Fondation Vincent.

At Cap-Rouge the Cowpea trial is still under evaluation; plants top growth were incorporated in half of the plots and the maize will be establish soon. Farmer participatory evaluation of this trial showed preference for the local line followed by IT89KD-391. However, different results were obtained at Matador were the more vigorous and yielding plant was the Cowpea line IT86D-716 followed by IT90K-277/2 as shown in Table 75. At this site, farmers have more tradition of Cowpea cropping and in the participatory evaluation carried out they showed preference for the IT86D-716 line for better growth, better plant cover, grain yield and color of the grain.

Table 75. Green foliage yields of Cowpea (*Vigna unguiculata)* lines established as grain and green manure in El Matador (Haiti).

IITA No.	Foliage green yield (kg/ha)
IT86D-716	3533.3 a*
IT90K-277/2	3000.0 ab
Local line	2033.3 bc
IT89KD-391	2000.0 bc
IT95K-1088/4	1666.7 c

*Means followed by the same letter are not statistically different ($P<0.05$)

Maize was planted after the incorporation of half of the Cowpea plots, as well as the establishment of maize with nitrogen fertilization as described in the experimental protocols, but it failed to establish and will be replanted the following planting season.

4.1.11 Enhancing beef productivity, quality, safety, and trade in Central America

Contributor: Federico Holmann (Tropileche Consortia)

Rationale

Beef is an important commodity in the economies of Central American countries. In 1995, regional beef exports realized more than US$ 126 M and imports cost nearly US$ 17 M. This is especially important to the low-income countries in the region. It is, for example, Nicaragua's most important agricultural activity accounting for 22% of the total Agricultural Gross Domestic Product (AGDP), with exports of fresh and processed meat to El Salvador, Costa Rica, Mexico and the USA among other countries. Guatemala, Honduras and Costa Rica export meat primarily to the USA and Mexico.

Total beef exports from the region are showing a declining trend although intra-regional exports increased by 45% in 1998 relative to 1997. However, increases in intra-regional exports are falling behind the total imports by the region. The lost of international markets, particularly the USA, added to the increasing presence of extra-regional competitors and the stagnation of the production and productivity in Central American countries are an indication of the crisis of the beef industry in the region, which affects smallholder producers.

Project Description

This a development-oriented project approved by the Common Fund for Commodities (CFC) expected to begin in November of 2002 which aims at alleviating poverty by raising smallholder farm productivity and enhancing trade in beef with improved meat quality and safety. This will increase the availability of affordable safe meat products for local consumers and make the Central American beef industry more competitive against imported beef. The project will be executed in the CFC member states Guatemala, Honduras and Nicaragua. Costa Rica and Panama will also participate when their membership is confirmed. Other countries that will participate when complementary funding is found include Belize and El Salvador.

This is a 4-year project with an estimated cost of US$5 million of which $3.5 millions are being funded by CFC and the remaining $1.5 million from co-funding allocated by counterpart contributions. The project executing agency will be the International Livestock Research Institute (ILRI) and the project leader Federico Holmann.

Co-executing agencies will be CIAT (Centro Internacional de Agricultura Tropical), SIDE (Servicios Internacionales para el Desarrollo Empresarial), IICA (Inter-american Institute for Cooperation in Agriculture), and CAC (Consejo Agrícola Centroamericano), Local partner organizations will be MIDA/IDIAP and ANAGAN in Panama; MAG-CORFOGA in Costa Rica, MAG-FOR and FAGANIC in Nicaragua, SAG and FENAH in Honduras, and MAGA and Federación Guatemalteca de Ganaderos in Guatemala.

The project's goal is to improve the livelihoods of smallholder producers, make quality safe animal-source foods affordable and available to low income consumers and increase the intra- and inter-regional beef trade in Central America. These activities will be conducted as part of the following project components: (1) Improving farm productivity; (2) Beef quality and safety standards and controls; and (3) Project monitoring, impact assessment and dissemination of research products.

The main activities of Component 1 (Improving farm productivity) will be:

(a) Identification of technical interventions to improve farm productivity;
(b) On-farm validation of best-bet technologies to improve farm productivity
(c) Implementation of disease surveillance and control measures; and
(d) Strengthening risk analysis capacity to prevent exotic diseases disrupting exports

The main activities of Component 2 (Beef quality and safety standards and controls) will be:

(a) Development of food safety and processing procedures along the beef production-to-consumption chain;
(b) Development of a carcass classification systems and regulations for meat quality and safety.

The main activities of Component 3 (Project monitoring, impact assessment and dissemination of research products) will be:

(a) Monitoring project progress and assessing the impact of the component activities and providing feedback to stakeholders;
(b) Dissemination of project outcomes and outputs to policymakers and official and private extension agents.

CIAT will participate in the co-execution of Components 1 and 3 in aspects related to increasing on-farm productivity through the introduction of improved-based forages aimed to increase weaning weights of pre-weaned calves and to improve the nutritional status of dams.

4.1.12 Collaboration with ETHZ-Zurich

Contributors: I. Rao and C. Lascano

The main objectives of the Swiss Center for International Agricultural Research (ZIL) are to: (i) identify and address priority research needs, for which the Swiss Federal Institute of Technology Zurich (ETHZ) has comparative advantage and a relevant contribution to make; (ii) promote coordination and interdisciplinary approaches to problem solving in relation to development relevant agricultural research; and (iii) raise awareness for the importance of investing into strategic research that addresses problems faced by developing countries. These objectives of ZIL are achieved through strategic research, capacity building and education, consolidation and diffusion of information, and the promotion of institutional partnerships with developing countries. ZIL is primarily supported by the SDC (Swiss Agency for Development and Cooperation/DEZA), by ETHZ and by its members.

We are currently collaborating with ETHZ through a three -year project (2000-2002) to mitigate methane emissions in ruminants with improved feeding systems. This project funded by SDC- ZIL is in its final year and the latest results obtained are summarized in this Annual Report (1.4 Assessment of the potential of tannins in legumes and saponins in tropical fruits to reduce methane in ruminants on grass diets).

For the funding period of 2003 to 2005 ZIL went through a priority setting exercise and identified “Livestock systems: Contribution to food security, human nutrition, income generation and environmental sustainability” as a research priority area (RPA). ZIL is aiming to receive 1.2 to 1.5 million Swiss Francs from SDC to support the Livestock systems RPA for the duration of 3 years (2003 to 2005).

To put together a research program around this new RPA on Livestock systems, ZIL organized a workshop at ETHZ, Zurich from 5 to 10 February, 2002 and invited and supported the participation of livestock researchers from ILRI and CIAT. Three scientists (Drs. S. Fernandez, G. McGrabb and A. Odenyo) represented ILRI, while C. Lascano and I. Rao represented CIAT. About 20 researchers including several ZIL members from ETH attended the workshop. The main objective of the workshop was to capture ideas for potential research activities under the RPA "Livestock systems" and to group them in order to advance the development of a coherent program that generates maximal synergies between the research outputs. The outputs of the workshop were: (i) a set of potential foci for RPA; (ii) participants' preferences on the foci; (iii) list of categorized project ideas; and (iv) assessment of the relevance of the project ideas.

Two of the project ideas identified in the workshop in which CIAT had participation were subsequently developed into research proposals and submitted to ZIL-SDC for approval in September 2002. This were:

1) *Adaptation of* Brachiaria *grasses to low P soils (collaborators: ETHZ and CIAT)*

In this project if approved, we will determine the role of root architecture and mycorrhizal association on P acquisition in *B. decumbens* and *B. ruziziensis* using isotopic approaches and the available methods to characterize root architecture (e.g. Winrhizo) (Output 1). The role of root exudation of protons, mobilizing organic acids, phenols and enzymes on P acquisition by *B. decumbens* and *B. ruziziensis* will be assessed using approaches developed at ETH and CIAT (Output 2). Candidate genes responsible for the low-P tolerance in *B. decumbens* will be identified at CIAT using a population obtained from a cross between *B. decumbens* and *B. ruziziensis* and classical methods from molecular biology (Output 3). Outputs 1 and 2 will be achieved by a PhD student based at the ETHZ (plant nutrition), output 3 will be achieved by 1 MSc student based at CIAT (plant nutrition, biotechnology). Collaboration with animal nutrition groups at CIAT and ETH will ensure evaluation of new hybrids with respect to their feeding value and their use in feeding programs by 1 MSc student.

2) Potential of tropical forage species to improve protein supply and to reduce methane emission in ruminants: Search for sustainable ways to cope with nutritional limitations in smallholder livestock (Collaborators: ETH, CIAT, ILRI and U Nacional de Colombia

The aims of this project are: (i) to identify tropical legumes adapted to acid, low phosphorus soils which have the potential to improve ruminant diets and to simultaneously reduce methane emission and (ii) to contribute to the understanding of the mode of action of tannins in ruminant nutrition. Legumes grown on contrasting soils both in Africa (ILRI) and South America (CIAT) will be used together in all experiments. These include the *in vitro* evaluation of promising tannin-rich forage legumes by testing how combinations of forage species with and without tannins affect protein degradability in the rumen, ruminal methane emission and protein release under conditions resembling the abomasum. Studies with ruminant livestock (mainly sheep) will be carried out focusing on microbial population shifts, the measurement of feed intake, rumen fermentation, methane release, post-ruminal protein flow, metabolic nutrient utilisation and nitrogen fertiliser value of the manure.

Work at the National University of Colombia will focus on tannin chemistry, ruminal protein degradability and the protein release simulating abomasal conditions (1 MSc. student). *In vitro* measurements will be carried out at CIAT (1 MSc, 4 undergraduate students). The *in vivo* trials will be performed at ILRI and ETHZ (1 doctoral student). ETHZ food chemistry will assist to describe the interrelationship with the carbohydrate pattern of the legumes.

The results of this work will provide a better understanding on how environmental factors affect tannin concentration and properties in forage legumes and how to use tannin-containing and tannin-free forage legumes as feed resource, in a way that they cause desired shifts in microbial populations associated with improved metabolic protein supply and less emission of methane.

4.1.13 Collaboration with U. Hohenheim-Germany

Contributors: R. Schultze-Kraft, University of Hohenheim, F. Parra, Corpoica, M. Peters, L.H. Franco and B. Hincapie

Development of multipurpose forage legumes for smallholder crop-livestock systems in the hillsides of Latin America: Proposal to Volkswagen Foundation. This initiative is being developed in partnership with the University of Hohenheim (Institute of Plant Production and Agroecology in the Tropics and Subtropics) and Corpoica (Corporación Colombiana de Investigación Agropecuaria). The aim is to develop multipurpose forage options for smallholder farmers in the hillsides of Latin America. Its ultimate goal is to improve livelihood of these farmers through increased income while at the same time conserving natural resources. In terms of forage technologies, in this context the focus is on shrub and fast-growing herbaceous legumes.

Given that there are limited numbers of high quality tropical shrub legume options adapted to infertile soils that are predominant in hillside regions, there is a need to identify additional forage resources for smallholders. *Desmodium velutinum* and *Leucaena diversifolia* have been chosen as potentially promising shrubs for such soils. However, there is very limited information available on variability among accessions in collections of the two shrub legume species held by CIAT. Thus a core collection will be evaluated for agronomic and morphological parameters and this information will the related to molecular markers and to information on origin using GIS to

Fast growing forages can exploit temporary and spatial niches not occupied by crops. Thus in the proposed work, we hope to evaluate collections of *Vigna unguiculata* and *Lablab purpureus* given that they can be used as feed, food and for soil improvement

Shrub and herbaceous legumes evaluated under controlled on-station conditions will be selected with farmer participation and disseminated through farmer-led field days. A strategy to support the scaling process will focus on the development of seed supply systems with active farmer participation.

It is anticipated that this work will have a direct impact on livelihood primarily in the selected research site in Cauca, Colombia, but also in Central America.

Activity 4.2 Evaluation with farmer participation of multipurpose forages in crop and livestock systems

Highlights

- Utilizing farmer criteria for selecting forage species, a sequence for data analysis was developed which helps to define profiles for forage species with high potential acceptance and adoption by farmers.

- Identified a number of entry points for forage-based technologies for farmers on steep slopes that have mostly crop and limited cattle method.

- Forage technologies that respond to farmers demand were identified and are being adopted by farmers in hillsides of Nicaragua and Honduras.

- Artisanal seed production of *Brachiaria brizantha* CIAT 261110 (cv. Toledo) and *Cratylia argentea* cv. Veraniega was initiated in Nicaragua (San Dionisio and Pantasm-Jinotega), and Honduras (Yorito/Sulaco/Victoria).

- In the Philippines, the adoption of improved forage systems have resulted in: (a) time saved for households (particularly women), (b) increased income from livestock from below the poverty line to twice the poverty threshold, and (c) reduced conflicts due to less herding which contributes to destruction of crops by animals.

- In Vietnam improved forage systems reduced time needed for herding ruminants and for collecting fodder for fish, with am mean of 150 days per household. Especially children benefited from reduced herding, who were able to attend school. Within three years, income from mixed systems of fish and ruminants fed on improved forage had increased by 265 %.

4.2.1 Development of participatory methods to enhance adoption of forages as feed resources and for INRM

Contributors: R. van der Hoek (University of Hohenheim), M. Peters, V. Hoffmann (University of Hohenheim), and H. Cruz Flores

Rationale

It is well recognized that forage-based technologies can play an important role in improving the environmental and socio-economic sustainability of smallholder production systems in the tropics, especially in situations with a fragile balance between the availability of natural and economic resources and their utilization. Forages can serve multiple objectives, such as provision of animal feed, enhancing soil conservation and maintaining and improving soil fertility. Forage species that are widely adapted, productive and palatable have been identified, but farmer adoption has often been low.

In this research existing participatory methods are applied in systematizing farmers' decision-making, learning, experimenting, visions and perceptions on their land use as well as in the development, testing and evaluation of forage based-technologies. Depending on the extent to which the available methods are suitable for these two purposes, they are adapted or replaced by new methodologies.

It is expected that using participatory methods for development of forage-based technologies will lead to:

- a better comprehension and insight of all stakeholders in the factors that play a role in the research, development, adoption and evaluation process;
- generation of ideas and initiatives from farmers in the design of experiments and development of other activities;
- a strong participation of farmers in the implementation of experiments (monitoring, "daily care");
- forage based technologies that are well adapted and adopted by farmers.

Most of the research is being carried out in the CIAT's reference site in the hillsides of Honduras with the financial support of BMZ/gtz, Germany. Although all categories are represented, activities have largely been focused at small farmers at higher altitudes, complementing work with cattle owners in lower areas. Expected outputs include a manual on participatory procedures for forage-based technologies, scientific publications and a Ph.D. thesis (Hohenheim University).

Materials and Methods

Farming system analysis includes: (1) Identification and priorisation of problems related to agriculture; (2) Selection of research topics and experiments with forages that contribute to the solutions of problems in agriculture, based on farmers' demand and taking into account the interactions within and outside the farming system; (3) Development of forage ideotypes (combinations of forage based technologies and their uses); and (4) Generation of other research topics by research results.

From the Farming Systems analysis we expect to get:
– Manual on the complete process (Out-, upscaling
– Farmers' forage solutions

The methodology that we will use includes the following elements:
- introductory meetings (about forages, farming systems)
- field visits (demonstration sites)
- meetings on problem ranking
- meetings on forage priorities
- meetings on research priorities

1. Analysis of existing farming systems

The analysis of a sample of 64 farmers indicates that altitude is a mayor determining factor in distinguishing different farmer categories (Figure 90), with the following results that were somewhat expected:

- The standard of living in the lower parts is higher than in the higher parts of the watershed, given farms hat are bigger, and maize yields are higher.
- Only 20% of the farmers in the watershed keep cattle, but most have other livestock like poultry, pigs and horses.

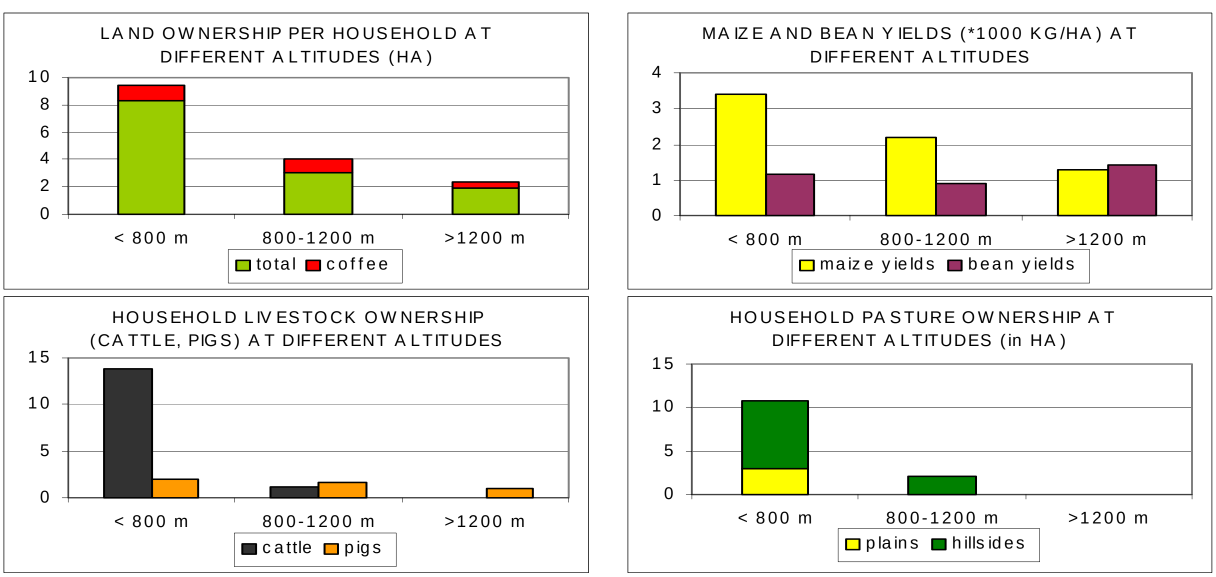

Figure 90. Farming systems analyis Yorito, Honduras. Effects altitude on land ownership, basic grain yield, livestock and pasture ownership.

Nutrient balances. In order to get more insight in nutrient availability and nutrient balances in different production systems, we carried out a study in six different farms and some results are presented here in two examples: La Patastera (1600 masl, 0.7 ha maize, 0.7 ha beans, 0.3 ha coffee) and Las Cañas (400 m asl, 2 ha maize, 0.7 ha beans, cattle, 7 ha pastures (Figure 91).

The following observations were made after examining the data:

- During the dry season crop residues are an important source of animal feed, but their use as feed affects soil fertility adversely.
- Although crop residues are used for animal feed, nitrogen balances in the lower parts of the watershed are less negative than in the higher areas, possibly as a result that more inputs are used, especially on maize. However, hardly any fertilizer is used on beans, whereas nutrient export is high (Table 76)
- Phosphorus balances are slightly positive in all farms examined.
- Potassium balances in all farms are highly negative due to export of crop residues.

Table 76. Fertilizer use for crop production, Yorito, Honduras

Fertilizer use (kg/ha)	< 800 m	800-1200 m	> 1200 m
NPK maize	85	20	13
Urea maize	115	25	0
NPK beans	20	0	0
Urea beans	5	0	0

Survey data 2001, n=64

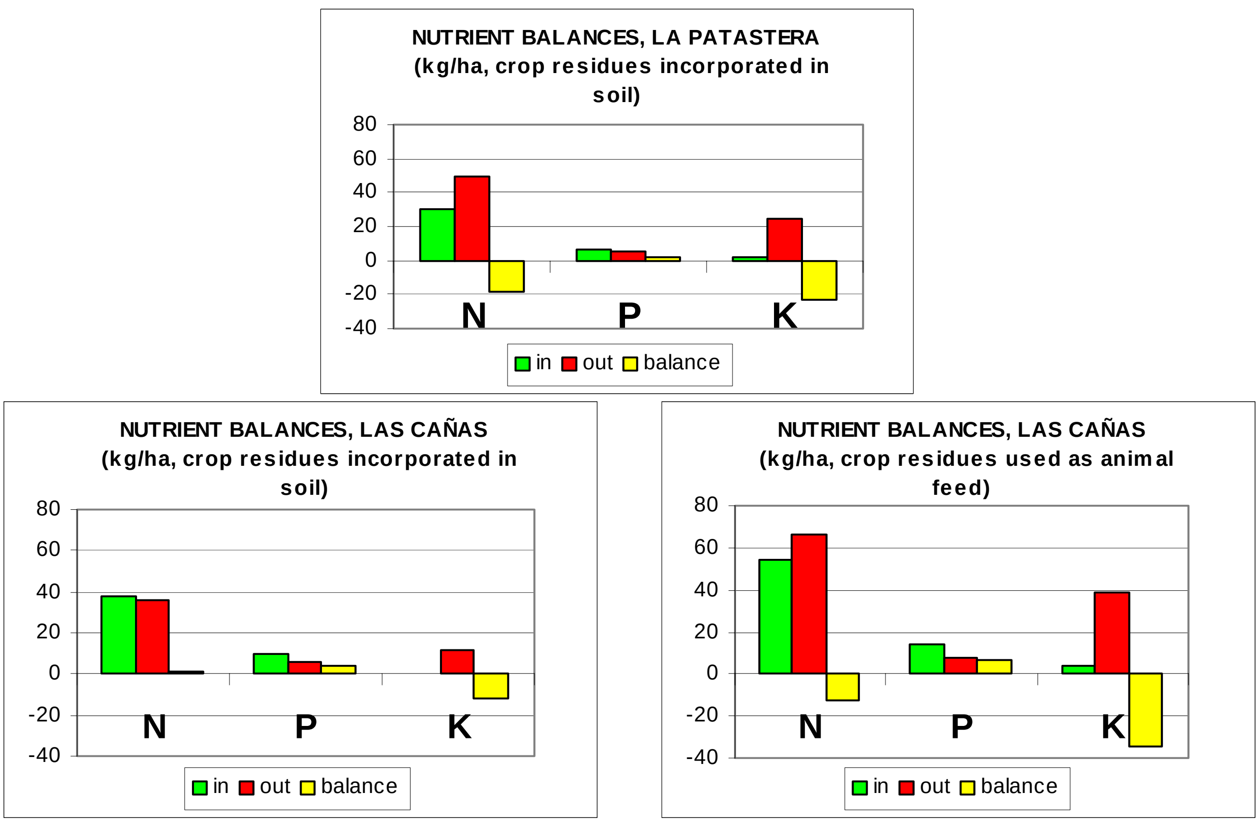

Figure 91. Nutrient balances in contrasting farms in Hillsides of Yorito, Honduras

Financial balances. A financial balances were made for some six farms and in Table 77 we show some results from farmer in the higher (La Patastera) and lower parts (Las Cañas) of the watershed.

Table 77. Financial balances of farmers from La Patastera y Las Cañas

	La Patastera 1600 masl	Las Cañas 400 masl		La Patastera 1600 masl	Las Cañas 400 masl
Maize primera	1 ha	2 ha	Coffee	0.3 ha	1 ha
Labor costs	1080	2895	Labor costs	0	3750
Total costs	1605	4675	Total costs	190	4480
Revenues	1600	11000	Revenues	2000	4000
Balance	-5	6325	Balance	1810	-480
Beans primera	0.5 ha	0.15 ha	Livestock	2 goats, 1 sheep, poultry	11 heads of cattle, 2 horses, 1 mule, 1 pig, poultry
Labor costs	850	250	Bought	0	4000 (2 steers)
Total costs	1433	430	Total costs	0	5080
Revenues	1200	1600	Sold	600 (2 goats)	8000 (2 heifers)
Balance	-233	1170	Balance	600	2920
Beans postrera	1 ha	1 ha	Total costs	3878	18215
			Total revenues	9600	30600
Labor costs	0	3150			
Total costs	650	3550	Total Balance	5722	12385
Revenues	4800	6000			
Balance	4150	2450			

Amounts in Honduran Lempiras (HNL), 1 USD equals approx. 16 HNL

Implications for forage-based technologies. Given the fact that most of the farmers do not possess cattle, we should target work not only "conventional" users of forage-based technologies, (i.e. cattle keepers) but also crop farmers.

- Legumes for green manure can be important especially for farmers at higher altitudes. There is also scope for forages as feed pigs and poultry, but the utilization of these forage has to be considered in combination with crop residue use. The fact that farmers in higher sites of the watershed tend to minimize risks (low input levels) and farmers at lower altitudes use inputs (labor, fertilizer, pesticides) to obtain higher production levels has implications for possible forage based technologies.

In general, the diverse production systems in the region provides ample scope (enough entry points) for forage-based technologies for farmers with cropping systems on steep slopes (forage crops for soil conservation and fertility) or farmers keeping small animals (forages for poultry, pigs, sheep, goats).

2. Identification and priorisation of problems related to agriculture

In Figure 92 we show the most important constraints and their relative weights as they came up during meetings with farmers. Soil fertility and soil humidity are of major concern everywhere. In the lower areas of the watershed lack of animal feed is problematic, whereas at the higher altitudes erosion and weeds are significant. In general, the preferences by farmers for forage can be described as follows for different parts of the watershed in Honduras which is being studied:

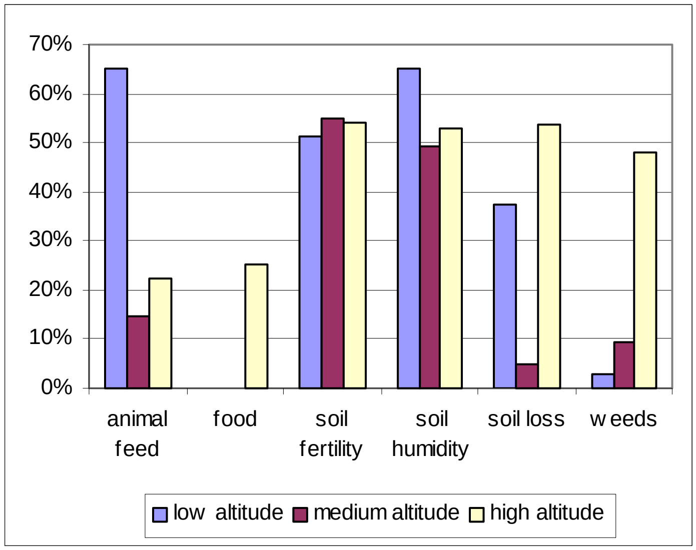

Figure 92. Nature and importance of agriculture related problems at different altitudes (expressed as percentage of a maximum score)

Lower altitudes (< 800 m asl)
1. grasses: grazing, cut and carry, also for soil retention
2. forage legumes: (green) manure, animal feed
3. leguminous shrubs, trees: animal feed, timber, fuelwood

Medium altitudes (800-1200 m asl)
1. legumines cover crops: green manure, animal feed
2. grasses: grazing, grass barriers for cut and carry and for soil retention
3. leguminous shrubs, trees: natural pesticides, insecticides, animal feed, green manure

Higher altitudes (>1200 m asl)
1. legumes: (green) manure, weeds, human food, animal feed
2. leguminous shrubs, trees: (green) manure, natural insecticides, timber, fuelwood
3. some grasses: soil retention, feed for sheep, goats, beasts of burden

One major aspect of our work this year was to establish experiments with forages that hopefully will answer problems that farmers have indicated are of high priority. In Table 78 we summarize the types of experiments proposed by farmers. Since June 2002, 40 experiments in more than 130 plots in 11 communities, and involving approximately 150 farmers (50% women) have been established. Some experiments are carried out by individual farmers, but most of them by groups of four or more persons.

Of the 40 experiments set up in farmers fields (Figure 93):
- 17 are focus on forages for animal feed: grasses - *Brachiaria brizantha* CIAT 26110, *Andropogon gayanus*; legumes – *Arachis pintoi, Stylosanthes guianensis*; leguminous shrubs – *Cratylia argentea*
- 6 focus on food production: cowpea (*Vigna unguiculata*), different accessions from IITA
- 14 focus on enhancing soil fertility: legumes – *Canavalia brasiliensis, Mucuna pruriens,* pigeon pea (*Cajanus cajan*), cowpea
- 2 focus on improving and maintaining soil humidity – Cratylia, Mucuna
- 1 focus on combating weeds

Table 78. Experiments with forage technologies proposed by farmers in Hillsides of Yorito, Honduras.

Grasses	In groups	Individually
Lower altitude	Compare different species, for grazing and for seed production	Different species for cut and carry, grazing, as poultry feed
Medium altitude	Compare different species, for grazing purposes	Compare different species cut and carry purposes
Higher altitude	Adaptation trial with one species	Adaptation trial with one species
Herbaceous legumes and (leguminous) shrubs		
Lower altitude		**- Test suitability as animal feed (poultry, calves)** - Green manure
Medium altitude	- Mixed cropping (maize, coffee) - Cocktail with grasses - Mixed cropping in gardens - Compare different cover crops (effect on soil fertility)	- Test different legume shrubs/trees - Mixed cropping (maize)
Higher altitude	- Compare different cover crops (effect on soil fertility) - Compare different species	- Adaptation trials with different species - Compare different species (mixed cropping with maize, barriers)

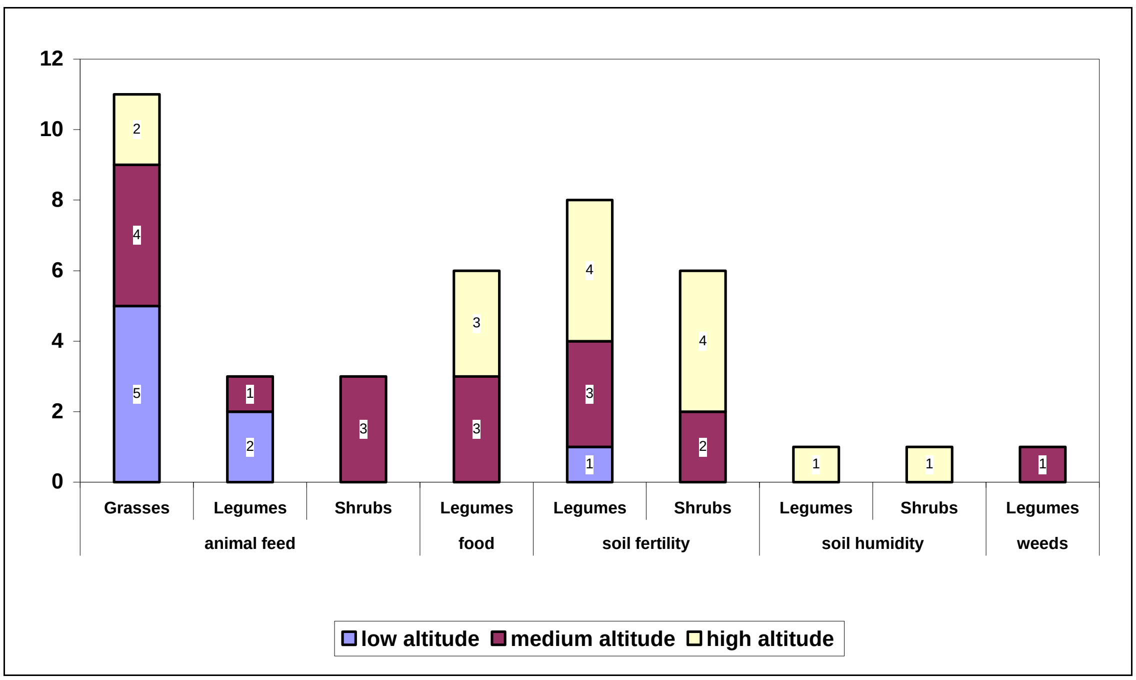

Figure 93. Number of forage based experiments for different farmers' objectives, forage categories and altitudes

In table 79, steps in the process of formulating and implementing experiments and interpreting research results with farmer participation are presented.

Table 79. Steps in the process of formulating and implementing experiments and interpreting research results

Step	Activities	Procedure	Output
Formulation of experiments with forage germplasm and other inputs	– Formulation of experiments with forage germplasm, crop residues, manure, using/testing participatory methods	– Based on the ideotypes, experiments are formulated – Carried out mainly by farmers	– Adaptation/development participatory methods on formulation/design of experiments – Protocols for experiments
Experimenting	– Experiments on management and utilization of forages and crop residues, use of manure, compost – Testing forage germplasm	– Experiments carried out "on-farm" (local conditions). Formal and non-formal experiments, depending on the expressed needs and local conditions – Experiments mainly carried out by farmers, in collaboration with researcher and local organizations	– Adaptation/development of participatory methods on implementation and monitoring of experiments – Research results on forage based technologies
Evaluation of experiments, interpretation of research results	– Technical and economical evaluations – Reformulation of experiments – Formulation of further (development oriented) actions	– Experiment results are evaluated and interpreted, further actions are developed, using participatory methods – Carried out by farmers, in collaboration with researcher and other stakeholders	– Adaptation of participatory methods on evaluation of experiment results – New experiments – Evaluation of forage based research – *Feedback to forage, soil, production systems, land use research*

During the coming months the on-farm experiments will be evaluated. According to the participatory procedures described in the above table the outcome of these evaluations will be used to formulate new experiments, of which the first will be implemented from September onwards (*Postrera* growing season). From May 2003 onwards a third cycle of experiments will be carried out.

The expected result from this exercise is the identification of a range of suitable forage based options for small farmers (especially for those living at altitudes beyond 800 m asl) based on their main problems and priorities within a farming systems context and taking into account their perspectives on land use. Some concrete examples of the results we expect are:

- use of different cowpea accessions for medium, high altitude to produce food and/or animal feed and/or green manure intercropped with maize or as a *postrera* crop;
- use of *Lablab purpureus* or Canavalia to restore soil fertility;
- use of *Brachiara brizantha* 26110 at higher altitudes as goat feed and soil conservation;
- use of pigeon pea as live barriers and to produce food;

We also plan to produce a manual on the use of participatory procedures for forage based technologies in Central-American hillsides.

4.2.2 Evaluation of forages for multipurpose use with farmer participation in hillsides of Central America

Contributors: L. A. Hernández Romero, M. Peters, A. Schmidt, L.H. Franco and G. Ramírez (statistician), with the collaboration of NARS and NGO partners, technicians and many farmers in Honduras, Nicaragua and Costa Rica (M. Posas, C. Burgos, H. Cruz, C. Davies, W.Sanchez, J. Bustamante)

Rationale

Forage germplasm in its multiple uses - for example as feed, for the suppression of weeds, maintenance and improvement of soil fertility and for erosion control - could play an important role in improving the well being of the small and medium size farmers in Central American hillsides. However, adoption - particularly of forage legumes - has been limited, possibly due to lack of direct interaction with the farmers. Therefore it is necessary to develop forage technologies with farmers, using participatory approaches. To address this issue, CIAT in collaboration with NARS, NGO's and farmer groups is identifying forage options with farmers participation. The work is anticipated to contribute to the development of an overall strategy to guide future research and to aid in the diffusion and adoption of forage-based technology by small farmers. The interaction with strong national partners – alongside the farmers – will be of paramount importance to the success of the approach. The work links closely with the TROPILECHE Consortia, using some of the same forage options. On the other hand, Forage germplasm selected from this work will be useful to TROPILECHE.

Results and Discussion

Database development and analysis

Using the sequence described in Figure 94, a procedure for analysis of forage species with potential for adoption by farmers was developed.

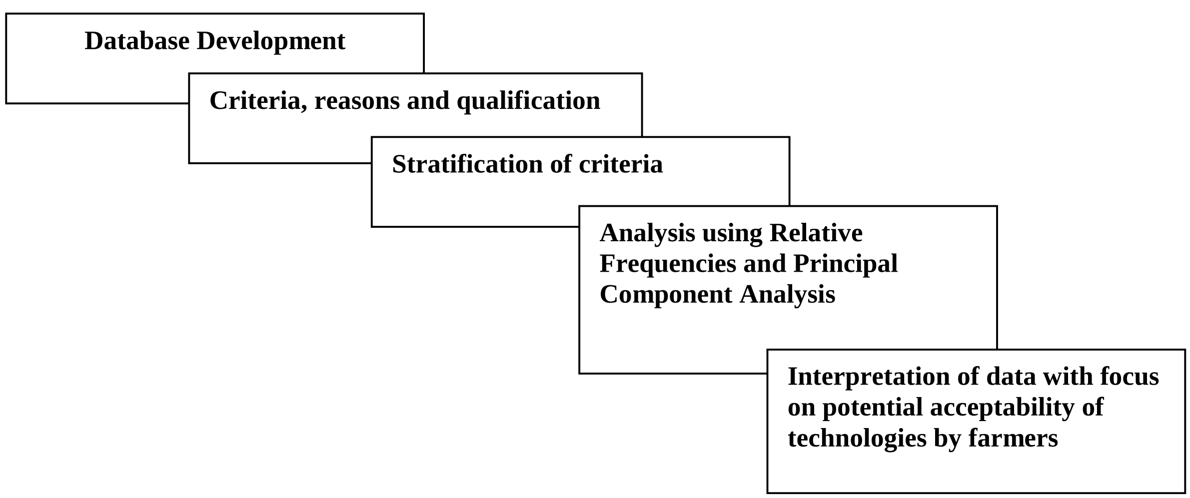

Figure 94. Sequence of analysis for participatory evaluation of forage technologies.

What follows is a brief description of the sequence:

- **Database development**. A central database which includes information from participatory evaluations in Honduras, Nicaragua and Costa Rica was developed in EXCEL. The information on the database includes: a) country, b) site, c) date of evaluation, d) season, e) forage technology, e.g. grasses and legumes, f) species, g) farmer criteria for assessment of forage technologies, h) reasons and explication of criteria, i) qualification of criteria using scales developed with farmers, j) frequency of criteria, k) preference ranking.
- **Definition of criteria** for forage technologies, based on farmer input. Farmer refer to these criteria for assessing forage technologies.
- **The criteria** are then *stratified* by grouping according to their similarity (i.e. homologues, synonyms and antonyms) and by doing this a qualitative analysis of information is possible.
- **Determination of importance of criteria** using relative frequencies and Principal Component Analysis (PCA) for the different forage technologies evaluated (i.e. grasses, herbaceous legumes, shrub legumes and green manures).
- **Final analysis and interpretation** of data to define technologies responding to farmers demands and consequently that are adoptable

Combined analysis including all forage technologies offered to farmers

Utilizing data from Honduras, a crosstabulation of frequencies with all forage technologies included (i.e. grasses, shrubs, herbaceous legumes, and green manures) indicated that, plant color was the by far most important criteria in the assessment made by farmers. Across seasons this parameter was given more importance in the dry season as an indicator of the ability of the plants to stay green and retain its leaves. Plant growth was the next important criteria, followed by cover, leafiness, competitiveness and production. In contrast to color all these parameters had a higher importance in the wet season.

Color was the most important criteria in all forage technologies. However, growth, especially in the establishment phase, was a more important criteria in grasses and shrub legumes, while cover was more important for herbaceous legumes and cover crops. Equally important for herbaceous legumes and cover crops were competitiveness, growth, leafiness and ability to function as green manure. For shrub legumes, possible use as (fuel), wood was another important criteria.

In conclusion, farmers selected forages based mainly on drought tolerance, ease/success of establishment and yield. Drought tolerance was the most important criteria indicating the demand and potential for adoption of dry season forage species.

All criteria were also analyzed using Principal Component Analysis. In the global analysis across technologies for the wet season, the first 3 PC (Principal Components) explained 64% of the variation, which is a high percentage when analyzing participatory work. In Table 80 Eigenvectors for the different criteria in selection of forage technologies in the wet season are shown. PC 1 is defined by criteria for establishment and stability/persistence, [i.e. CRCOB = cover, CRCOM = competivity, CRPROD = production (DM yield) and CRCREC = growth (biomass, vigor and spread - mainly establishment phase]. PC 2 is defined by criteria for drought tolerance, production and stability, [i.e. CRCOLOR = color (healthy plant, in dry season as well drought tolerance), CRFOLL = foliage (quantity of leaves), CRMADER = wood, CREPROD = capacity for reproduction (seeds, stolons), CRCAPREB = regrowth capacity]. PC 3 are related to the foliage of the plant, [i.e. CRMACO = vigor and spread, CRABÓN = green manure effect, and CRHOJ= leaf growth].

The analysis of dry season data across forage technologies show a similar level of confidence, with the first three PCS explaining 66% of the variation. PC 1 is defined by CRCREC = growth, CRCOLOR =

color, CRCOM = competitivity and CRSUA = tactile softness, an indicator for palatability of the forage. PC 2 is defined by CRCOB = cover (.59) and PC 3 by CRABON = green manure effect, CRPROD = production and CRFOLL = foliage.

In the dry season less criteria are related to the selection of forage technologies by farmers, possibly as a reflection of the major importance of few parameters, responding to particular constraints for farmers in this time of the year (Table 81).

Principal Component Analysis

Eigenvectors

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CRABON	0.061960	0.279153	**-.454800**	-.005722	0.437197	0.109790	-.118927
CRCAPREB	-.350573	**0.366230**	0.014438	0.066499	0.033664	0.395176	0.096861
CRCOB	**0.418509**	-.027395	-.251376	0.315134	-.298388	-.041494	0.085038
CRCHOJ	0.225885	-.109230	**0.410788**	0.467462	0.238581	0.179566	0.009926
CRCOLOR	0.107306	**0.447170**	-.162064	0.126096	0.014569	0.055664	0.479595
CRCOMP	**0.392438**	0.108686	-.284225	0.204823	-.090126	-.324029	0.166495
CRCREC	**0.305984**	0.215723	0.270761	0.020026	-.321944	0.605767	0.041836
CRMACO	0.280763	0.060545	**0.464713**	-.080321	0.244347	-.157474	-.011671
CRPROD	**0.340937**	0.144272	-.205053	**-.428305**	-.062630	0.259181	-.510341
CRFOLL	0.023195	**0.439528**	0.124819	0.251230	0.430591	-.184588	-.327455
CRMADER	-.299658	**0.391215**	0.123153	-.126343	-.225894	-.179175	0.213021
CREPROD	0.122870	**0.378982**	0.285554	-.182545	-.348504	-.405728	-.210650
CRSUAV	0.295706	-.051157	0.101772	**-.563172**	0.358144	0.002243	0.502097

Table 80. Principal Component (PC) Analysis of criteria for selecting forage technologies by farmers, using results from Honduras. (Data for wet season).

Principal Component Analysis

Eigenvectors

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CRABON	-.007256	0.343933	**0.558121**	0.324481	0.155223	-.104893	0.399541
CRCOB	0.143834	**0.598185**	-.109366	0.240832	-.099309	0.082254	-.033382
CRCHOJ	-.138005	-.241400	0.255903	0.708498	-.288388	0.237784	-.403995
CRCOLOR	**0.374681**	-.093888	0.020924	-.021589	-.266911	0.371303	0.505659
CRCOMP	**0.358349**	0.257920	-.267953	0.224088	0.155321	0.341760	-.025788
CRCREC	**0.391641**	-.197591	0.065073	-.029910	-.309856	-.020711	0.040658
CRMACO	0.333661	-.259232	-.174473	0.092453	0.365513	0.261877	0.022456
CRPROD	0.294268	-.128644	0.087559	0.247617	0.612922	-.394374	-.047090
CRFOLL	0.337422	-.314955	**0.351235**	-.133257	-.001917	0.031861	-.247685
CRMADER	-.313888	-.253537	0.290909	-.020670	0.175475	0.280416	0.443191
CREPROD	0.062738	0.306696	**0.466293**	-.431546	0.198599	0.419694	-.395960
CRSUAV	**0.351841**	0.099703	0.266556	-.094279	-.334647	-.436685	0.027560

Table 81. Principal Component (PC) Analysis of criteria for selecting forage technologies by farmers, using reults from Honduras. (Data for dry season).

In Table 82 the most important criteria of farmers for the selection of forages, disaggragated for the wet and dry season. For farmers (in Honduras) it is most important that regardless of season forages have a good establishment and growth and compete effectively with weeds. In the wet season good cover, regrowth capacity and reproduction are additional criteria of high importance. In the dry season the outstanding criteria for forage selection by farmers is drought tolerance, expressed in color as an indicator for staying green and retaining leaves. Additional parameters of high importance to farmers are softness of leaves, likely related to palatability, and reproductive capacity. Specific criteria for applying to particular forage technologies are wood production (shrub legumes) and green manure effect (green manures).

Table 82. Summary table for most important criteria for selection of forages by farmers in the wet and dry season (Honduras). The most important criteria are highlighted in bold.

PC[1] (criteria)	Wet Season	Dry season
1	**Cover** **Competitivity** Production **Growth**	**Growth** **Color** **Competitivity** Softness of leaves
2	**Color** **Foliage** Wood production **Reproductive capacity (Persistence)** Regrowth capacity	**Cover**
3	Establishment, vigor and spread **Green manure** Growth of leaves	**Green manure** **Reproductive capacity (Persistence)** **Foliage**

[1]Principal component criteria.

Utilizing the same sequence described above, a separate analysis for each forage technology was executed and is summarized below.

Analysis disaggragated according to specific forage technologies (i.e. grasses, shrub legumes, herbaceous legumes and cover crops/green manures)

In Table 83 we present a summary that describes the most important criteria used by farmers for the selection of forages and the technologies identified based on these criteria. In the wet season color, competitiveness, foliage and cover were defined in PC 1, while leafiness and reproductive capacity were defined in PC 2. In the dry season competitiveness with weeds was related to PC 1, foliage, establishment/vigor/spread, cover and softness related to PC 2 and color and growth to PC 3.

It is anticipated that the selected technologies – from the basket of options offered - respond best to farmers' demands and hence have the highest likelihood of acceptance and adoption. The selection criteria vary slightly according to season and these differences need to be taken into consideration when defining perennial options. Over seasons *Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato were the grasses that best responded to farmers criteria, the latter having a particular high acceptance in the dry season. Other options considered by farmers are *Brachiaria dictyoneura* and for wet season performance, *Panicum maximum* cv. Camerún.

Table 84 shows results for herbaceous legumes. In this case 2 PCs explained 93% and 87% of variation in the wet and dry seasons, respectively. In this context, *Centrosema pubescens* CIAT 15160 had the highest overall attributes related to farmer's criteria. *Centrosema plumieri* and *Desmodium ovalifolium* CIAT 33058 were options that best responded to dry season criteria. For the latter it needs to be stressed that farmer criteria were based on visual and tactile criteria, and quality of *D. ovalifolium* may be negatively affected by drought conditions. *Stylosanthes guianensis* CIAT 11844 and *Arachis pintoi* CIAT 22160 were identified as potential options for use in the wet season, with *A. pintoi* being able to survive the dry season and regrow very quickly once rains commence.

Table 83. Summary table for most important criteria for selection of grasses by farmers in the wet and dry season and identification of technologies with high potential acceptance based on these criteria (Honduras).

	Grass species		Grass species
PC (criteria)	Wet season	PC (criteria)	Dry season
1		1	
Color	Bb 26110 "Toledo"	Competitivity	Bb 26110 "Toledo"
Competitivity			
Foliage	Pm Camerún		Bh 36061 "Mulato"
Cover			
2		2	
Leafiness	Bd 6133	Foliage	Bb 26110 "Toledo"
		Establishment/vigor/spread	
Reproductive capacity	Bb 16322	Cover	Bd 6133
		Softness (Palatability	
	Pm Camerún		Pm 16031
3		3	
Establishment/vigor/spread	Bb 26110 "Toledo"	Color	Bh 36061 "Mulato"
		Growth	
Growth	Bh 36061 "Mulato"		Ag (local check)

Bb- *Brachiaria brizantha*; Pm – *Panicum maximum*; Bd – *Brachiaria dictyoneura*; Bh – *Brachiaria* hybrid; Ag – *Andropogon gayanus*

Table 84. Summary table for most important criteria for selection of herbaceous legumes by farmers in the wet and dry season and identification of technologies with high potential acceptance based on these criteria (Honduras).

	Legume species		Legume species
PC (criteria)	Wet season	PC (criteria)	Dry season
1		1	
Color	Cp 15160	Color	Cp 15160
Competitivity	Sg 11844	Competitivit	Do 33058
Green manure		y	
Cover		Growth	
Foliage			
2		2	CP DICTA
Growth	Ap 22160	Cover	
		Production	

Cp - *Centrosema pubescens*; Sg - *Stylosanthes guianensis*; Ap – *Arachis pintoi;* Do – *Desmodium heterocarpon* sp. *ovalifolium*; CP – *Centrosema plumieri*

Results for shrub legumes are presented in Table 85. Three PCs explained 96% and 74% of variation in the wet and dry season, respectively. The most preferred species across seasons were *Leucaena leucocephala* CIAT 17263 and *Cratylia argentea* CIAT 18668. *Leucaena macrophylla* had appeal for farmers in the dry season and *Calliandra calothyrsus* CIAT 22316 was the most preferred accession.

In the dry season *C. calothyrsus* CIAT 22316 and *C. argentea* CIAT 18668 were additional options preferred by farmers.

Table 85. Summary table for most important criteria for selection of shrub legumes by farmers in the wet and dry season and identification of technologies with high potential acceptance based on these criteria (Honduras).

	Shrub Legume		Shrub Legume
PC (criteria)	Wet season	PC (criteria)	Dry season
1 Color Growth Regrowth capacity	 Ll 17263	1 Foliage Color Wood	 Ca 18668
2 Green manure Foliage	 Cc 22310 Ca 18668	2 Growth	Ll 17263 Lm 47-85
3 Wood	Cc 22310 Cc 22316	3 Wood	Ca 18668 Cc 22316

Ll – *Leucaena leucocephala*; Cc – *Calliandra calothyrsus*; Ca – *Cratylia argentea*; Lm – *Leucaena macrophylla*

In Table 86 we show the most accepted cover/green manure legumes based on farmer criteria. Three PRINs explained 94% of variation in the wet season. Due the annual nature of most species, dry season results are not available. *Mucuna pruriens* was the accessions that best marched to farmer criteria followed by *Lablab purpureus* and *Pueraria phaseoloides*.

Table 86. Summary table for most important criteria for selection of cover/green manure legumes by farmers in the wet season and identification of technologies with high potential acceptance based on these criteria (Honduras).

	Legume species
PC (criteria)	Wet season
1 Production Color Green Manure	1 Mp IITA BENIN
2 Foliage Growth Competitivity	2 Mp IITA BENIN Lp DICTA Pp 7182
3 Cover	3 Mp IITA BENIN Pp 7182 Lp DICTA

Mp - *Mucuna pruriens*; Lp – *Lablab purpureus*; Pp – *Pueraria phaseoloides*

In Table 87 profiles for each technology (grasses, herbacesous legumes, shrub legumes, cover/green manure legumes) based on the criteria for selection of the most promising species and accessions are presented.

Table 87. Profiles based on farmer criteria of forage species/accessions with the highest potential of acceptance

Forage Technology	Criteria Wet season	Criteria Dry season
Grasses		
"Toledo"	color, competitivity, cover, foliage, leafiness, reproductive capacity, establishment/vigor/spread	competitivity, establishment/ vigor/spread, cover, softness, color, growth
"Mulato"		establishment/ vigor/spread, cover, softness, color, growth
Herbaceous legumes		
Cp 15160	color, competitivity, green manure, cover, foliage	color, competitivity, growth
Shrub legumes		
Ll 17263 Cc 22310 Cc 22316 Ca 18668	color, growth, regrowth capacity	foliage, color, wood
Cover/green manure legumes		
Mp IITA BENIN Lp DICTA Pp 7182	production, color, green manure, foliage, growth, competitivity, cover	

With the development of a sequence for statistical analysis a participatory methodology for selection of forages was completed. Based on farmer criteria, it is now possible to define profiles for forage technologies with high likelihood of acceptance by farmers and identify forage technologies that match to farmer criteria. The sequence for analysis will now be validated in other sites. Comparisons between scientists knowledge and farmers' knowledge will be carried out to define commonalities and discrepancies.

Scaling of forage options in Hillsides of Honduras and Nicaragua

In Table 88 we show results from early uptake of forage options in the CIAT reference sites in Honduras and Nicaragua. In Honduras now about 170 farmers are testing and adopting forage options on their farms. Taking into account an estimated 500 to 600 livestock keepers in the area (H. Cruz, pers. comm) this equals 28% to 34% the total. The preferred forages so far have been *B. brizantha* CIAT 26110 (cv. Toledo), *Brachiaria* hybrid Mulato and *Cratylia argentea*. In addition, work is extending to the higher altitudes of Yorito, with an additional 150 farmers involved in testing forage options (see 4.2.1).

In Nicaragua uptake by farmers has also began with *B. brizantha* CIAT 26110 (cv. Toledo) and *Brachiaria* hybrid Mulato being the options preferred by farmers.

In addition, in collaboration with farmers, 12.5 ha of *Brachiaria* hybrid Mulato were established between July and October 2001 in different regions of Nicaragua, including Chinandega, Poneloya – León, La Patriota – Matiguás, Paiwitas - Matiguás Las Limas – Rio Blanco and Santo Tomás –

Chontales. In Matiguás, half of the area, (i.e. 1.75 ha) was established in association with *Arachis pintoi*.

Table 88. Distribution of forage materials in Honduras and Nicaragua (2002).

	Honduras								Nicaragua	
	June 2001		Update December 2001		January – August 2002		Total		2002	
Material	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)
A. gayanus 621	0.24	5	0.00	1			0.24	6		
B. dictyoneura 6133	0.12	8	0.19	0	0.25	5	0.56	12		
B. decumbens									0.16	4
B. brizantha 26110	0.56	17	8.37	14	8.47	13	17.40	44	1.48	37
B. hibrido 36061			12.30	6			12.30	6	1.76	44
P. maximum 16031	0.64	11			0.20	5	0.84	17		
P. purpureum cv. Camerún	0.64	16	0.27	0	0.24	6	1.15	19		
A.pintoi 22160	0.04	1					0.04	1		
C. argentea 18668	0.50	12	1.73	11	2.10	16	4.33	39		
C. pubescens 15160	0.04	2			0.02	1	0.06	2		
L. leucocephala 17263	0.55	9			0.15	3	0.70	11		
L. purpureus	0.10	1					0.10	1		
S. guianensis 184	0.12	5			0.10	5	0.22	6		
M. pruriens IITA BENIN	0.10	5					0.10	1		
V. unguiculata (Verde Brasil)			0.14	2			0.14	2		
Total	3.7	92	23.0	34	11.5	54	38.2	167	3.4	85

4.2.3 On-farm forage seed production in Nicaragua and Honduras

Contributors: A. Schmidt (CIAT), A. Blandon (INTA), M. Mena (INTA), M. I. Posas (SERTEDESO), G. Giraldo (CIAT), C. Burgos (DICTA), C. Davies (CIAT), H. Cruz (CIAT), L.H. Franco (CIAT), P. Argel (CIAT) and M. Peters (CIAT)

Growing interest in *Cratylia argentea* and *Brachiaria brizantha* cv. Toledo within farmer groups at the CIAT references sites in San Dionisio, Nicaragua and Yorito, Honduras led to an increasing demand for forage seed. Since market seed prices of *Brachiaria brizantha* cv. Toledo (in Nicaragua 32 US$/kg) are well beyond the economic ability of farmers and/or are scarce or not available at the market, farmers decided to initiate their own seed production schemes for *B. brizantha* cv. Toledo and Cratylia. The objective is to increase seed availability for themselves, the communities and the national market. Furthermore, results from these production efforts will complement our knowledge about the seed production potential of the two species under the prevailing environmental conditions.

For training purposes, especially for harvest/post-harvest and for marketing, the farmer seed production group was connected to the recently formed Smallholder Seed Production Enterprise (PES, it’s acronym in Spanish), which has the objective to produce high-quality seed of beans and maize varieties and has access to seed processing equipment for post-harvest handling.

In Nicaragua, seed produced by the forage Seed Farmer Group will either be sold directly or to the local seed bank managed by the local farmer organization Campos Verdes, which then will sell the seed. A similar scheme exists in Yorito, Honduras where seed is either directly marketed through an NGO, SERTEDESO. Schemes to involve ‘Cajas rurales’ are also explored.

In Nicaragua, in the case of *Cratylia argentea*, a total of 7 farmers dedicated small areas of land to the planting of the shrub legume. Together with a small area at the SOL site, the total area for seed production of *C. argentea* adds up to 3.5 ha (Photo 25). Establishment began at the end of June 2002 and farmers were provided with seed, fertilizer and written technical information. Seed and fertilizer will be returned to the project upon harvest in order to facilitate more farmers with seed. Farmers are given technical backstopping. In collaboration with INTA-Matagalpa a group of farmers at Pantasma-Jinotega picked up the production of *B. brizantha* cv. Toledo. This group has experience with the production of *B. brizantha* cv. Marandú. In Nicaragua, first results will be available early next year. Further effort in seed production will be included in the FONDEAGRO project (see section 3.4.8 of this AR) and additional farmers throughout Nicaragua. Recently work began in collaboration with INTA on the formulation of regulations regarding forage seed production, import, sale and quality issues.

There is a need for an integral strategy for seed production within Central America in order to meet farmer demands for high quality seed of legumes and grasses. Different approaches might be developed for different species. Vegetative propagation should be taken into account in early stages. However, it remains to be seen if production levels in grasses will allow for competitive prices on the seed market. In contrast, legume seed seem to enter a non competitive market offering good opportunities.

Photo 25. *Cratylia argentea* seedlings transplanted for seed production at San Dionisio

In Honduras we are also promoting seed production by farmers in close collaboration with DICTA. In Table 89 we show costs for the production of *B* brizantha cv. Toledo seed in Yorito, Honduras. Though the initial investment is relatively high with 300 USD/manzana (mz; 1 mz about 0.7 ha) the potential net return with 615 USD/mz is also high. Since it is 10 times greater than the net return with maize (61 USD/mz) (G. Giraldo, per. comm.). In consequence farmers have embarked in seed production of cv. Toledo, with about 400 kg of seed produced by farmers expected in 2002. In view of the high profitability of forage seed production, farmers also are diversifying with the production of *Cratylia argentea* seed.

Table 89. Production costs for Toledo seed in Yorito Honduras

Activity	Cost (1 USD = 16.4 Lps)	
	Lps	USD
1. Land preparation (labor, supplies, machinery)	1520	93
2. Planting (labor, supplies, machinery)	1180	72
3. Maintenance during establishment (weeding, fertilizer, labor)	808	49
4. Seed harvest (labor, bags)	1058	65
5. Postharvest (Drying, cleaning, labor, packing)	355	22
Total cost of production	4921	301
Reserve (10%) for unforeseen events	492	30
Total	5413	331
Cost of rent of land	500	30
Grand Total	5913	361
Brut return = kg clean seed produced * price/kg = 80 x 200 = 16,000 Lempiras (= USD 976)		
Net return = Brut return – Total cost = 16,000 - 5,912.5 = 10,087.5 Lempiras (USD 615)		
Cost per kg seed = Total cost/kg clean seed produced = 5913/80 = 74 Lempiras/kg (= 4.5 USD/kg)		

4.2.4 On-farm evaluation of green manures in hillsides of Nicaragua

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Rationale

Farmers are becoming ver concerned with the increasing prices of external inputs. At the same time soil fertility in farmer fields is decreasing and weeds are becoming a larger problem over time. In order to overcome these limitations and backed up by CIAT, the local farmer organization "Campos Verdes" initiated a project to introduce, evaluate and promote the use of cover crops and green manures (CCGM) in the communities of San Dionisio in 2001. CCGM legumes may significantly contribute to enhanced soil fertility, water and soil conservation and weed suppression. Some of these green manure crops show high drought tolerance and can be used as forage or even for human consumption. Growing CCGMs may result in a smaller amount of applied agrochemicals, which are already contaminating the scarce water resources of the people in San Dionisio.

While plant adaptation/management and technical feasibility are important factors, economic viability is considered decisive for adoption. Therefore, cost-benefit analyses are one of the main objectives of the project in order to compare the current management including N-fertilizer and agrochemical application with the use of cover crops. Further objectives are to demonstrate the multiple uses offered by CCGM including drought tolerance, and their management within the local community.

Materials and methods

A workshop was held in San Dionisio in April 2001 to which all members of Campos Verdes had been invited. A total of 27 farmers assisted the event and the proposed project was presented and discussed. Sites with different soil and climate conditions throughout San Dionisio were identified and CCGM options discussed. Farmers selected *Mucuna pruriens*, *Canavalia ensiformis* and *Lablab purpureus* as CCGMs for the experiment. At the end of September 2001 the experiments were established on 8 farms in different communities of San Dionisio. The experiments consisted of seven treatments, which were arranged in a randomized block design with 3 replicates at each site. The treatments are summarized in Table 90. CCGM legumes were sown in maize plots (4 x 4 m) at the traditional bean sowing distance (0.4 x 0.4 m).

Table 90. Treatments included in on-farm cover crop/green manure experiments in San Dionisio, Nicaragua.

Treatment	Year 2001*	Year 2002
1	Maize	Maize without N-fertilizer (Control)
2	Maize	Maize with low N-fertilizer level
3	Maize	Maize with high N-fertilizer level
4	Maize	Maize with very high N-fertilizer level
5	Maize with *Mucuna*	Maize without N-fertilizer
6	Maize with *Canavalia*	Maize without N-fertilizer
7	Maize with *Lablab*	Maize without N-fertilizer

* Cover crops/green manures were sown into existing maize plots in September when the maize was entering its mature stage.

In September 2002 a German student from the University of Hohenheim initiated a survey on local soil organic matter management techniques in farms of key collaborators in San Dionisio. Farmers using any kind of practice to improve soil organic matter contents are interviewed and their experiences and techniques documented. In collaboration with the PE-2/TSBF –Project additional information on farm nutrient balances will also be collected. A workshop in December 2002 among all informants will try to identify local organic matter indicators for a selected number of materials used in San Dionisio.

Results and Discussion

The absence of rainfall at the beginning of October 2001 affected the establishment and biomass production of the CCGMs in all sites which resulted in reduced fertility effects on the subsequent maize crop. The effect was minor at higher-altitude sites such as Ocote arriba and Carrizal and the Susuli sites on the more humid eastern zone of San Dionisio.

As a result of what happened in 2001, this year three *Vigna unguiculata* accessions (cowpea) (identified as promising for their biomass production potential under drought conditions in San Dionisio in 2001) were chosen to replace the selected green manures species at sites where biomass production was insufficient in order to have an influence on subsequent maize crops. The cowpea accessions where sown in early June 2002 and mulched after 2 months before maize plots were established. At all other sites maize was established at the end of May.

Table 91 shows plant height, soil cover and biomass production of all green manure species prior to the maize crop. Maize harvest will take place in November and data will be available for the final workshop in December 2002 and for further promotion and dissemination of CCGM legume species. Given the high interest of farmers in San Dionisio, it is expected that a growing number of farmers in San Dionisio will adopt CCGMs technologies for growing maize in the future and will show further interest in other legume species. The farmer association “Campos Verdes” in collaboration with CIAT will be glad to support this task. The first step was the creation of a seed fund for green manure seed managed by Campos verdes. Five farmers applied already for the fund and were supplied with Mucuna and Lablab seed this year.

Table 91. Plant height, soil cover and biomass production of green manure species prior to maize crops at San Dionisio (15 weeks after establishment)

Location	**Species**	Plant height **(cm)**	Soil cover **(%)**	Biomass **(g/m^2)**
Ocote arriba	Mucuna pruriens	30	100	456
	Canavalia ensiformis	55	100	325
Piedra colorada	*Mucuna pruriens*	30	75	178
	Canavalia ensiformis	55	40	68
	Lablab purpureus	55	60	103
Susuli arriba	*Mucuna pruriens*	42	100	388
	Canavalia ensiformis	67	100	299
Carrizal	*Mucuna pruriens*	40	100	255
	Canavalia ensiformis	57	75	108
	Lablab purpureus	15	40	89
Corozo	*Mucuna pruriens*	25	50	122
	Canavalia ensiformis	40	60	77
	Lablab purpureus	12	40	60
Jicaro*	IITA 637-1	41	80	389
	IITA 284-2	39	60	175
	IITA 719	36	50	194
Piedras largas*	IITA 637-1	51	80	299
	IITA 284-2	44	100	234
	IITA 719	46	80	253

*Data recorded 9 weeks after establishment

4.2.5 Evaluation of *Cratylia argentea* as live barrier to control erosion and as a source of feed in Costa Rica

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Rationale

In the Central Sur Region of Costa Rica smallholders dominate agricultural production. Farms are characterized by short heavy rains, a pronounced dry season and steep slopes. Soils are of low fertility and depth, vegetational cover is low and utilization of soil conservation practices is limited. Hence soils are highly vulnerable to degradation. Double purpose cattle on pastures is the dominant activity, but the prolonged dry season and degradation of soils limit productivity. To respond to these constraints, to increase income and recuperate areas through reforestation, small and medium sized livestock farmers often keep their cattle in confinment or there is a combination of grazing and confinment mixtures of grazing and stables. In such systems, livestock diet are based on sugar cane and king grass, pastures and supplements such as chicken manure and molasses. Productivity per area is much higher than under constant grazing. However the highest cost in such systems is supplementation with chicken manure, contributing to as much as 50% of the overall cost of feeding. Under these circumstances *Cratylia argentea* has been identified as a suitable option for dry season supplementation (substituting chicken manure), leading to higher environmental and economic sustainability of these production systems. *C. argentea* may also have potential as a live barrier to control erosion thus further improving sustainability. As little is known about the suitability of *Cratylia* as a live barrier on steep slopes, this on-farm experiment is conducted with the following objectives: (a) Measure erosion with and without *Cratylia* live barriers, and (b) Evaluate forage production from *Cratylia* live barriers.

Materials and Methods

Two livestock farms were selected in Acosta and Bocana, respectively in Puriscal.

Two planting arrangements of *Cratylia* live barriers are compared, rectangular and triangular, with two planting densities.

Treatment 1:	triangular, planting density 1 * 1 m, between plants inside rows and between rows, respectively
Treatment 2:	triangular, planting density, 0.5 * 1 m, between plants insides rows and between, respectively
Treatment 3:	rectangular, planting density 1 * 1 m, between plants inside rows and between rows, respectively
Treatment 4:	rectangular, planting density, 0.5 * 1 m, between plants insides rows and between, respectively
Treatment 5:	Control (without barrier)

Two replications per treatment are used, with three live barriers per treatment per repetition. Each live barrier has three rows and a longitude of 30 m. Distance between barriers is 12m. In between barriers *Brachiaria brizantha* cv. Toledo is established.

In addition, to the live barriers about 0.5 ha of erect grasses such as *B.* brizantha cv. Toledo and areas for vegetative propagation/seed production of grasses of prostrate growth habit such as *Brachiaria decumbens* were established, with the aim to compare effects on soil conservation with and without *Cratylia* barriers.

The following variables will be measured: Nutrient content of soil; Organic Matter content; Soil density; Erosion; DM yield of *Cratylia argentea*; and liveweight gain for animals supplemented with *Cratylia agentea.*

Results from this study which involves two farmer organizations, (the Asociación de Productores de Bocana de Puriscal-21 farms, and the Asociación de Bajo de Jorco de Acosta-30 farms) will be reported next year.

4.2.6 Studies on indigenous fodder trees and shrubs in N. Vietnam

Contributors: R. Roothaert and T.T.T. Thuy

Rationale

The 'Forages for Smallholders Project' has been working with farmers in Tuyen Quang since 1998. Although the focus of the project is to improve feed resources for livestock through improved forage species, it has been observed that farmers use a wide range of local tree and shrub species to feed their livestock. Many of these shrubs and trees grow in forest areas that have been allocated to individual farmers. The contribution that this local feed makes to the animal's diet is not clear, neither is the nutritive value of these species. From studies in Kenya and Nepal, however, it was shown that farmers have good knowledge about these local feed resources. Knowing more about the situation in Vietnam would help researchers and development workers to better advise farmers on complementary feeding practices.

The objectives are:

- To assess the use of local woody species that farmers use to feed their cattle and buffaloes.
- To assess the contribution of this fodder compared to other feed resources.
- To assess the nutritive value of the most commonly used fodder trees and shrubs.

Materials and Methods

Discussions were held with two groups of farmers in Song Duong and Yen Son districts. Farmers were invited who had experience with cattle rearing. Questions were asked about general agricultural activities, livestock problems, feed resources, exotic and indigenous fodder trees on farm. A preliminary report is presented here.

The study will continue with a formal survey in two communes. Botanical samples of local tree species will be collected for identification. Feed samples will be collected for nutritive analysis. The samples will be air dried and analysed in a laboratory in Hanoi

Results and discussions

According to the farmers, most of land in Tuyen Quang is privately owned farmland; they are allowed to use their farm for a long period, 50 years or more. The land usually consists of:

- Forest land, which includes natural and man-grown forests.
- Cultivated lands, mainly for food crops and fruit trees. Some is used for forage trees on hills and alluvial lands.
- Fallow land, small proportion, for cattle grazing in the forest and the hills.

The main income of the households in the districts comes from rice, maize, sweet potatoes, cassava, vegetables, fruit trees, tea and coffee. Livestock keeping generates a smaller part of the income. Animals kept include cattle, buffaloes, pigs, poultry and fish. Cattle are mainly for traction, ploughing fields, and production of organic fertilizer for the crops. Herding livestock is considered a good occupation for elderly people and children, who cannot do hard farm work. This might jeopardise children's chances to attend school though. Grazing and tethering are the main systems, although a few farmers practice stall feeding.

Fodder for cattle and buffaloes in Tuyen Quang consists of natural grasses and shrubs, exotic trees, and crop by- products. Farmers said that there was enough food for the animals in spring, summer and fall (rainy season). During the rainy season, the main fodder for their animals are natural trees. They always lack feed for cattle in winter. The way of solving this problem is different in each zone. For example, in Yen Son District, where the farmers grow a lot of exotic trees, winter diet includes natural grasses, exotic trees and by-products of crops. In Son Duong farmers feed natural grasses, sweet potatoes, cassavas and by-crop products during winter.

According to the local people, many exotic fodder trees and shrubs species have been recommended and planted in trial fields, but few species were adopted by farmers. Households with few members are interested in cultivating fodder trees, as they don't have labour for herding or tethering in the mountains. They grow exotic trees in the small pieces of land around the home compound, fallow land or free fields between main crops. Characteristics of exotic fodder trees that are appreciated are:

- High green yields.
- Growth through the year (especially in the winter).
- Good growth and regrowth.
- Palatability for cattle.
- Suitable to others animals, e.g.fish.

Since the amounts available are very little, it is only fed strategically; young animals for fattening, pregnant animals and ill cattle benefit from it. Farmers are looking for seeds to plant more, and want more information.

Farmers told that there is an abundance of local fodder trees and shrubs species in Tuyen Quang. All species have local names. The trees establish naturally, and are found in many places such as mountains, forests, hills, alluvial land, and scattered within crops. They are often fully developed in the rainy seasons (spring, summer and fall), and some are available in winter as well . The big trees can be propagated by seeds and roots, but the shrubs mostly by cuttings.

4.2.7 Socio-economic evaluation of forage technologies in Philippines and Vietnam

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Rationale

The Forages for Smallholders Project – Phase II (FSP–II), started in January 2000. The Asian Development Bank has committed funds to the project for a period of three years. The goal of FSP was to improve the livelihood of resource-poor farmers in upland farming systems in Asia by: developing sustainable forage technologies; increasing livestock production; conserving soil; and enhancing nutrient management.

Since the adoption of new forage species, farmers had expanded their use to contour lines or cover crops and new livestock production systems had developed. However, no reliable data on the socio-economic benefits of the forages in the Philippines and Vietnam, were available. The goals of the study were to assess the financial and social benefits of forage technologies developed through participatory research with the Forages for Smallholders Project.

Background

Philippines. The introduction of new forage species selected by CIAT in Mindanao, began at the end of 1995 with participatory diagnostics and experiments. Demonstration plots were established in Malitbog. Initially only fodder banks were established. The municipality of Malitbog was located in old forest concessions, clear-felled by loggers between 1960 and 1980. Soils were poor and acidified quickly after slashing and burning. Cagayan de Oro City included a vast rural area comprising several communities and the density of settlement was much higher. In both provinces farmers sometimes cropped land with slopes steeper than 50%, very susceptible to soil and water erosion. In Cagayan de Oro, most farmers used new forages through cut and carry from fodder banks while other farmers established pastures, border lines and contour lines. In Malitbog, the establishment of contour lines became more frequent after the pilot project (1992-1994).

Vietnam. The introduction of new forage species selected by CIAT in Tuyen Quang Province started in 1997, with participatory diagnostics and experiments with 12 species by the Vietnam Sweden Mountain Rural Development Programme. Demonstration plots and farmer experiments were established in 6 communes, amongst which were: Phu Lam; My Bang in the district of Yen Son; and Duc Ninh in the district of Ham Yen. These 3 communes continued experimentation in 1998 and in 1999 the program extended to their district level and to 3 other districts. The first stage of FSP ended in 1999 and FSP–II financed continuation of the forage research activities.

Materials and methods

Philippines. The study was carried out on the island of Mindanao, the Philippines, from April 25 to May 22, 2002. As the numbers of households to be interviewed and herd sizes were small, the inputs and outputs of the entire herd, including ruminants, horses and swine, were considered. To assess the financial benefits of new forages two groups were sampled: early adopters and recent or non-adopters. Several methods and tools (table 1) were used to collect the data in three stages: 1) meetings with large group of farmers; 2) farmer focus groups and 3) household surveys. All farmer groups were convened two times. Data on labour needs, finances, feeding practices and gender labour distribution were collected in individual households using structured interviews. Market and saving objectives and land issues were addressed during group meetings. Interest, insurance and inflation rates were collected from literature or key informants.

Social effects of new forage introduction were researched using semi-structured interviews in focus groups, and in structured interviews at household level. Semi-structured interviews with women's focus groups enabled the assessment of women’s opinions of the benefits of new forages. Information obtained from the women and from household surveys revealed the gender labour distribution. Household surveys were conducted in wealth-stratified sub-samples of the focus group participants. To assess the financial impact of new forage introduction, two groups of FSP participating farmers were interviewed: early and recent adopters. Goat owners that adopted forages before 2001, and cattle and buffaloes owners that adopted forages before 2000 were classed as early adopters. Interviews with early adopters lasted about one hour and for recent adopters the time needed was about half an hour.

In Malitbog, a total of 27 farm households were interviewed: 17 early adopters and 10 recent or non-adopters. In Cagayan de Oro, a total of 26 household surveys were conducted: 15 early adopters and 11 recent or non-adopters. All interviewed farmers used in the analysis own at least one herbivore (cattle, buffalo, horse or goat). Total production from livestock production systems includes: direct income from livestock; home-consumption of products; benefits from manure and labour on farmers' own fields; transport for own household; and benefits from insurance and financing of the animals. Direct income from the sales of trust animals was adjusted according to the share held. Evolution of the monetary value of livestock was taken into account.

According to the methods proposed for production systems with restricted markets for land, capital and labour, the costs of these three production factors are presented as returns from land, capital and labour, after calculation of the net value of output (Bosman *et al*, 1997). Total production minus total cost gives the net value of output from livestock production, i.e. the amount farm households consider as their income from their livestock production. Net value of output was separated in returns to land, capital and labour. Return to labour from the household was calculated as net value of output minus returns to land used for forage and fishpond and minus returns to capital value of the livestock.

Returns to land represents the cost of land, which in conventional financial methods is considered part of total cost. Similarly, return to capital represents capital cost. These variables are presented in the following formulas:

Net value of output =	total value output - total cost
Return from land =	area * market value * real interest rate
Return from livestock capital =	market value of livestock * interest rate on savings
Return from labour =	total value output – total cost – return from land – return from capital.

The cost of land for forage was not taken into account for recent adopters. For early adopters, only the parts planted with new forages were accounted for as returns from land. Daily household income from livestock production system was calculated by dividing return from labour by the total estimated number of workdays for livestock husbandry.

At the time of study, the exchange rate was 1 US$ = 50 Philippine Pesos.

Vietnam. This study was conducted in North Vietnam from May 25 to June 22, 2002. In Duc Ninh, a total of 30 farm households were interviewed: 20 early adopters and 10 recent adopters. In Phu Lam, 6 early adopters and 1 recent adopter were interviewed. Twenty household surveys from Thang Quan contributed to data collection for the sample of recent adopters. A total number of 14 households from Duc Ninh, Phu Lam and Thang Quan were interviewed specifically about their pig production system. The contribution of new forages to poverty reduction was estimated by comparing the income of the sample participants with the standard income for the community, available from local government services. The total income of farm households was calculated with the estimated contribution of livestock to livelihood. Ranking of the relative contribution of livestock to livelihood and the contribution of improved forages to general feed resources was carried out according to methods proposed by Cramb and Purcell (2000).

The time needed for fodder collection for ruminants and fish was recorded for both the dry and wet seasons: November to February and March to October, respectively. It was too complicated for farmers to distinguish shorter intervals for fish fodder collection. Moreover, farmers mostly cut fodder for fish and ruminants at the same time. A weighting using a total of 20 grains of seed for both wet and dry season, allowed farmers to repartition the time spent and fodder used for the 2 production systems. This method also allowed the allocation of the cost of land cropped with forages for the 2 systems. The value of concentrated feeds produced on-farm was accounted for at the market value estimated by the farmers. Other tools and methods were the same as the ones used for the Philippines. The exchange rate for 1 US$ was VND 15,000.

Results from the Philippines

Livelihood resources and activities in Cagayan de Oro. Among the interviewed farmers one was a tenant, one rented land and one used land over which someone else claimed ownership. All other farmers participating in FSP activities were landowners, most having profited from the Agrarian Reform. Wealth ranking during the group meetings proved difficult as farmers tended to want to define the ranking criteria in such a way that all farmers present were of the same class: in Tagnagni, “tabagonom”; in Pagalungan, “regular” and in Dansolihun, "pobre" (Table 92). Criteria such as ownership of appliances or means of transport were not functional. The poor only owned a radio and the middle class could only afford one appliance, which could be anything between a radio and a washing machine. The poor sometimes could not afford to pay for transport, while the middle class either had enough money to pay for transport, own a cart or even to buy a motorbike. Household size did not provide a clear distinction either, accept in the case of the rich who had less children, but they were already clearly distinguished by their level of education.

Table 92. Classes of wealth for three barangay, with estimated representation according to and essential criteria attributed by farmers in Cagayan de Oro.

	Rich	Normal	Poor
Tagpagni	hayahay: 10 %	arang arang: 20 %	tabagonom: 70 %
Pagalungan	dato: 5 %	regular: 80 %	pobre: 15 %
Dansolihun	hamogaway: 1%	pobre: 70 %	kabus / timawa : 29 %
Food	eats 3x /day plus snacks	eats 3x /day	eats 2x / day
Land	owns > 5 ha land	owns 1-5 ha	is tenant or landless
Education	has professional occupation	elementary school and sometimes college	only elementary school, or did not finish
Livestock	plenty cattle & buffaloes, but does not take care	cattle and/or buffaloes & owns poultry and pigs	owns poultry and pigs
Housing	in concrete	GI sheets & good lumber	indigenous materials
New forage adoption	not, as he does not take care of animals.	adopts forage	if landholder, sometimes for contour line and for sale.

The comparison of the relative importance of livestock for livelihood resources, between owners and non-owners of cattle or buffaloes, showed that keeping large ruminants affected the need to do non-farm activities such as off-farm labouring, firewood selling and small trading. In Lumbia, a barangay involved in FSP activities since 1996, livestock were estimated to contribute 37.5% of livelihood, close to the 35% estimated in Dansolihun, where FSP activity started in 2000. In Lumbia and Pagalungan, cattle and buffaloes contributed to consumption as they provided milk for the farm household. In Lumbia, off-farm labour or small trading was not a source of income, indicating that agriculture provided enough employment and income.

The effect of the particular livestock species on income was important. In Tagpagni, farmers without cattle or buffaloes only made 10% of their total livelihood from small livestock, while this figure increased to 24% for cattle or buffalo owners. The large livestock owners estimated that livestock (small and large) contributed 32% toward their total livelihood. In Pagalungan, the difference between owners of cattle or buffaloes and those having them in trust (*alima*) was almost as large at 30% and 12% of total livelihood coming from livestock, respectively. The differences in the contribution of livestock to livelihood between villages were probably related to goat, cattle and buffalo dispersal to the FSP program. In Dansolihun, there was goat dispersal and the farmers had profited from several years of a very flexile and elastic payment schedule from NGO credit programs for livestock. This explained the relatively high contribution of livestock to livelihood for this barangay where FSP activities were only recent. After cattle dispersal, Lumbia and Pagalungan profited from dairy dispersal programs from the National Dairy Authority and the Philippines Carabao Centre from the Department of Agriculture (DA). In Tagpagni, dispersal programs had only started recently, after the first FSP activities.

Participants in FSP program confirmed the relationship between the contribution of livestock to livelihood and the adoption of new forage. According to them, the majority of farmers not having ruminant livestock would not adopt forages, however, some participated in the FSP program with the hope of acquiring an animal through a dispersal program.

The large range of animals that profited from the new forages included: cattle, buffaloes, horses, pigs, goat, rabbit, guinea pigs and chickens. The most important expenses for livestock were concentrates (bran of corn or rice, or residues from root crops), minerals (salt), rope and troughs. Drugs and vitamins were provided and administered by government agents for free.

Income from livestock was used for several purposes. Most important were human medical cost and savings. The only investment mentioned was in small stores or trade. In Dansolihun village, some farmers still needed to buy food from their livestock income. Local alternative income resources were limited, but some temporary work in construction or driving was available to some. In nearby cities and in Manila, girls could easily obtain short and long term domestic jobs.

The market value of animals varied over the year. Buffaloes were expensive from August to December. The prices of other livestock were low from March to July, due to increased sales at the end and the start of the school year when farmers needed to pay school fees (school holidays were from May to June). Milk prices were constant over the year—20 Peso/kg for buffalo milk and 5 Peso/kg for cow's milk.

Amongst the interviewed early adopters, nine out of 15 farmers had fodder banks plus border lines or contour lines, three had only fodder bank, and three had only lines. Of the nine recent adopters, one had only a fodder bank, five had only contour or border lines and three had established both. Plots for grazing were rare.

Livelihood resources and activities in Malitbog. The inventory of the focus groups showed that they were almost entirely composed of people who owned or used cattle or buffaloes. The relative contribution of livestock to livelihood varied between the villages and in relation to the ownership of large ruminants. The contribution of off-farm labour, craft and small trading was more important for farm households without large ruminants. The comparison between owners and non-owners of cattle or buffaloes, of the relative importance of livestock for the livelihood resources, showed that keeping large ruminants affected the need to do non-farm activities. Also, income from smaller livestock increased when a household raised large ruminants. For those who had cattle or buffaloes, the relative contribution was close to 25%. This figure was much lower for non-owners of large ruminants, 15% only.

The figures were much higher than the 10% published by Cramb and Purcell (2001). They considered all livestock together and the partitioning of livestock into three categories in this study might have led to an overestimation. However, data from the benchmark survey show that the mean contribution was 13% for all farmers and 19% for those owning livestock. A check amongst the interviewed farmers present at the validation meeting again showed much lower figures. For further calculations, the figure of 15% was used for non-owners of large ruminants (including the poor as well as recent adopters) and for those with cattle or buffaloes, the mean contribution of livestock to livelihood was retained at 20%.

Cattle, buffaloes, horses, pigs, goats and poultry, all benefited from the new forages. Poultry, for home-consumption and marketing, grazed *Arachis Pintoi*. Goats were mainly for marketing. Pigs were home slaughtered and the meat not used for home-consumption was sold in the neighbourhood or exchanged for staple food. Cattle, buffaloes and horses were only sold when urgent needs arose or when they needed replacement. Buffalo milk was either home consumed or sold. Ruminants, horses and swine could be shared in *alima* (a traditional system whereby care of livestock is contracted to someone alse, and benefits from those animals are divided among owner and caretaker).

The most important expenditures for livestock were concentrates (bran of corn or rice, or residues from root crops), minerals (salt), rope and troughs. Drugs and vitamins were provided and administered by government agents for free. A large variety of crop and fruit residues, such as sweet potato and cassava leaves, banana trunk, rice and corn straw, and residues of coconut, pineapple and local sugarcane, were periodically fed to animals.

The income from livestock was used for several purposes. Of particular interest for the financial analysis were the human medical costs and the investment in motorbikes to do paid transport of people. Some farmers still needed to buy food from their livestock income. Land for agriculture was mostly bartered against large livestock. Local alternative income resources were restricted to farm jobs and handicrafts. In the nearby cities and in Manila, young females could easily obtain short and long term domestic jobs. Of the early adopters interviewed, 10 out of 17 farmers had fodder banks plus border or contour lines, 2 had only fodder banks, and 3 had only lines. At least one farmer used plots of *Paspalum* and *Setaria* for grazing.

Effects on production system and livelihood in the Philippines. Two types of grazing were distinguished: *tigway*, or tethering close to the homestead and *bakero*, herding mostly on distant range lands. *Bakero* was only practised in the short dry season. Before the introduction of new forages by FSP, tethering was the most important means of providing forage to animals. Some farmers cut and carried natural forage on days when rainfall limited the grazing time of animals. In Cagayan de Oro, some farmers already had some guinea grass and napier for this purpose.

According to the farmers, the increase in work for forage cropping and cutting was small compared to the decrease in herding time. The increased time required for forages occurred during specific times during the year, but time management prevented labour demand conflicts with other crops. The reduction or disappearance of herding on faraway pastures had social effects. In Cagayan de Oro, since the adoption of new forages the number of animals increased and thus time needed for livestock activities. As a result, total labour demands increased in stead of decreased.

In some villages, crop residues from rice and maize were harvested to feed animals, while in others tethered ruminants grazed on them. When forage was lacking in dry season, farmers chopped banana trunk and mixed it with various grasses that were available, or with salt. In Cagayan de Oro this practice was generally abandoned after the new forages were available in sufficient quantity.

Some farmers in Cagayan de Oro already cultivated forages like Napier and a local variety of *Panicum maximum*, for soil protection and animal feeding. Most of these farmers were early adopters. The first available varieties of Napier were steadily replaced by the dwarf variety that were more vigorous, needed less maintenance and produced more. Moreover, animals also consumed the stem of the dwarf Napier but refused the stems of the older varieties. The observations of one farmer were contrary to this; his cattle do not appreciate the new Napier as much as the old ones. Several farmers in Malitbog (San Migara) used a local variety of *Glyricidia* and other species that were consumed by their animals before the introduction of new forages.

Farmers mentioned the following as benefits of new forage introduction: improved body condition and overall health of animals (a shinier coat made this obvious); increased length and quality of work by draught animals; greater pig and poultry production; larger amounts of available manure due to reduced herding time and an increase in the number of animals; control of soil and water erosion; production of firewood; and increased income due to various factors, such as time saving through reduced herding and a reduction in the number of conflicts. Benefits of N-fixation was not always mentioned.

Some farmers that did not own any animals, planted new forages just to control erosion in contour lines. Farmers cropped maize, root crops and several high value vegetables between contour lines. Banana was sometimes integrated into the contour line and *Leuceana* was intercropped with cassava. The control of water erosion of soil and nutrients was estimated to increase crop production by 10 to 25%. The variation was due to the slope of the land—the steeper the land, the higher the gain.

In a village in Cagayan de Oro, areas planted with maize decreased, due to rat infestation and poor local soil fertility. Farmers estimated that they could make more money from dairy production and reduced the area planted with maize so that production just covered household needs. The exchange of the maize-planted areas for dairy production had positive economic trade-offs through farm diversification and the use of market for the exchange of goods.

Not all interviewed farmers in Malitbog collected manure. Some farmers practising zero grazing at their homestead, just swept the manure under their flowers where it washed away during the heavy rains. In Lake village, all farmers cropping vegetables applied manure. Without manure application the soil became infertile within two years. If farmers did not have manure from their own animals, they bought poultry manure.

The improvement in animal feed quality and quantity was very important. Before the introduction of new forages, little cutting and carrying was practised. They suffered from insufficient feed in the dry season, due to low quality, and in the rainy season, due to high water content and limited grazing time. Several species of livestock often fed upon *Arachis pintoi*, but most farmers used the new grasses for cutting and carrying. The majority of farmers that adopted new forages did not own cattle, buffaloes or goats previously. Most farmers acquired cattle through government dispersal systems or private trust (*alima*). Goats were generally paid for in cash, but several villages also had government goat dispersal systems. The effect of new forages upon the reproduction of cattle or buffaloes was impossible for these farmers to estimate, as they had no pre-forage comparison. Farmers observed increased numbers of offspring and shortened anoestrus after parturition: from 3 to 1 month in goats; 1 year to 3 months in cattle; and 10 to 4 month in buffaloes.

According to farmers in Malitbog, the growth of their swine increased when they fed them *Arachis pintoi* or *Setaria splendida.* In Cagayan de Oro farmers did not observe an increase in pig production but sometimes used easily accessible forage to keep the pigs calm or as a complement to the expensive concentrates. Before farmers increased their animal numbers, the new forage technologies saved them time. In a village in Malitbog, time spared was estimated at 2 hr/day during part of the dry season, and in general, farmers said life was more relaxed as it was easier to plan with the animals in zero-grazing. The reduction in time needed in the wet season was limited as the forage for cutting and carrying was a long way from the village. In other villages, farmers' compounds were more spread out, and time was saved all year round. In another village, the mean time spared was estimated at 30 min/day. This time was used for the extension of other farm activities, such as tending to vegetables and fishponds, off-farm trading, and roadside food selling. Farmers could also make themselves and an animal available for hire for off-farm work or use the time to attend meetings and training sessions, or to pursue administrative problems. More time and energy was available for social activities inside the household and in the community.

The reduction or disappearance of tethering and herding affected livelihood by increasing time availability and reducing the destruction of crops. Consequently the production of maize, banana and vegetables, and the income from animals' work outside the farm, increased. The rope of tethered animals often damaged young plants but when a rope was not used the animals went onto the maize. Most farm households that had ruminants prior to forage adoption increased their herd size after using new forages. The increased number of livestock required more labour input on the farm and as a consequence the time available for non-farm work decreased. The increased need for on-farm labour had a negative trade-off on the income from firewood cut in the forest area, as time was not available. However, this was positive change for the environment. The income from livestock sales and milk marketing increased, as did the production of most crops. Some farmers mentioned the production of banana had decreased, due to a new type of banana suffering from fungal infestation in the hot, humid climate.

The positive affect of new forages upon livelihood resulted in more farmers adopting them and greater areas being planted. To ensure FSP's self-supported progress, new farmers received a small amount of planting material or two seedlings/cuttings of trees for free. In most villages, an alliance organised the new forage distribution. Members of the alliance helped each other with the initial work of land preparation and planting. In one village, 100% of the farmers adopted forages, but some only had fodder banks. The high adoption level was due to encouragement by the landowner (the local religious leader) and the availability of goats and cattle from his own sharing system and the government dispersal program.

The discussion revealed three major reasons for farmers not adopting the new forage systems proposed by FSP, if they had: no animals; no land or tenant or caretakers; or no awareness of the forages. Some farmers were simply not motivated to obtain a higher income through forages, for example: large land owners receiving plenty of income via tenancy rights and *alima* shares; elderly childless people who made enough to meet their needs; or people who received sufficient allowances to survive adequately.

Farmers that did not own, or have in trust (*alima*), a goat or large ruminant would generally not adopt new forages, even if the effect on soil erosion control was claimed to make it worth their while. The rate of adoption FSP technology was very dependent on the government program of livestock dispersal.
Unfortunately, the adoption of new forage depended highly upon the dispersal and credit schemes for goats, cattle and dairy buffaloes. These distribution schemes could not keep up with the autonomous rapidly growing program of new forages for feeding ruminants. An easy to manage dispersal system with an avalanche effect is required to keep pace with the expanding FSP program. Enthusiastic farmers who expected to receive an animal and were then disappointed by the unavailability, would often stop maintaining their new forage crop.

Another obstacle to the success of FSP was the traditional animal trust system (*alima*). In most *alima* systems half of the offspring and income from products were returned to the owner. However, the current production level from livestock (partly due to deficient feeding causing low reproduction rate) was too low for recipients in the *alima* system to generate enough money to buy their own animal in the short term. The low return for their labour investment did not motivate them to improve their land quality and animal reproduction through new forage adoption. Performing off-farm work better rewarded their labour. The exception was when an *alima* animal was trusted, by parents, to their married children. This situation provided a social support system for the parents, whose labour availability decreased after their children’s marriage. However, young married couples that received an *alima* cow, often had to sell the first animal that they owned (their first share was the second calf) to pay the school fees of their eldest child. Such systems did not contribute to sustainable improvements in the production system and livelihood of farm households. When farmers cropping new forages received animals in *alima* they should have the right to ask for the first calf as their share, as the returns from the trust animal will be higher. Tenants or caretakers and people that did not own land were not likely to adopt new forages unless they had an area available for their own annual crops and a good contract.

About half of the farms did little animal management. Together with low market prices, this resulted in low financial benefits. Local goats produced only 1 kid per pregnancy and usually only once a year. In Cagayan de Oro, some farmers mentioned that their cow was not pregnant after more than a year. This was partly due to the unavailability of fieldworkers from the Veterinarian Department to do artificial insemination. Some expensive upgraded dairy cows had already stopped lactating and were not yet pregnant. The delay of a new gestation until after the drying of the cow strongly affected the productivity of the dairy system and also endangered the continuation of the dairy dispersal program. If artificial insemination is the only strategy being used by farmers with dispersal cows, a government agent should not perform the procedure. Some upgraded cows showed signs of heat stress. This had a negative impact on animal welfare and efficiency. Farmers should be advised to do backcrossing with the local breeds and to strive for crossbreeds with less than 50% exotic blood. In one village, DA workers spent their time treating only the livestock from dispersal programs, and in other villages, animals died because not enough agents were available for health care. The privatisation of veterinarian services should be given priority.

Financial benefits in the Philippines. The estimated income per labour day, of farm household livestock production systems, increased significantly after involvement in FSP. The average income from livestock of early adopters was about 2/3 of the wage for one day of agricultural labour (75 Peso), and about equal to the daily wage of youngsters (Tables 93 and 94). However, for recent adopters this income was only 20% of the adult labour wage and close to half of the wage or youngsters. The level of standard deviation showed that some farmers do much better. In Malitbog, the 9 highest income earners had a mean household income from livestock of 77 Peso/day (sd=46). Their labour income per worker was equal to the agricultural wage for an adult. In Cagayan de Oro the top 7 made 89 Peso/day (sd=49), an income higher than the daily wage. The farm households that specialised in dairy earned 72 Peso/day (sd=65), about equal to the daily wage.

Table 93. Means and standard deviation (sd) of the partial livestock budget[1] of early adopters and recent adopters of new forage farm households in Cagayan de Oro (in PhP), with p value for the difference between early and recent adopters.

	Early adopters (15)		p	Recent adopters[2] (11)	
cost of hired land	mean	sd		mean	sd
Cost of housing	1,964	5,670		89	128
Cost of inputs	4,306	7,792		3,122	4,148
Total cost	6,270	13,326		3,211	4,138
Increase of value	32,431	47,015		5,261	3,752
Net income animals sold –bought	-17,039	45,988		-1,563	3,083
Income from marketed milk & meat	9,325	17,744		0	0
Home-consumption of animals and products	1,949	2,585		350	924
Income ploughing and hauling off-farm	322	876		1,541	3,015
Ploughing and hauling for own farm	2,155	4,548		517	806
Manure on crops	8	11		9	8
Benefit insurance value herd (i = 4 %)	2,095	2,275		476	400
Benefit of financing by animals sold (i=12%)	198	222		16	34
Total value output	31,444	44,021		6,608	4,560
Output – input	25,174	33,650	0.025	3,397	2,874
Return from land (i = 6 %)	548	965			
Return from herd capital value (i = 6 %)	1,571	1,706		357	300
Return from labour (income from livestock)	23,055	31,953		3,040	2,642
Labour days invested in livestock (days/year)	279	229		173	67
Household income from livestock / labour day	67	62	0.012	20	17

[1]The share of *alima* was applied on the value of the animals present in herd, and marketed.
[2] Labour input used in this table for recent adopters is the time needed for tethering and herding animals before new forage use, in stead of new forage establishment and cut and carry.

To assess the effect of contour lines farmers were asked to estimate: the quantity of crop harvested before and after their use; the quantity of artificial fertiliser used before and after; and the relative change in area covered. The mean area covered with contour lines and crop was 0.43 ha. The net increase in yield for the 8 farmers alone was 1210 Peso, equivalent to 220 kg of maize, and an increase of 500 kg/ha. Income per labour day increased for these farmers. Their net increase was divided by the number of days needed for maintenance, plus one fifth of the days invested in the establishment, of the new forages. The cumulative labour income of 59 Peso/labour day (sd=61) was intermediate between the wages for adults and youngsters (Table 94).

Table 94. Means and standard deviation (sd) of the partial livestock budget[1] of early adopters and recent adopters of new forage farm households in Malitbog (in Peso), with p value for the difference between early and recent adopters.

	Early adopters (n=17)		p	Recent adopters[2] (n=10)	
	mean	sd		mean	sd
Cost of housing	375	943		383	722
Cost of inputs	2,617	3,856		2,405	2,906
Total cost	2,992	3,831		2,788	3,087
Increase of value	3,259	4,431		1,958	1,974
Net income animals sold - bought	1,631	4,441		53	814
Income from marketed milk & meat	0	0		160	320
Home-consumption of animals and products	1,147	2,221		693	1200
Income ploughing and hauling off-farm	2,211	4,400		360	1,080
Ploughing and hauling for own farm	1,894	4,165		2,000	3,192
Manure on crops	209	319		60	98
Benefit insurance value herd (i = 4 %)	386	224		208	174
Benefit of financing by animals sold (i=12%)	103	126		19	22
Total value output	10,853	11,068		5,510	3,632
Output - input	7,861	10,262	0.032	2,722	1,152
Return from land (i = 6 %)	221	289			
Return from herd capital value (i = 6 %)	241	140		130	109
Return from labour (income from livestock)	7,398	10,134		2,591	1,080
Labour days invested in livestock (days/year)	164	84		138	45
Household income from livestock / labour day	53	56	0.028	22	15
Labour income including benefit contour lines	59	61	0.017		

[1]The share of *alima* was applied on the value of the animals present in herd, and marketed.
[2] Labour input used in this table for recent adopters is the time needed for tethering and herding animals before new forage use, in stead of new forage establishment and cut and carry.

The sharing of animals reduced the positive effects of new forage, particularly in Malitbog. When considering the system without *alima*, the labour output of the early adopters increased by 20% (compare Table 94 and 95). The total productivity of livestock per labour day after new forage adoption in Malitbog reached the level of the labour wage: 68 Peso/day (sd=57). The comparable labour income from livestock, including the part going to the owner, was the same in Cagayan de Oro and Malitbog. However, the total benefit of improved forages in Malitbog was higher due to saved time and the contribution of contour lines to the control of soil erosion. These changes yielded 1825 Peso/year and 1125 Peso/year, for saved labour and erosion control respectively. The mean improvement was 3 Peso/day/household for each effect. The lower labour needs for the establishment and maintenance of forage in cropped land also contributed to a comparable income. This was also reflected in the labour productivity per hectare of forage. Though the proportion of farmers having fodder banks was about equal, the area covered was much larger in Cagayan, 0.58 ha on average, than in Malitbog, where it was only 0.34 ha. The larger amounts of feed in Malitbog were due to the ease of establishment and maintenance of contour lines.

Table 95. Adjusted total livestock productivity, not considering share for owner, influenced by sharing *alima* (in Peso).

	Early adopters (17)		p	Recent adopters (10)	
	mean	sd		mean	sd
Cagayan de Oro					
Total value output	31,664	43,935		6,921	4,387
Return from herd capital value (i = 6 %)	1,627	1,690		387	284
Return from labour (income from livestock)	23,219	31,892		3,324	2,620
Household income from livestock / labour day	68	62	0.014	22	18
Malitbog					
Total value output	13,352	11,177		6,087	4,364
Return from herd capital value (i = 6 %)	319	156		179	169
Return from labour (income from livestock)	9,820	10,510		3,120	1,818
Household income from livestock / labour day	68	57	0.006	25	17

Social and gender effects in the Philipines. The reduction or disappearance of tethering (*tigway*) and of herding (*bakero*) had neutral and positive social effects. The neutral social consequence of the reduction of herding on distant pastures was only experienced in villages that had a communal grazing area. Bakero herders would meet on distant pastures. The adoption of forage cut and carry reduced the social contact between sexes and diminished the temptations that often resulted in suspicious partners. The number of early marriages of youngsters decreased. Young people would instead meet at the house, in the church or during other social activities.

Children's nutrition improved due to the increased availability of milk, increased vegetable and fruit production and improved income from livestock. The increased availability of milk contributed to the reduction of malnutrition in some households. The whole community benefited from increased social involvement as farmers met more frequently and organised activities. Environmental benefits affecting the community were reduced firewood cutting, control of soil erosion and improved fallow.

According to farmers the attitude and approach of fieldworkers had changed. In the past, the fieldworkers were only concerned with animal health, after FSP began they became more interested in animal feeding and breeding. The fieldworkers had improved their extension services by making them more frequent and reliable. Farmers received more training and were more often involved in workshops. Previously, only selected farmers from a region were invited for meetings and/or training in an often distant village. However, due to the project, meetings were being held in more villages enabling more farmers to attend. Fieldworkers had contact with more farmers as they visited the villages and went to the fields to demonstrate the work. FSP brought a technology that lasted and provided ongoing benefits to farmers. FSP agents also taught farmers about soil conservation and crop rotation and made them aware of new technologies in other fields through cross projects visits.

The participatory monitoring of innovations was highly appreciated as these methods encouraged farmers and allowed them to learn more from each other. Some incorporated preference ranking in their way of thinking during the process of decision making in the family and in their seasonal crop rotation. Farmers in one village claimed their awareness or boldness had increased. They now dared speak to higher authorities and asked for legal contracts. In the words of a female farmer in Lumbia (now studying to become a teacher), "they lost their complex of inferiority." The need to organise the distribution of plants and seeds, as well as the monitoring and evaluation, made them aware of the strength of co-operation.

Farmers mentioned the following changes in the attitude and approach of state agents resulting from the FSP method, they: listened to farmers more; taught farmers to monitor and evaluate; encouraged farmers; and asked farmers to make decisions. Field trips and cross-visits were highly appreciated as they allowed farmers to learn from each other and to see other innovations. As they listened to farmers, the fieldworkers also learned more about the farmers, and more from them. For example, fieldworkers learned about planting according to the moon and tide calendar and the use of rocks for erosion barriers.

Women's focus groups acknowledged all of the positive effects previously mentioned but some also said that they benefited especially from the increased availability of firewood from *Leuceana*. The women saved time from by reducing tethering and herding. In Malitbog, the introduction of new forages reduced the involvement of young and women in tasks like herding and cutting and men executed more tasks than before (Table 96). In Cagayan de Oro, as overall labour input decreased, the men, women and children all profited from the saved time as they had all been involved to the same degree. The saved time was invested in work in alliances for forage cropping, manure collection and cutting and carrying. Residual time saved was spent on their preferred activities: cropping and selling of vegetables; making brooms and mats; and various trade and roadside food selling activities. The increased working time of draught animals, saved time and the availability of extra income, were judged to be more positive to men.

Table 96. Gender involvement in animal husbandry tasks of farm' households in Mindanao.

	After adoption			Before adoption		
	young	men	women	young	men	women
Cagayan de Oro	30	43	27	28	47	25
Malitbog	25	47	29	39	26	34

According to women, they had a say on the financial benefits received from livestock. Women in Mindanao had kept much of their crucial authentic role, that they would have lost according to Illo (1997). Women represented in the survey sample were owners of all animal species. In general, husband and wife partnerships managed household resources in common agreement. Early adopter women in Pangalungan were firm about their decisive power.

Increased labour productivity on farms, prevented young men and women migrating to town to seek an income. Increased labour needs due to animal care and milking had created permanent on-farm jobs. This change was apparent at the family level: the eldest children still went to town to make money but the youngest stayed on the farm once there were cows to take care of. Direct control by youngsters of part of the household resources was not a general custom or practice, but according to key-informants, depended upon the availability of livelihood resources. When a farm household had enough resources, adolescents would be given some to prepare for their future

Contribution of forages technologies to poverty reduction in the Philippines. To evaluate the contribution of FSP to poverty reduction, the estimated total income per active household worker was compared with the mean provincial expenditures of 2001. The total income was estimated from the output-input (farmers' perception of income) and the relative contribution of livestock to the livelihood system. Relative contributions for recent and early adopters in Malitbog were 15% and 20% and in Cagayan de Oro 10% and 20 %, respectively. For dairy farmers in Cagayan de Oro the contribution was assumed to be 30%.

In Malitbog, the income of recent adopters was far below mean expenditures. The estimated income from traditional systems was about 15% of mean expenditures, and was below the poverty limit of 1 US$/day, i.e. 18,250 Peso/year. Income from early adopters was about double mean expenditures and slightly above the poverty limit. Without accounting for the part of trust to be returned to the owner total income, total income was still close to the poverty limit.

The estimated total income for early adopters in Cagayan de Oro was more than half the mean expenditures and almost triples the estimated total income for recent adopters. Old livestock production systems only generated an estimated income equal to roughly 20% of mean expenditures. However, without accounting for the part of the trust to be returned to the owner, the income of recent adopters was also close to the poverty limit. The adoption of new forage technologies allowed farmers in Cagayan de Oro to cover more than 60% of mean expenditures, and to have an income double that of the poverty limit. Dairy farmers had an estimated income close to the level of the mean expenditures. FSP played an important role in poverty reduction.

Compared to East Kalimantan[1], Indonesia, the benefits of new forages for farmers were low. This was due to several factors that hampered the autonomous adoption of forages. The effects could increase as farmers make better use of animals' manure. Reorganisation of the animal dispersal program could help a self-supporting expansion of the new forages proposed by FSP. Adoption of new forages demanded at least two conditions: ownership of animals and good conditions for the use of land.

Results from Vietnam

Livelihood resources and activities in Tuyen Quang. Livelihood resources in Thang Quan were diverse. The variety of handy-craft products was large, including things such as chairs, brooms, tooth sticks and woodcarvings. Farmers in Duc Ninh mentioned only carpentry as income from handy-craft. The processing of products at farm level was frequently a source of income. Income from transport was obtained by using buffaloes or cattle, as well as small tractors. The wage of an agricultural labourer varied from 12,000 to 15,000 VND/day. Wages were highest in Phu Lam. Off-farm jobs like fishpond digging or labour in construction were better paid at between 16 000 and 20,000 VND. Mean rural wage was considered to be 15,000 VND/day. A working day for hired labourers was at least 7 hours, but most jobs were done by task.

The estimation of the contribution of livestock to total livelihood resources varied between genders and wealth strata. Amongst the lowest wealth classes, men estimated the contribution of livestock at 24% and women at 40%, a mean value of 32%. The effect of the species of livestock owned amongst the classes of high income was low. The estimation of those having only a fishpond was a contribution of 35% whilst those also owning cattle and/or buffalo estimated the contribution at 27%, a mean of 30%. This figure was confirmed in Phu Lam.

Farmers fed the improved forages to cattle, buffalo, fish, rabbit and pig. Poultry also fed on the available new forages. Some farmers planted already green fodder for fish and swine. The most

[1]In East Kalimantan, mean income from livestock systems with new forage was 14.500 Rp/day, an equivalent of 1.5 US$/day; mean result in Mindanao (53 Peso/day) was close to 1 US$.

commonly planted new species were Napier (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), *Paspalum atratum*, *Brachiaria decumbens* and *B. ruziziensis* and *Setaria spacelatha*.

According to the farmers, the quantity of forages they gave to their swine was too small to be estimated. Almost every farmer fed green fodder to their pigs because they knew that the animals needed them to improve their health and digestion. Before the introduction of new forages by FSP, farmers planted several kinds of feed for swine: sweet potatoes for leaves and roots; bamboo grass (*Arundinaria pusilla*); *Bohemia nivea* (Lá gai); or used residues from banana and maize. Several new forages were used, but *Stylosanthes guianensis* and *Trichantera gigantea* were specially planted for swine. Young pigs did not eat the improved grasses.

Chickens and moskovy ducks fed on the improved forages but farmers did not plant or cut these just for poultry. Before the introduction of new forages, poultry fed on the naturally occurring forages. Poultry preferred to feed on the new forages and did not use the natural forages once the new ones were available. Farmers were not aware of an increase in poultry production. However, several effects due to the availability of new fodder were mentioned: healthier animals, apparent through their better feather cloth; heavier animals and eggs; improved colour of egg yolk; higher quality of eggshell and reduced cannibalism.

Almost every farm household had a fishpond, 91% in the survey sample. Seven of the 51 fishpond owners only produced fish for household consumption. No farmers produced fish purely for marketing. The survey sample contained 2 producers of fingerlings. Fish could be marketed all year round whenever a farmer needed cash. Strongly market-oriented farmers emptied their fishpond once a year, usually in January, just before the Vietnamese New Year (Tet). Most of these farmers bought new fingerlings after 1 to 3 months but others bought bigger fish later. Some farmers had several fishponds and produced and fed fish all year round. Fodder for fingerlings was chopped during the first month. Natural grasses as well as new-planted grasses produced large quantities of fodder from July to mid September. After this, farmers had to complement these fodders with bamboo leaves, cassava leaves and chopped banana trunk, and this took more time. Some farmers planted bamboo grass for the dry season on plots beside the pond. Gradually fodder was replaced by ground cassava for the final fattening. Most cassava was produced on farmers' own land. Before feeding cassava to fish, it was chopped, dried and ground. The fattening period corresponded with the wintertime when the feed consumption of the fish was reduced due to the cold.

The demand for manure was high and in most villages it was not readily available. Consequently prices were high, especially close to town or factories. In Phu Lam all manure was marketed. Buffaloes were mostly used for ploughing and hauling. Cattle were used for hauling and for ploughing dry land. Farmers who did not have buffalo or cattle could hire either a buffalo together with the person operating it for 40,000 VND/sao [2] or, if available, a machine for 30,000 VND/sao.

The prices of cattle and buffalo did not fluctuate over the year, but prices for poultry and pigs were low in July and August and good between September and February. The price for cattle and buffalo had increased exceptionally during the previous 2 years, due to improved demand and decreased availability. The market price for tea increased in 1999 and a lot of farmers sold ruminants to invest in tea plantations. This caused low ruminant prices in 2000 due to the large numbers available but by 2002 prices had improved greatly. Crossbred cattle were mostly the result of artificial insemination with Red Sindhi. The best fish prices were paid between November and February. In the commune of recent adopters, staple foods, clothing, fertilisers and government taxes were also paid for using the

[2] 1 ha = 7 sao

income from livestock. In all 3 communes, the income from livestock was used for productive investments, e.g. in rice threshing machines.

Cropping of forages for cattle and buffaloes was an innovation as farmers were only used to cultivating forages to feed fish. Not all villages irrigated the newly planted forage. Children did most of the herding. Tethering was not very frequent probably because grass cover was too restricted.

Time was a well-developed concept in Tuyen Quang. All of the households visited had a clock and several had specific television programs they liked to watch. Time estimation could be considered highly reliable, though the short time spans sometimes seemed to be underestimated. The number of days estimated for the cropping of new forages as well as for sweet potatoes varied largely.

Effects on production system and livelihood in Tuyen Quang

The benefits of new forages mentioned by farmers included:

- higher yield than natural grasses;
- low fertiliser and pesticide requirements compared to other crops;
- better use of barren land or land with low soil fertility;
- control of soil erosion;
- faster growth of fish, cattle and buffalo;
- higher price received at marketing due to increased body fat;
- better body condition and hence working capacity for cattle and buffaloes;
- enabling of expansion of livestock activities
- direct income from forage products;
- increased availability of manure;
- saved time from cut and carry for fish and from herding for ruminants.

The high yield of the new forages allowed farmers to keep animals and to improve their livelihood. Most farmers had to reduce their herd after the reduction of communal land, while others sold animals when their children had to go to secondary school and no labour for herding was available. The new forage technology allowed them to keep animals in zero grazing. Improved forages allowed other farmers access to animals as they were able to produce sufficient fodder from their small plots. Within 5 years of acquiring an initial animal in trust or on credit, several farmers succeeded in repaying the trust or credit, selling at least 1 animal and growing their own herd to at least 4 cattle. The productivity of fish farming increased. According to 1 farmer, the period until marketing was also reduced from 11 months to 9 months due to the availability of new forages.

Most farmers planted the forages close to the fishpond or the animal shed to reduce labour time. Planting on the fishpond border was increasingly frequent and allowed farmers to use a previously useless area . Other previously unused land (of low fertility or steep slope) was also planted with forages, allowing farmers to make better use of their small plots. Fodder banks were mostly situated on pond banks. Other large areas were mixed with fruit trees. Improved forages often replaced beans or cassava as intercrops between tea plants. Cassava hampered tea culture and farmers were glad to have a replacement crop, particularly one that required very little investment and maintenance, and helped to conserve soil on the slopes where tea was usually planted. When new forages were cropped on an area previously cropped with a staple food, cash crop, vegetables or cassava for animal feed, the original crops were planted in areas further from the pond or shed, e.g. cassava on forest land.

The net yield of the previous crops was low). Moreover, they were replanted each year, so seeds and fertiliser had to be purchased and they also required pesticides. All of the replaced crops had a labour productivity lower than 2/3 of the agricultural wage. In Thang Quan, farmers mentioned sugarcane yields of 190 VND/kg, whilst tillers and cuttings of new forages were sold for 2,000 VND/kg. One interviewed farmer irrigated his fodder bank and another used humid land to ensure green fodder all year round.

Financial benefits in Tuyen Quang. Most farm household characteristics of early and recent adopters were not significantly different, however, the area planted with new forages was higher for early adopters. After 1 to 2 years, recent adopters still had less than half the area of new forage of early adopters. This difference partly explained the lower financial benefits recorded by recent adopters. The herd size of recent adopters at the time of this study was only slightly smaller than that of early adopters, but the recent adopters had increased their herd size in the last 2 years from an average of 1.7 to 2.1 ruminants. The herd size of early adopters was quite stable at 2.4 animals.

The market for forestland was restricted in Tuyen Quang. Cropland, fishpond and homestead (with an owner certificate—red book) could be traded but forestland (green book) could not. Prices of owned land varied according to its value for production and location (e.g. proximity to main road). The Government fixed land prices and taxes on land according to 6 classes and market prices were generally double this amount.

For the calculation of the financial benefit of saved time, the number of days per year saved was multiplied by the mean income/labour day. Mean labour day income was averaged from a wage of 15,000 VND/day and the labour productivity of rice, maize, cassava, sugar cane and beans—12,000 VND/day.

Buffalo and cattle production system in Tuyen Quang. Saved time was an important benefit for most farmers keeping ruminants. However, this was not the case for those having significantly increased their number of animals or having started keeping buffalo or cattle only after new forage adoption. The number of labour days per year required for the early adopters of new forages was much lower than the figure for recent adopters before their planting of new forages: 149 and 258 day/yr, respectively (table 6). Using the mean labour day income as the multiplier, a mean saved time of 120 day/yr corresponds to a gain of 1.44 million VND/yr for a farm household. Only 50% of saved time was attributed to adults (table 9), and only two-thirds of this saved adult time was used for productive farm activities. Family members used the remaining saved time for leisure, training and study. Therefore the financial benefit of saved time was estimated at 400 000 VND/month per household, or 82,500 VND/month/member.

Without accounting for saved time, the labour income from the ruminant systems of early adopters is significantly higher than that of recent adopters, due to increased labour efficiency (Table 97). The mean agricultural wage in the province was 15,000 VND/day, and the labour income from livestock before adoption was one third of that. After adoption, however, it was higher than the mean agricultural wage.

Income from marketed animals and the off-farm labour of ruminants was significantly higher for early adopters than for recent adopters: 1,571 VND/yr (sd=1479) compared to 843 VND/yr (sd=1,144), respectively ($p<0.1$). However, the herd value increased more for the recent adopters. This greater increase in herd value for early adopters was entirely due to the improved market value of ruminants. The net value of output from the ruminant production system, i.e. farm households' cash income, was not significantly different between early and recent adopters, mainly due to the small sample size. After correction for the increase of herd value of early adopters, the net value of output was about 13% higher than for recent adopters (Table 97).

It is likely that recent adopters had already profited from the new forages. However, this did not have much affect upon the financial results.

Labour income from the crops previously planted on forage area was higher than income from livestock for recent adopters. The labour income after adoption of new forages was twice as high compared to the crops previously planted. Not accounting for the increased value of the herd nor returns to land, the productivity of land for ruminant production was estimated at 12.3 million VND/ha (sd=15.6). This was almost twice as much as the estimated productivity of lowland rice.

Table 97. Means and standard deviation (sd) of the partial budget of the ruminant production system for early and recent adopters of new forage in Tuyen Quang (in 1000 VND), with p value for the difference between early and recent adopters.

	Early adopters (20)		p	Recent adopters (26)*	
	mean	sd		mean	sd
Cost of housing	67	35		65	53
Cost of inputs	170	129		238	187
Total cost	239	161		297	213
Increase of value	670	1,399		980	1,217
Net income animals sold and bought	1,180	1,053		367	1,118
Income ploughing and hauling off-farm	61	261		18	90
Home-consumption ploughing and hauling	396	368		450	282
Manure on crops	23	11		8	14
Benefit insurance value herd (i = 3 %)	180	80		132	65
Benefit of financing by animals sold (10.8%)	82	82		45	60
Total value output	2,922	1,349		2,458	1,729
Net value output (cash income)	2,352	1,264	0.113	1,703	1,384
Return from land (i = 10 %)	45	28		33	18
Return from herd capital value (i = 6 %)	361	160		264	130
Return to labour	1,949	1,178		1,401	1,277
Labour days invested in livestock (days/year)	149	112	0.001	258	60
Household income from livestock / labour day	17	15	0.004	5	4

* Labour input used in this table for recent adopters is the time needed for tethering and herding animals before new forage use, instead of new forage establishment and cut and carry.

Fish production system in Tuyen Quang. Maintenance of fishponds was performed regularly. Commercial producers cleaned their ponds each year. Small producers, including those producing only for home-consumption, cleaned their ponds once every 2 or 3 years. Some farmers hired labourers or machines to do the job. Pond banks were more frequently repaired: 43 out of 52 fishpond owners spent time on it in 2001. The maintenance cost of fishponds was calculated using the labour cost of 18,000 VND/day for all farmers.

Labour time spent on fish production by early adopters was slightly less than half the labour time needed by recent adopters before they planted forage, a difference of 71 days/year. The mean number of saved days for all early adopters is 30 day/yr. The mean saved time of 30 labour days/yr corresponded to about 40 min/day. Almost 80% of this was attributed to adult labour (table 9) and this saved adult time was used to supplement income with 290,000 VND/yr or 25,000 VND/month per household, i.e. 5,000 VND/month/person.

Only the data from farm households marketing fish were considered for financial assessment. Estimations of production for household consumption appeared not to be very reliable. Three fish producers, early and recent adopters, experienced lowered yields due to fish diseases. The total value of output, i.e. farm households' cash income, was not significantly different between early and recent adopters. The claim of some farmers that they could finish in 9 months instead of 11, corresponds to a 20% higher return to labour. Variation in this small sample was far to high to show this as a significant improvement. The net value of output from the fish production systems of early adopters was 18% higher than for recent adopters (Table 98).

Table 98. Means and standard deviation (std) of the partial budget of the fish production system for early and recent adopters of new forage in Tuyen Quang (in 1000 VND), with p value for the difference between early and recent adopters.

	Early adopters (22)		p	Recent adopters (18)*	
	mean	sd		mean	std
Maintenance cost of fishpond	629	595		609	464
Cost of inputs	689	477		750	785
Total cost	1,318	943		1,359	1,156
Income from marketed fish	2,465	1,936		2,237	2,060
Home-consumption of fish	725	511		602	265
Total value output	3,190	2,097		2,839	2,155
Net value output (cash income)	1,871	1,481		1,580	1,778
Return from fishpond (i = 10 %)	519	493		495	389
Return from forage land (i = 10 %)	54	77			
Return to labour	1,298	1,257		1,084	1,761
Labour days invested in livestock (days/year)	53	41		124	73
Household income from fish per labour day	32	27	0.008	12	15

* Labour input used in this table for recent adopters is the time needed for tethering and herding animals before new forage use, instead of new forage establishment and cut and carry.

As with the ruminant production system, the principal reason for the increase in efficiency was the reduction in labour time (Table 98). Before the adoption of new forages, return to labour from fish production was close to the highest rural labour wage of 12,000 VND/day. The income per labour day of fish production with new forages was almost triple that amount and 2.5 times the labour productivity of lowland rice, 32,000 VND/day.

Swine production system in Tuyen Quang. The swine production systems were well organized. All 5 interviewed farmers with sows used artificial insemination. Two of them raised piglets but did not fatten them. Their sow, of a fertile local breed, was inseminated with a sire of a lean European breed, e.g. Yorkshire, Large White or Duroc. The piglets were either all sold or the farmers just sold the surplus that they could not fatten themselves. The farmers fattened 4 to 14 piglets a year in 2 rounds of 5 to 6 months. Housing was always on a closed concreted floor. Compartments were constructed either from bamboo or concrete and the roof was made mostly from leaves but some tiles were also used. The cost of construction and maintenance of the pig houses varied according to the materials used (Table 99).

Swine were fed with bran from rice and maize and with various feeds available from the market, such as cassava starch, soybean, fishmeal and concentrates. Most farmers also cropped sweet potatoes in rainy season and a large variety of herbs and grasses for the dry season. The sweet potato leaves could either be cooked together with their roots, the bran and the starch or fed directly to the pigs. Some farmers used new forages instead of sweet potatoes but most only used these if there was a shortage of sweet potato. They stated that the advantage of sweet potato was its double use: fresh (leaves) and cooked (leaves and roots). Not all farmers cropped forages for their swine.

Table 99. Mean and standard deviation (std) of yearly return to labour of 2 rounds of swine fattening by 12 farm households in Duc Ninh and Thang Quan (x 1000 VND).

	Not using new forages (6)		Using new forages (6)	
	mean	sd	mean	sd
Total cost *	2,977	1,446	4,120	1,138
Total value output	4,688	1,706	6,883	1,488
Return from forage land**	1	1	2	2
Return from capital	117	47	158	35
Return from labour	1,644	757	2,719	1,296
Total days of labour	123	55	187	77
Days of labour per pig	35	30	39	14
Income per labour day	19	17	16	7

*The cost of housing was standardised to reduce variability in the small sample.
**Real interest rate of 4% and land value of 1,000,000 VND/ha.

According to farmers having replaced sweet potatoes with the improved forages, the use of *Setaria, Brachiaria brizantha* and *Stylosanthes* for example, allowed them to save time. This was not confirmed in the small sample of 12 pig fatteners, of whom 6 also used new forages. Replacing sweet potatoes with new forages increased the need for manufactured feeds, as roots were not available, thus increasing cost. Farmers did not observe any change in production when they had replaced other green fodder with new forages. Mean income per labour day in swine production is 18,000 VND (sd=12,000), high compared to traditional ruminant and fish production.

Social and gender effects in Tuyen Quang. Most positive gender and social effects resulted from the reduced time needed for cutting and herding. According to the female focus groups in Duc Ninh, the effects of new forages were even more positive for women and children than for men. Mostly women and children (after school) were in charge of herding (Table 100). Women in Phu Lam did not frequently herd prior to new forage introduction and their men did most of the forage cutting for fish. Forage cutting for ruminants was limited to the period around calving. They estimated women's and men's saved time to be equal. Children were able to study and rest more and to help their mother with household tasks. Farmers in Phu Lam suggested that increased rest improved their children's health. The reduction in time spent herding reduced exposure to the sunlight, which may have positive effects on skin cancer, but was more directly appreciated by women, as their skin stayed clear. A clear, non-tanned skin is very important to women in Vietnam. Women were often also responsible for the cutting of natural grasses for fish and swine and the time needed for this task was decreased. Adults, rather than children, mostly did the new tasks of planting and maintaining forages. Moreover, the 15 day/sao/year cost needed for these tasks was small compared to the mean saving of 1 hr/day for cutting and 2 hr/day for herding. The general level of involvement of men, women and children in livestock activities did not change after adoption of new forages, except for a slight tendency of men and children to replace women for cut and carry (Table 100). In Vietnam, increased wood production was not a direct consequence of FSP, as leguminous trees were hardly planted.

The saved time of men and women was invested in other farm jobs and housekeeping, but also in study, training, meetings, and enjoying more leisure activities. Leisure activities mentioned were resting and television watching. Watching television was a preferred leisure activity and had educational advantages as farmers also watched programs of the provincial extension services. According to women, forages had a positive effect on other crops due to soil conservation and manure availability. Labour saved from livestock was used to better manage crops, resulting in higher yields. Higher yielding crops then required increased labour time for harvesting and processing. Saved time was invested in a range of farm activities including cash crops like rice, cassava, beans, sugarcane and fruit trees. Activities women appreciated more were planting forages and feeding fish.

Table 100. Gender involvement in animal husbandry tasks of farm' households in Tuyen Quan.

	After adoption			Before adoption		
	Young*	Men	Women	Young*	Men	Women
All livestock activities	23	50	27	25	51	25
Herding	46	21	33	49	21	30
Cut & carry	24	50	25	22	48	30

* Considered as young are persons going to secondary school or below 16 years.

According to women, the increased farm household income from livestock, forages and crops was invested in expenditures for the family, for example, in household equipment including motorbikes, in children's' education, in purchasing drugs, cloths and inputs for agriculture. These results were in accordance with those from the general meeting. Women put a high priority on household equipment, and a means of transportation. In about 90% of households, women kept the money. This supported their claim to have a say on expenditure.

The social changes mentioned in the general meeting were: more time for leisure, easier transportation due to the availability of bikes, and a decrease in the number of conflicts due to less crop destruction by grazing animals. Mixed gender focus groups mentioned 3 general advantages: an improvement in hygiene, as less dung was deposited on communal grounds; a greener environment; and a reduction in the use of pesticides. Reduced herding time decreased the number of conflicts due to crop destruction by unsupervised animals and this improved relationships between neighbours.

The study tours FSP organised were a new experience for most farmers and highly appreciated. The tours not only allowed them to learn about new forages but also about other farm activities and methods, so helping them to increase the efficiency of their farms. FSP fieldworkers spent much of their time in the field as planting grasses was a new activity for farmers. The increased number of meetings and training courses along with the reduced number of conflicts improved village unity. The participatory approach stimulated households to exchange knowledge on all aspects of farming.

According to farmers, they assisted the fieldworkers from FSP in developing methods for plant reproduction, planting methods on contour lines, and in identifying species suited to local soil quality. Farmers experimented successfully with the reproduction of some species, using tillers when seeds were lacking and helped to find the optimal spacing between lines and plants of forages for local conditions. At first, forage plants on contour lines died after 1 year due to water logging. Farmers experimented by planting without a soil ridge and plants survived.

In Vietnam, the adoption of new forages seems to only be hampered by a classical extension phenomena: observe before trying. Once the farmers saw the enormous benefits they were keen to plant new forages but planting materials were lacking in every village. The price of 2,000 VND/kg of planting materials was high compared to many crops. Farmers came from other districts, not yet contacted by the FSP program, to buy their first planting material and receive on the spot farmer-to-farmer training. However, farmers not having fish or ruminants or those owning only a small area of land, e.g. only irrigated land and a homestead, would not adopt new forages. Structures for cattle dispersal existed and worked effectively. The adoption of new forages did not depend on it as most farmers bought their first ruminants from their savings. This contrasted with the situation in Mindanao and East Kalimantan where a strong relationship was identified between livestock distribution schemes and the adoption of new forages.

Contribution of forages technologies to poverty reduction in Tuyen Quang. In Tuyen Quang province, 4 classes of wealth were distinguished according to mean monthly income per person in the household (Table 101). Village and commune leaders classified households using secondary information and data. In Duc Ninh, the lowest level had been increased to 100,000 VND/month, as the category of poor was almost empty. It was suggested that the major cause of the difference in wealth was the area of land available and the stage of evolution of the household. Some farmers did not ask for more land from the government during the land distribution scheme. Other plots were split amongst the children when parents stopped farming. Amongst the wealthiest though were people with small land areas who increased their income with well paid off-farm jobs like fishpond digging or construction labouring, which both paid between 16,000 and 20,000 VND/day. All poor households of Duc Ninh comprised young married couples with small children that could not yet help with farm and household work. In Duc Ninh, farmers of all 4 classes adopted forages.

Table 101. Classes of wealth in Vietnam according to income per month (x 1000 Dong).

Category	Poor < 82	Acceptable > 82 <200	Convenient > 200 < 400	Good > 400*
Duc Ninh	3	60	20	17
Thang Quan	15	82		3

* 400.000 Dong/month = 320 US$/year, normal expenditures for electricity: 40.000 Dong/month

In Phu Lam, a minority of people had an income below 200,000 VND/month, and most of them did not have a fishpond or large livestock and did not need to plant forages. In Phu Lam, the poorest group did not crop forages as it seemed they knowledge as well as capacity to organise and to analyse their situation. The contribution of livestock to livelihood was inversely related to wealth. This corresponded with the farmers' belief that differences in land area explained the income disparity between farmers. People were better off with a larger area of land that allowed them to get more income from upland crops and wood. The rich consumed and acquired more income from poultry and swine and their total contribution was estimated at 32%. The large middle class acquired more income from fish, cattle and buffalo. They estimated overall contribution at 34% but seemed to have underestimated the contribution of other livestock. The lower class, mostly recently married couples and/or recent adopters, estimated the contribution of livestock at 39%, the larger part coming from livestock not using new planted forages. The contribution in Phu Lam was estimated at 30%. The increase in income due to new forages was calculated by data derived from individual interviews. The total increase in income from saved labour for a mixed system was 87,500 VND/month/person. The monthly income from livestock per household member, of early and recent adopters, was estimated at 257,000 (sd=216,000) and 97,000 (sd=111,000) VND/month, respectively. This was equivalent to a mean increase of 160,000 VND. The total estimated increase due to new forages, including saved labour, was 247,500 VND/month/person (160,000 + 87,500).

4.2.8 Participatory evaluation of *Brachiaria* Mulato

Contributors: R. Roothaert, T.T. Khanh, C. Phaikaew

Rationale

New *Brachiaria* hybrids are needed in Southeast Asia, that can produce better seeds. *Brachiaria* Mulato and other hybrids have recently been developed in latin America by CIAT in collaboration with partner institutes. Some seeds of *Brachiaria brizantha* CIAT 265560, *Brachiaria brizantha* CIAT 26424, and *Brachiaria* hybrid 36061 were released to national coordinators of the Forages for Smallholders Project in Lao, Thailand, Philippines, Indonesia, Vietnam, and China.

Update. In Thailand, 200 plants of Mulato were planted at the research station at Pakchong, for the purpose of seed production. No flowering has been observed yet. In Vietnam, the different accessions were planted at farmers fields at Daklak. All accessions were equally producing green material, two months after planting. No flowering was observed yet.

Activity 4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed

Highlights

- Over 1 Ton of forage seed (mostly Brachiaria, Cratylia, Arachis and Desmodium) was sent to 12 countries from the CIAT-Palmira Seed Unit

- A total of 879 kg of forage seed was either multiplied or procured for dispatch to 10 countries in LAC.

4.3.1 Multiplication and delivery of selected grasses and legumes

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Seed Unit of Palmira

Advanced experimentation and effective adoption of new forage plants is critically dependent on initial seed supplies. A small unit at CIAT headquarters attempts to meet seed demands for experimental purposes and diffusion.

Seed multiplication plots are established at CIAT-Palmira CIAT-Quilichao, and CIAT-Popayán. Seed of 156 distinct accessions representing 17 genera were multiplied (Table 102). Total production was nearly 900 kg, with major volumes of *Brachiaria*, *Cratylia*, and *Vigna* seed produced. Diffusion of new forage plants is most efficiently accomplished via seed. A small seed multiplication unit exists to supply seed requirements of CIAT projects and those of collaborators, both within Colombia and internationally. Seed in excess of these needs is distributed at no cost in small quantities to bonafide researchers, or in larger quantities is sold to private persons or entities. Seed requests are addressed as possible according to seed supplies. A total of 520 individual seed samples were dispatched in the 12 months period ending 30 September 2002. This represents a seed volume of over one ton of seed, sent to 12 different countries (Table 103).

Table 102. Seed multiplication at the CIAT-Quilichao, CIAT-Popayan, and CIAT-Palmira experimental stations. (September 2001 to September 2002), totals by species.

Genus	*Species*	Number of Accessions	Harvest (kilograms)
Brachiaria	*brizantha*	12	**129.350**
Brachiaria	*decumbens*	1	13.000
Brachiaria	*lachnantha*	1	6.500
Calliandra	*calothyrsus*	3	11.250
Calliandra	*sp.*	1	1.100
Canavalia	*brasiliensis*	1	13.000
Centrosema	*pubescens*	1	21.000
Cratylia	*argentea*	38	196.045
Desmodium	*heterocarpon*	4	40.100
Flemingia	*macrophylla*	25	2.003
Lablab	*Purpureus*	32	16.818
Leucaena	*diversifolia*	1	0.140
Leucaena	*leucocephala*	2	55.400
Mucuna	*Sp.*	8	12.291
Rhynchosia	*schomburgkii*	2	4.650
Stylosanthes	*guianensis*	1	1.300
Vigna	*unguiculata*	23	341.050
Total		156	864.997

17 Genera; 156 distinct genetic materials (accessions), for a total of 864.997 kg

Table 103. Seed dispatched from CIAT forage seed multiplication unit (September 2001 to September 2002).

Genus	Kilograms	Number of samples	Genus	Kilograms	Number of samples
Andropogon	0.074	2	*Flemingia*	1.321	8
Arachis	172.738	37	*Galactia*	0.035	1
Brachiaria	572.932	158	*Lablab*	0.098	2
Cajanus	5.804	3	*Leucaena*	28.969	20
Calliandra	2.319	12	*Mucuna*	2.200	4
Canavalia	10.110	6	*Nenonotonia*	3.000	1
Centrosema	2.456	33	*Panicum*	0.150	2
Clitoria	24.200	2	*Pueraria*	1.174	9
Codariocalyx	0.008	1	*Stylosanthes*	2.989	24
Cratylia	174.120	102	*Zornia*	0.048	2
Crotalaria	0.031	1	*Vigna*	47.300	33
Desmodium	72.411	57			
Total kilograms :	**1,124.487**				
Total Number of samples: 520					

These samples were sent to twelve (12) countries: Costa Rica (2); Germany (9); Ecuador (1); Honduras (8); Kenya (1); Nicaragua (30); El Salvador (1); Tanzania (10); USA (1); Venezuela (16); Virgin Islands (1) and, Colombia distributed: CIAT (194), FIDAR (6), Universities (69), Particular (112), NARs: Corpoica (2), Agroamazonía (3).
A total of 520 samples, representing 1,124.487 kg were distributed.

Seed Unit of Atenas

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT).

Seed multiplication activities continued at the Atenas Seed Unit (Costa Rica) in collaboration with the Escuela Centroamericana de Ganadería (ECAG). The seed produced is destined to support advanced evaluations and promotions of promising forage germplasm both by CIAT's projects and regional research/development institutions.

From August 2001 through August 2002 a total of 879 kg of experimental and basic seed was either produced at Atenas or procured from associated collaborators. The bulk of the seed was formed by *Brachiaria* hybrid cv. Mulato (476 kg), *Cratylia argentea* (112 kg), *Brachiaria* spp. (99 kg), *Arachis pintoi* (63 kg), *Leucaena* spp. (26 kg), *Centrosema* spp. (13 kg), *Desmodium heterocarpum* spp. *ovalifolium* (6 kg) and *Paspalum* spp.(6 kg). Small quantities of experimental seed was produced of *Panimum maximum*, *Desmodium velutinum* and *Chamaechrista rotundifolia* spp. *grandiflora*..

During the period August 2001-August 2002 a total of 944 kg of experimental and basic seed were delivered by the Seed Unit of Atenas (Costa Rica). Table 104 shows that 92 seed requests were received from 10 countries, where more than half of the requests came from Costa Rica. However, a significant amount of seed was delivered to Nicaragua (149 kg), a country involved in forage projects with the assistance of CIAT. A high amount of experimental seed of *Brachiaria* species was delivered, particularly of Brachiaria hybrid cv. Mulato, which is being promoted regionally with the assistance of the private sector. A total of 36 requests were delivered of *Brachiaria* hybrid cv. Mulato.

Table 104. Countries, number of requests and amount of experimental/basic forage seed delivered by the Seed Unit of Atenas (Costa Rica) during the period August 2000-August 2001.

Country	No. of Requests	Forage species (kg)			
		Brachiaria spp	*A. pintoi*	*C. argentea*	Other species
Colombia	2	20.03		0.30	0.55
Costa Rica	76	255.06	111.10	111.00	12.35
El Salvador	1	4.00			
Haiti	3	0.20	6.00	0.80	4.80
Honduras	2	55.04	6.00	0.04	0.77
México	1			1.00	
Nicaragua	3	127.60	9.00	5.00	7.04
Panamá	2	50.00	11.00		
Puerto Rico	1		10.00		
Dominican Republic	1	72.10	50.00	10.10	2.10
Total	**92**	**584.30**	**203.10**	**128.24**	**28.61**

Activity 4.4: Expert systems for forages biodiversity by linking geographical information with biological data

Highlights

- Forage database Version 1 was released for the use by researchers and extensionists working with tropical forages.

- Interactive tool for incorporating Expert knowledge on forage in a GIS-based targeting tool was developed.

4.4.1 Development of a database and retrieval system for the selection of tropical forages for farming systems in the tropics and subtropics, SoFT

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Rationale

The demand for livestock products from both ruminants and monogastrics in the developing world is increasing at 6-8% per annum. This situation provides a unique opportunity for smallholder farmers in developing countries to increase income by satisfying that demand, and in some cases will enable them to advance to market-oriented production for the first time. Income obtained from market-oriented livestock production will impact on cash flow and purchasing power, and hence act as a driver for sustainable intensification. The challenge facing all farmers is to develop strategies to meet the feed demand of their animals and the market demands for livestock products of specified quality.

Sown tropical forages can provide part of the feed base that will support this market expansion. Forages can be used to provide improved feed quality and quantity in a range of farming systems, including those based on maize and rice cropping systems, as well as systems that depend almost entirely on continuous grazing of native grasslands. The benefits of including well-adapted sown forages in the diets of animals in these systems has been well documented, but adoption has been limited for a number of reasons, including poor access to appropriate information.

Farmers depend very heavily on advice from extension specialists, development agencies, researchers and seed companies, but their knowledge is often limited because of inexperience in a region, the difficulty in harnessing the expertise of others, and poor access to information. Much of the important information is fragmented, unpublished or published in media of limited circulation. This project intends to synthesize and interpret this information and so overcome this limitation to the wider adoption of forages.

This project will bring together in one knowledge system (SoFT – Selection of Forages for the Tropics) much of the accumulated information on species adaptation, its use and management of the last 50 years from across the tropical world. Information will be sourced from scientific literature, the plethora of reports, and from unpublished information gleaned directly from agronomists with extensive tropical experience. The completed product will be a computer-based system that can be used to select "elite" forage accessions for a range of uses, farming systems and environments. This synthesis of information on the use (e.g. fish, pigs, mulch) and application (e.g. ley pasture, under trees, relay with rice) of forages, in addition to the conventional genotype x environment information, is unique and made possible by the development of new information management systems.

The specific objectives of the project are:

- To develop a knowledge system for the identification of forages suitable for specified niches within smallholder farming systems.
- To promote the system within the "communities" who are using tropical forages.
- To develop a strategy for maintenance and updating the knowledge system.

Materials and Methods

To develop a knowledge system for the identification of forages suitable for specified niches within smallholder farming systems. The LUCID database platform will be adapted for use in this forages application, including GIS capabilities. The development of criteria for database design for these features will be carried out in an incremental fashion, with the first model being developed in partnership with project members and Australian extension specialists and then modified in response to inputs from workshops of potential users identified in SE Asia (Thailand, Laos) and scientists who have expertise in delivery of information to smallholder farmers. This "user group" will also be invited to evaluate the system to ensure in the final stages of the project to ensure it meets their requirements and is sufficiently user-friendly. Members of the user group have been selected because they have known expertise in forage use in the various farming systems. Information for the database will be acquired from two major sources, i.e. from the literature, including various project reports with limited circulation, and through small, focussed workshops attended by invited specialists. Data from literature will be sourced at four locations, Australia, Africa (ILRI), CIAT and Europe. Europe has been included because of the vast amount of data residing in Universities and Funding Agencies – primarily in Germany and France but also in the UK. Best results will almost certainly be achieved by employing experienced (perhaps retired) agronomists who already are aware of the current available data sources. Information will also be sourced from workshops. A workshop will be held in Africa, in Asia and in the Americas (covering south, central and north America including the USA), in Europe (Germany) in Australia. The prime aim of these workshops is to acquire information and knowledge of forage use and adaptation that has not been published. However, the experienced tropical forage agronomists attending these workshops will also be asked to assist in identifying further sources of published information and other agronomists who should be contacted at a later date. Information arising from both the literature reviews and the workshops will be evaluated prior to inclusion in the database. One of the components of the database will be a comprehensive bibliography. An information/fact sheet will be written for each forage in the database. Many of these already exist in other databases or in text format, such as facts sheets from the Queensland Department of Primary Industries, ILRI, CIAT, FAO, PROSEA, etc. Where appropriate, permission will be sought to use existing fact sheets at least as a starting point. However, it is envisaged that in most cases information gleaned from forage specialists will necessitate the review of existing sheets and more likely, a complete re-write to accommodate the wider scope of this knowledge system. Similarly, photographs and line drawings of forages will be sourced from existing publications where possible but new material will also be sourced from participating agronomists. On completion of the Alpha version of SoFT the system will be evaluated to ensure that selections made by the system are appropriate and that users find it user-friendly. It is planned that the final testing of technical quality of the outputs (i.e. the selection of forages, the GIS outputs, the associated bibliographies) will take place at a workshop of approximately 6 agronomists with broad international experience. After this initial evaluation, and subsequent adjustments made to the system, a broader cross section of forage agronomists will be asked to evaluate the system and feed information back to enable errors to be corrected or modifications made. After this final technical review, the user groups who assisted in the initial design Production will take place in the last 6 months of the project, with the system initially being produced in English. Translation of the knowledge system into other languages is outside the scope of the current project, although translation into some Asian languages is considered desirable.

To promote the system within the "communities" who are using tropical forages: The database will released at the International Grassland Congress, Dublin, 2005. This will be associated with a contract session focussing on the adoption and effectiveness of forages in smallholder and commercial systems. The project will also publicize its use within the four regions via key NARS and NGOs and through research and development agencies. It will be made available on both the WEB and on CD/DVD.

Availability and publicity via the CGIAR Virtual Systemwide Livestock Initiative and its associated newsletters and networks, will ensure wide awareness and distribution. Members of the project team and their associates will use opportunities while visiting developing country institutions and during regional conferences and meetings to demonstrate and promote the product. The ownership of the project developed by the users groups will be utilized for promotion of the system within their domains. During the course of project development formal linkages with CAB International will be negotiated to establish linkages with the Animal Health and Production compendium. Opportunities to link the knowledge system with the Virtual Colombo Plan initiative will also be explored during the conduct of the project in association with ACIAR. Such penetration into the education market could have a positive effect on the longer-term use of the system by tertiary graduates.

To develop a strategy for maintenance and updating the knowledge system: The database will be updated from time to time as new information becomes available. Such updates are anticipated every 3-5 years. Funding for this activity will come from sales of the knowledge system. Although copies of the CD/DVD will be available at no cost in developing countries, potential users and libraries in developed countries and development agencies will be charged. Similarly for WEB users, a password will be supplied at minimal cost to users in developed countries while developed country users will be fully charged for the password.

This activity recently began with a survey of potential users on the requirements for SoFT. This survey will be used a background information for a design and planning workshop to be held in Asia in October 2002.

4.4.2 Development of a forage database

Contributors: Fabian Barco, Manuel A. Franco, Luis H. Franco, Belisario Hincapie, Carlos Lascano, Gerardo Ramirez and Michael Peters

The International Center for Tropical Agriculture (CIAT) developed a Forages Data Base to facilitate the selection by researchers and extensionists of adapted and productive grass and legume genotypes for different land-ecosystems in tropical areas and by doing this benefit a large number of farmers.

To develop de Database CIAT relied on results from forage germplasm evaluation carried out since 1978 in South America, Central America, the Caribbean area and in West and Central Africa, in collaboration with National Agricultural Research Institutions.

The Database being released this year and which reflects the efforts of many forage researchers contains information on the following subjects:

1. Agronomic characterization of 5.374 accessions of grass and legumes, evaluated in experimental stations of CIAT in Colombia
2. Evaluation for adaptation of 2.209 forages accessions in 8 mayor screening sites representing the savanna, forest margins and hillsides ecosystems
3. Performance of forage accessions in 230 sites distributed in tropical America and Africa, which represented different environmental conditions.

A high proportion of the results included in the Database came from formal Forage Evaluation Networks and G x E Trials, such as:

1. RIEPT (International network of evaluation of tropical pastures)
2. RABAOC (Reseau de Recherche en Alimentation du Bétail en Afrique Occidentale et Centrale

3. Centrosema Network
4. Colombian Brachiaria Network
5. Multi-location trials of Arachis and Desmodium collections in Colombia

All results in the Database are presented by forage accession (CIAT No) and by evaluation site using basic statistical methods like average, standard deviation, and maximum and minimum values.

The platform of the Forage Database was developed by CIAT in 2000 using Microsoft Access trough an application in Visual Microsoft InterDev. As a result information contained in the forages Database, could be included in a CD-ROM (Photo 26) and Internet.

Photo 26. Forage Database in a CDROM

4.4.3 Incorporating Socio-Economic Data and Expert Knowledge in Representation of Complex Spatial Decision-Making

Contributors: Rachel O'Brien (CIAT, Curtin University), Robert Corner (Curtin University), Michael Peters, Simon Cook, Thomas Oberthür (CIAT)

Rationale

The overall approach which intends to integrate agro-ecological, economic and social information, is based on the following two main assumptions:

- A wealth of information on the agro-ecological adaptation of forage germplasm is available in CIAT-held and other forage databases. However, the access and hence utilization of this information needs to be improved. In addition data is often uncertain or missing, and methods are needed to combine existing data with expert knowledge to provide better analysis.

- In previous evaluations of forage germplasm adaptation to environmental conditions, agro-ecological information is separated from socio-economic factors influencing forage germplasm adoption.

Based on these assumptions, the targeting of forage germplasm is intended to enhance the utility of existing information and, in future, to integrate environmental and socio-economic adaptation of forage germplasm for multiple uses. It is anticipated that this approach will allow a more accurate and client-oriented prediction of possible entry points for forage germplasm.

One product of this research will be a fully functional web-based or CD-ROM tool, primarily designed for targeting forage germplasm in Central America. The primary target users are NGO's, development agencies, national research institutes and decision-makers in government. In conjunction with farmers, these users will be able to more effectively target suitable locations for new forages, with the aid of the tool. This will result in more informed choices being made, thus allowing more effective use of public funds dedicated to agricultural development and natural resources conservation.

Tools to better target forages will also help improve the wellbeing of smallholders by assisting them to more effectively utilize their resources in sustainable ways. The addition of carefully selected forages to a farming system has a plethora of benefits both for the farmers and for the environment as well as the wider community. These benefits derive both from the direct influence of forage planting, and the indirect increase in cattle production and cropping system improvements, and include for example improved sustainable intensification, reduced erosion and alleviation of protein and micronutrient deficiencies in the community.

Materials and Methods

Literature and model review. Review of literature and of existing similar models and software is ongoing. Possible approaches to representing spatial decision-making with particular emphasis on incorporating socio-economic data and expert knowledge are under investigation. Existing tools are being evaluated to determine their effectiveness in representing expert spatial decision-making processes and in particular in targeting forage germplasm. Bayesian modeling has been identified as the most appropriate method, especially with sparse and uncertain data.

Case study – design and develop a tool. As a case study, a decision support tool to target forage germplasm is being designed and developed, using geographical information system (GIS) technology. This targeting consists of identifying which forages would be suitable or successful in a particular location, given data and/or knowledge about the forages and about the location in question.

The forage data is in the form of spatial coordinates for a number of locations at which accessions have performed successfully. Success is defined from data available in the CIAT forages database, other data sources and input from experts, including adaptation, production and adoption. It is recognized that definition of success may vary from species to species, and may also depend on the unique characteristics of the farmer. In particular, a risk-averse farmer may require a high level of certainty that the selected forage will be successful according to his or her criteria, whereas in other situations a lower level of certainty but a higher rating of success may be desirable.

The scope of the final tool will be decided in conjunction with end users. The majority of the research is being carried out at CIAT in Cali, Colombia. CIAT holds an extensive forage germplasm database, as well as various climate and other biophysical GIS databases. CIAT also is providing expertise on forage adoption and GIS technology. Data has been collected for Central America, focusing on the San Dionisio region in Nicaragua and the Yorito region in Honduras. The tool will then be provided to users in other areas for further testing and verification.

Consultation. Prior to and during the development of the tool, there has been consultation with potential users. These potential users are representatives from CIAT, and development agencies,

NGOs, national research institutions and ministries of agriculture and natural resources in Central America, with focus on Honduras, Nicaragua and Costa Rica.
Testing and validation. Because a large part of the inputs to the model are based on expert knowledge, it is difficult to validate the model using data-based techniques such as bootstrapping and jack-knifing. Expert opinion will be necessary to judge the accuracy and effectiveness of the model. In addition the model will be applied to locations where forage trials are currently underway, but not included in the databases used to specify the model. Case study data will also be applied to alternative existing software packages, in comparison to the tool being developed here.

Implementation. The tool is being implemented using Delphi in conjunction with MapObjects LT. In this way the user requires no proprietary GIS platform.

Results and Discussion

Potential users and stakeholders. A number of potential users and stakeholders in development agencies, NGOs, national research institutes and ministries of agriculture and natural resources in Central America were visited in early 2002. In particular MAGFOR Nicaragua, INTA Nicaragua, CATIE Costa Rica and CIAT field sites have shown interest. Potential users will be involved in the testing of early versions of the model. In addition the model is being adapted for use in tropical fruits targeting.

Data. There is a variety of data available on forages (RIEPT, Brachiaria, Arachis, Desmodium, RABAOC and experimental stations) as well as GIS data for climate, elevation, soils, population, accessibility, etc. One of the main sources for forage data is the RIEPT database, containing data on adaptation, establishment and production of forage species in Latin America. There are data on 2539 trials of 929 accessions in 28 locations in the CIAT RIEPT database for Central America (Figure 95). An initial analysis of RIEPT elevation, temperature and rainfall data against 1km grid GIS data shows poor correlation for sites in Central America (ranging from $R^2 = 0.08$ for mean August rainfall to 0.75 for mean December rainfall). However analysis shows that much of the data is fairly strongly correlated with some obvious outliers. Removing these outliers improves correlation greatly, however the outliers are not always the same for all months.

Different reasons for outliers. Mislocation. Site AL04 Turrialba (Costa Rica) lies at 9°55’21” N 83°49’40” W according to the RIEPT database, and at 600 m asl. However on the 1km DEM this location lies at 2064 m asl. There is also a large discrepancy between temperature and rainfall data, suggesting the coordinates are wrong.

Missing data. Site AE15 Comayagua (Honduras) has an elevation of 0 m asl in the RIEPT database, but 555 m asl on the 1km DEM. This suggests the elevation data is simply missing in the RIEPT database.

Internal inconsistencies. Sites AL16, BL86 and AL29, amongst others, lie on the same coordinates in the RIEPT database. Yet they all have different entries for temperature.

Detecting errors and deciding which data to trust. A procedure could easily be written to compare temperature and rainfall data between RIEPT and GIS data, where both sets of data are available. Discrepancies could then be earmarked for manual follow-up. These will in general fall into three categories:

Mislocation: coordinates in RIEPT database are wrong. Solution: adjust coordinates by comparing with other resources. GIS data coarseness: coordinates may be correct but the 1km DEM and climate surfaces may not represent adequate variation within the 1km. However this will not explain strong outliers. Solution: decide whether RIEPT or GIS data is likely to be more accurate. Data errors: typing errors, copying errors, etc in the RIEPT database. Solution: use GIS data. Missing data: blanks in the RIEPT database. Solution: complete using GIS data. In addition GIS data and RIEPT data will obviously never agree completely, so we need to decide which to use. It makes sense to use the soil data from the RIEPT database, as this is likely to be more accurate than anything we can get from GIS data. But should we use any RIEPT data at all for elevation, temperature and rainfall, or should we rely entirely on GIS data.

Figure 95. RIEPT database sites in Central America

Models and algorithms. Literature on similar models and algorithms has been reviewed, as well as existing software packages. Bayesian methods were identified as the most useful way to deal with uncertain data and expert knowledge. Bayesian modeling has been applied to core forage species using climate and soil data and expert knowledge (Figure 96).

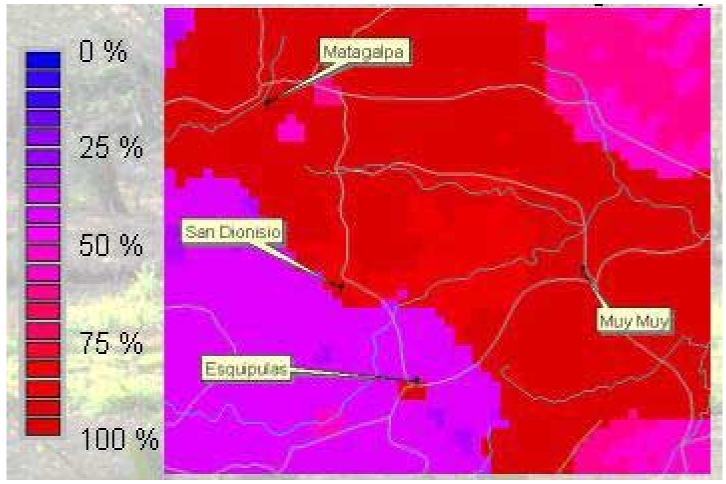

Figure 96. Bayesian modeling applied to *Stylosanthes guianensis* using elevation, annual rainfall and length of dry season to predict the probability of adaptation being excellent or good.

Tool development. The project is linked to SoFT (Selection of Tropical Forages, see section 4.4.1) with the developed tool separate but complimentary to their work. SoFT is an international project started in 2002, in which CIAT is involved, which aims to collect and make accessible expert knowledge about tropical forages. The SoFT database will be an input to the tool, along with the RIEPT database and other expert knowledge. A demonstration tool has been implemented in Visual Basic showing the various steps of the model: choosing a location, filtering out species, updating prior probabilities, calculating joint probabilities, ranking species for a given location, viewing and querying map output (Figure 97).

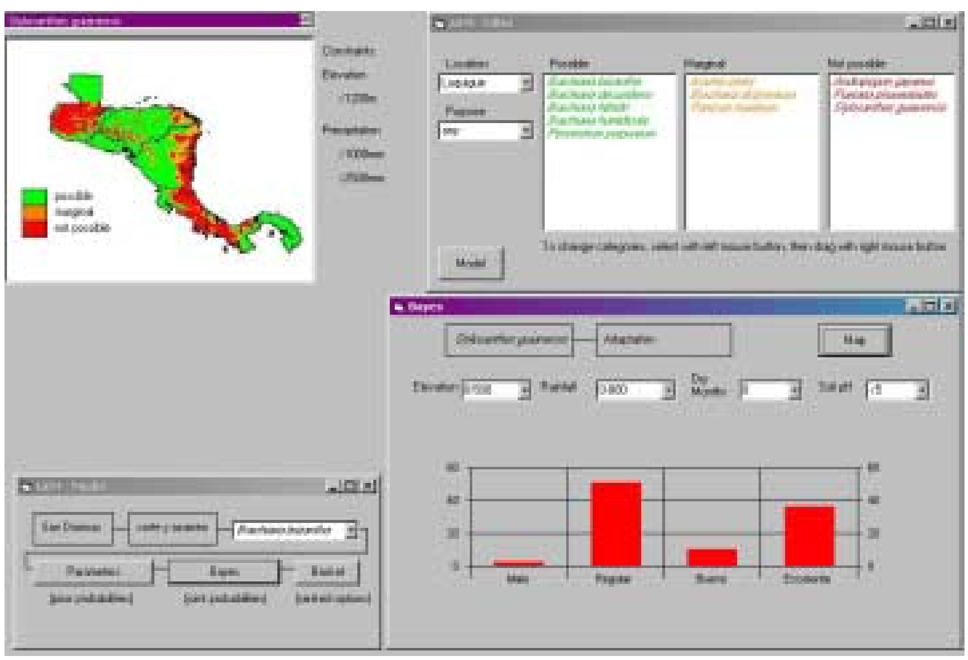

Figure 97. Demonstration tool showing steps in the model.

A prior probability tool has been developed in Delphi to elicit expert knowledge to update data-derived prior probabilities. The expert can manipulate probabilities with simple point-and-click operations and examine via bar charts and tables the impact on all other probabilities (Figure 98).

Figure 98. Tool to elicit expert knowledge to update or create prior probabilities for use in Bayesian modeling.

This tool will be used to create prior probability tables for all species as input to the Bayesian modeling phase of the tool.

Future activities

- A Beta version of the tool will be presented at a forages expert meeting in Bangkok in October 2002.
- Socio-economic data and knowledge, including management systems and access to market, will be incorporated into the tool, either at the same time as biophysical data, or as a separate hierarchical layer.
- Real time mapping functionality will be added to the tool.
- The model will be adapted for other commodities, including production of a tropical fruit-targeting version of the tool.

- The forages tool will be extended to include all forage species for which data and/or knowledge is available, and also some species will be further subdivided into accessions where this is deemed useful by experts.

- The forage tool will be evaluated by collaborators in Central America and their feedback used to update the tool.

- Methodology will be developed to assess the accuracy of the tool, in particular in comparison to alternative methods of targeting forages.

Actvitity 4.5 Facilitate communication through newsletters, journals, workshops and internet (IP5 Staff)

Highlights

- A new and improved version of CIAT's Forage Web Site was launched this year.

- Newest information on the bioecology and management of grass-feeding spittlebugs transmitted in five field days to 293 stakeholders

- Newest information on the bioecology and management of spittlebugs summarized in technical bulletin being prepared for publication

- The Journal Pasturas Tropicales celebrated its 20th Anniversary this year. To assist in the consultation of and Index of Authors and Subject Matter was published.

4.5.1 Development of a Forage Web Site

Contributors: Simone Staiger

The revamped Web site of CIAT's Tropical Forages Project, launched in January of this year, is the result of teamwork between all Project members, under the general Web site coordination of the Communications Unit and with the support of both the Systems and the Information and Documentation units.

The Web site has allowed us to disseminate our research results extensively and to promptly communicate important news, for example the release of a variety of *Cratylia* (Photo 27).
Characteristics of the Web Site. The site, accessible under the URL http://www.ciat.cgiar.org/forrajes/index.htm, allows users (universities, research institutes, partners, donors, and the scientific community in general) to:

- Consult the forages database.

- Consult databases on the extensive genetic resources of tropical forages conserved in the CIAT gene bank.
- Make on-line requests of samples of unimproved germplasm, which is available free to researchers and farmers.

- Contact CIAT plant breeders to obtain samples of improved germplasm.

- Browse the easy-to-use catalog of electronic and printed products, many including useful tools and methods.

- Download PDF files containing the full text of recent publications and documents.

- Obtain specific information about each research theme: germplasm, highly nutritive grasses and legumes, genetic improvement of *Brachiaria*, pathology and endophytic fungi, spittlebug bioecology, host plant resistance to the spittlebug, and adaptation to abiotic stress.

- Access additional information resources, such as a publications list, the full text of newsletters, background documents, annual reports, and general information about the project (project description, staff list, links to partners and donors).

- Keep up-to-date on the latest advances through Homepage's news section.

Photo 27. CIAT's Forage Web Page

Statistics Illustrate the Initial Impact of the New Web Site. The visits to the site are definitely on the rise, with more than 1800 visits in August (Figure 99).

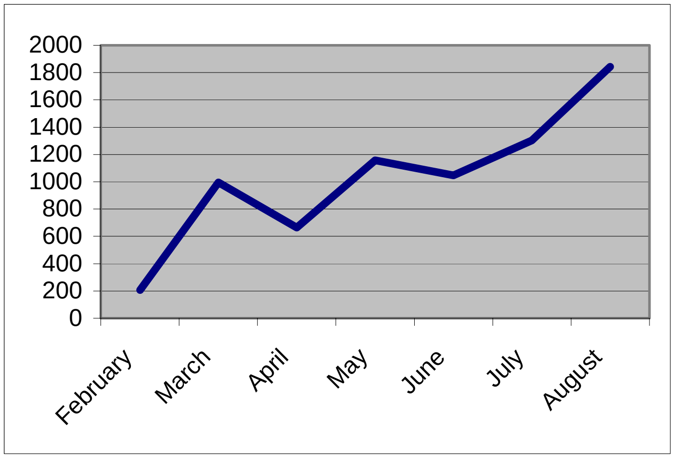

Figure 99. Visits per Month 2002

Hits per Page. Besides the project's homepage, the most visited pages were the ones on released varieties, the publication list, and the background document. The pages relative to the 'Products' list are not included in these statistics because they belong to the general CIAT Product Database—the most visited section of the Center's Web site (Figure 100).

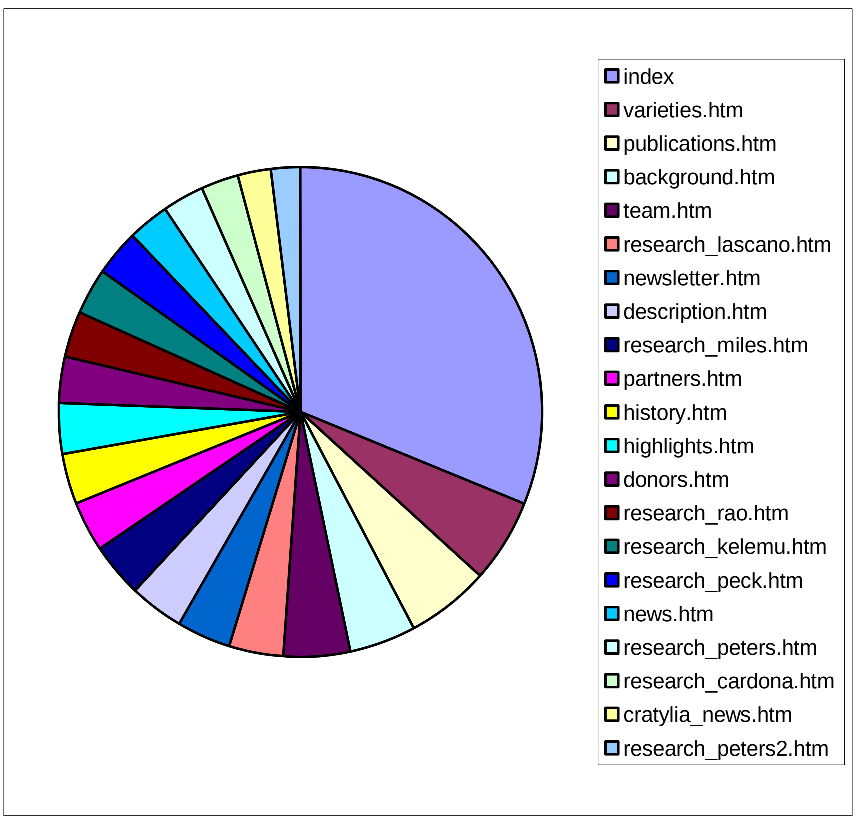

Figure 100. Most visited pages the Forage web site

Downloads. To date, the most popular documents downloaded by users have been news relative to Cratylia (*Cratylia argentea* cv. Veranera, a output of the agreement between Colombia's MADR, CIAT, and CORPOICA) and the report submitted to the Colombian Government. There is also a large interest for the Annual Report (Figure 101).

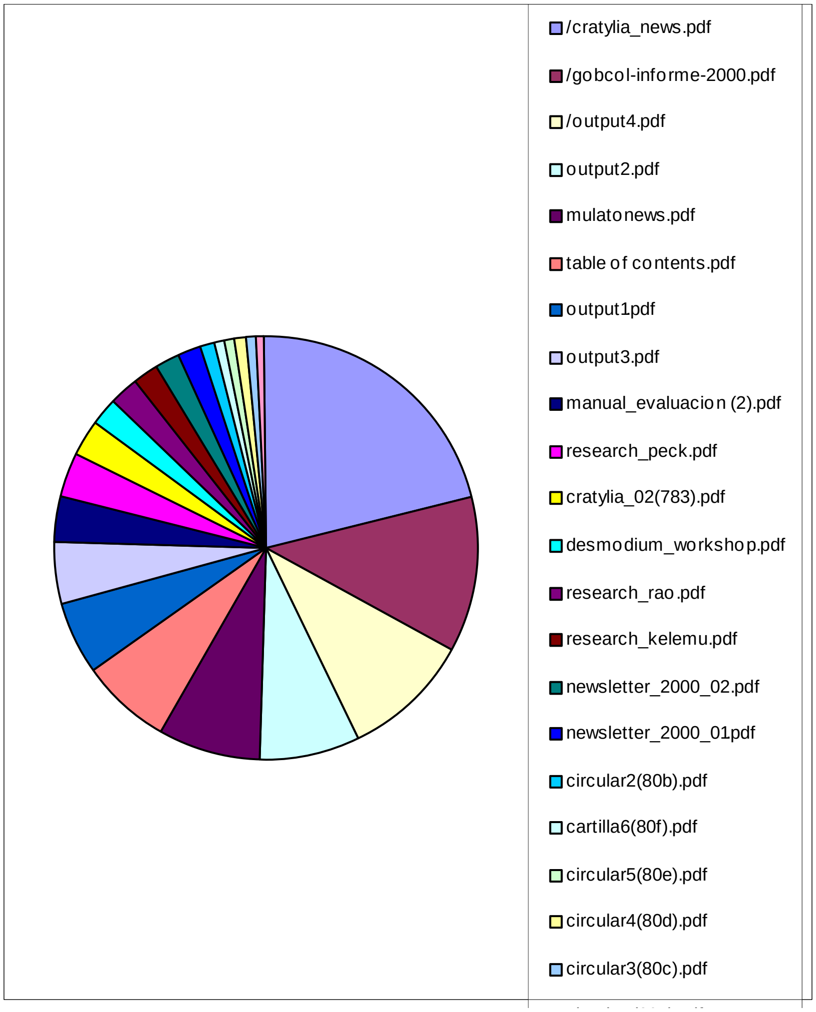

Figure 101. Downloads from CIAT Forage Web Page

Perspectives. The project aims to continuously update the information on the Web site, specifically the Research Themes, and include more downloadable documents in PDF format. Plans are to include an interactive map showing the global reach of Project activities.

4.5.2 Updating FAO Grassland Index

Contributors: R. Schultze-Kraft, M. Peters, A. Schmidt and M. Andersson

It is of high importance that up to date information on forage germplasm is available to research and development practitioners in an accessible form. The FAO Grassland an pastures group is therefore updating basic information from two FAO publications "Tropical Grasses" (1990) by Skerman, P.J. and F. Riveros and "Tropical Forage Legumes" (1988) by Skerman, P.J., Cameron, D.G. and F.

Riveros. CIAT and collaborators are contributing to this effort in writing profiles for species where CIAT has a high expertise.

The following species profiles were prepared:.

- SCHMIDT, A. PETERS, M. AND SCHULTZE-KRAFT, R. (2001) *Desmodium heterocarpon* (L.) DC. subsp. o*valifolium* (Prain) Ohashi. FAO Grassland Index, Rome, Italy.

- PETERS, M. AND SCHULTZE-KRAFT, R. (2002) *Centrosema brasilianum* (L.) Benth. FAO Grassland Index, Rome, Italy.

- ANDERSSON, M. SCHULTZE-KRAFT, R. AND PETERS, M. (2002) *Flemingia macrophylla* (Willd.) Merrill. FAO Grassland Index, Rome, Italy.

- PETERS, M. AND SCHULTZE-KRAFT, R. (2002) *Cratylia argentea* (Desv.) Kuntze. FAO Grassland Index, Rome, Italy.

- PETERS, M. AND SCHULTZE-KRAFT, R. (2002) *Aeschynomene histrix* Poiret

In addition corrections and additions have been made in a number of other species profiles

4.5.3 Twenty years of Pasturas Tropicales

Contributor: Alberto Ramirez (Editor)

The Journal Pasturas Tropicales first appeared as a CIAT publication in 1981 as an upgrade of the Informative Bulletin on Tropical Pastures that was first published in 1979. Pasturas Tropicales was CIAT's response to the need at the time of having a high quality and periodical publication that would allow researchers participating in the International Pasture Evaluation Network (RIEPT) to publish there work. From the beginning and up to know Pasturas Tropicales is recognized as high quality publication and as a result it is a preferred journal for many researchers in Latin America who are committed to developing improved forages and in seeking better management strategies for pastures to solve livestock production constraints of farmers in tropical regions.

Up to now (Number 3 -Volume 23 of 2001) a total of 474 documents have been published in 2342 pages. The scientific articles and research notes were submitted by 563 authors and collaborators from different national institutions of Brazil, Colombia, Costa Rica, Mexico, Panama, Peru, Venezuela, Paraguay and Argentina (Table 105).

A distribution of articles submitted to Pasturas Tropicales by country, indicates that many of the researchers were from low tropical areas of Brazil and Colombia where large areas of land are dedicated to pastures/livestock production (Figure 102).

To assist in the consultation of articles published during the last 20 years (1981-2001) in Pasturas Tropicales and Index of Authors and Subject Matter was produced and it is now available in the Web page of CIAT Tropical Forages Project (Photo 28).

In spite the good reception of the Journal among forage researchers in the region, a problem that still needs resolution is funding for the publication. About 50% of the cost of publishing and distributing Pasturas Tropicales comes from subscriptions and the rest is subsidized by CIAT. Given that this arrangement is not sustainable there is an urgent need to obtain alternative funds (i.e. Advertisement) or to reduce distribution costs by publishing Pasturas Tropicales as an e-Journal.

Table 105. Main national institutions publishing research results in the magazine Tropical Pastures between 1981 and 2001.

Country	Institution
Brazil	Embrapa-CNPq, Embrapa-Cerrados, Embrapa-CNPGl, Embrapa-CPAC, Empresa de Pésquisa Agropecuária de Itajai (EPAGRI), Centro Estadual de Pesquisa Aplicada em Sericicultura (CEPAS), Embrapa-CPATU, Embrapa-UEPAE, Embrapa-EMPASC, UNESP-Botucatu, Ceplac, Embrapa-Cenargen, UFLA, FEALQ, Epamig, ESALQ, Instituto de Zootecnia-Nova Odessa, Embrapa-Amazonia Oriental, UNESP, IPA
Colombia	CIAT, CIAT-CIRAD, ICA, Secretaría de Agricultura de Antioquia, Universidad Nacional sede Medellín, Universidad Nacional sede Palmira, Universidad de Antioquia, Universidad de la Amazonia, CORPOICA
Mexico	Universidad Veracruzana, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Fonaiap, Inifap, Colegio de Posgrado-Chapingo
Costa Rica	CATIE, MAG, Universidad Nacional, Instituto Tecnológico
Bolivia	IBTA, Universidad Mayor de San Simón
Panama	IDIAP
Peru	Universidad Mayor de San Marcos, IVITA, INIA, Universidad Agraria La Molina
Venezuela	Universidad Central, Instituto de Zoología Tropical
Argentina	Estación Experimental Obispo Colombres, Universidad Nacional del Nordeste, INTA, Universidad de Buenos Aires,
Cuba	Instituto de Ciencia Animal

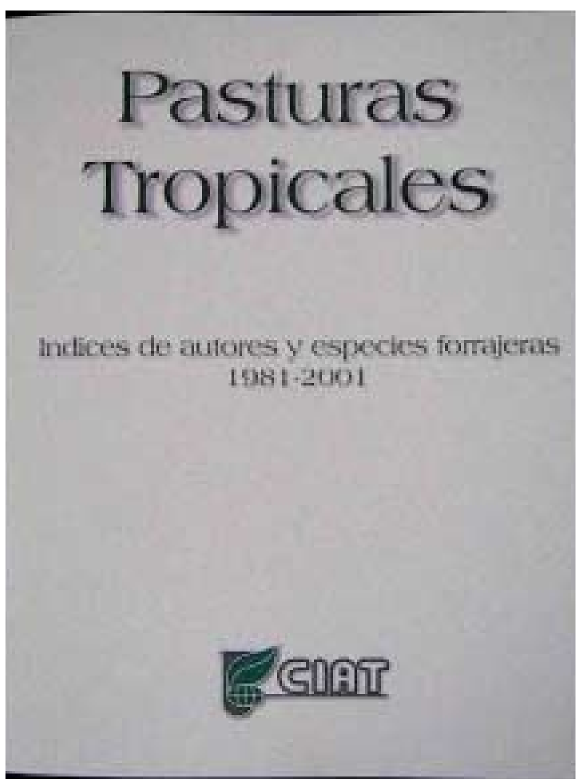

Photo 28 . Index of Authors and Subject Matter and it is now available in the Web page of CIAT Tropical Forages Project.

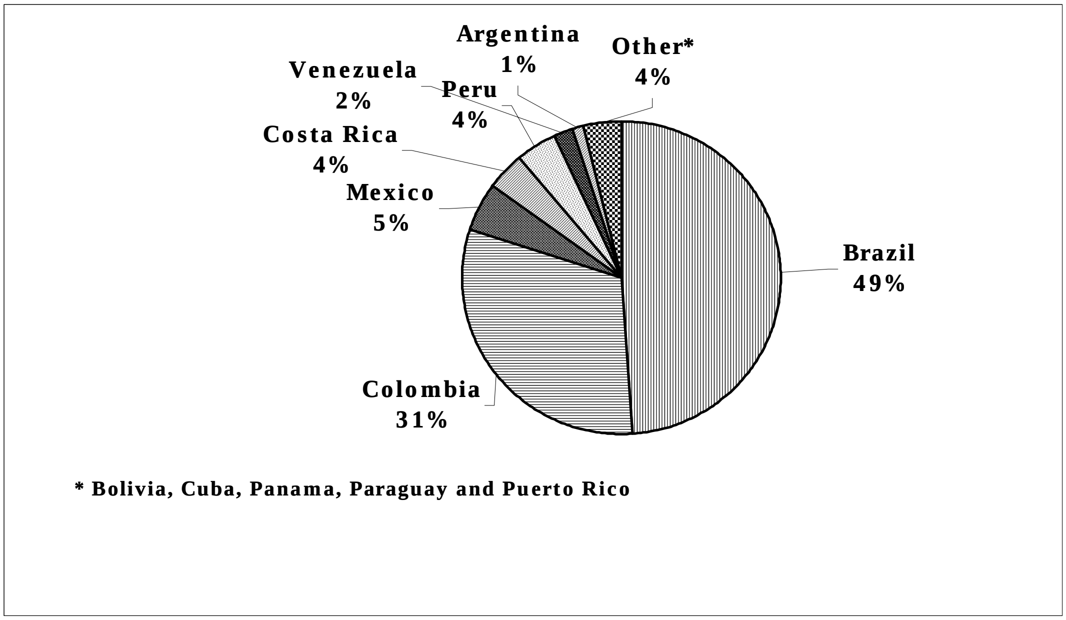

Figure 102. Distribution (percentage), by country, of the articles published in Psturas Tropicales between 1981 and 2001.

4.5.4 CIAT field day on spittlebug biology and management

Contributors: Anuar Morales, Ulises Castro, Jairo Rodríguez, Francisco López, Daniel Peck

Five field days on spittlebug biology and management were carried out to transmit the most recent research results throughout Colombia. With organizational help from local collaborators, four CIAT support staff traveled to these sites to present a series of talks and field visits.

The field day was designed for a diverse group of stakeholders including professionals, technicians, and students of agronomy, biology and veterinary sciences, as well as ranchers, administrators and farm managers who desired to learn more about this group of insects and strategies and tactics for their management. A total of 293 people received training in these events:

- Universidad del Sucre, Sincelejo (Sucre), 31 October 2001, 60 participants
- Universidad San Martín, Barranquilla (Atlántico), 2 November 2001, 35 participants
- Universidad de la Amazonía, Florencia (Caquetá), 6 November 2001, 136 participants
- C. I. La Libertad, Villavicencio (Meta), 9 November 2001, 18 participants
- CIAT, Palmira (Valle del Cauca), 11 March 2002, 44 participants
- Universidad Nacional, Palmira, 26 September, 2002, 104 participants

4.5.5 Prepare technical bulletin on spittlebug bioecology and monitoring

Contributors: D. C. Peck, U. Castro, F. López, A. Morales and J. Rodríguez

To further transmit recent research results on the bioecology and management of grass-feeding spittlebugs, a first draft of a technical bulletin was prepared with the following citation:

- Peck, D. C., U. Castro, F. López, A. Morales & J. Rodríguez. El Complejo Salivazo de los Pastos en Colombia. Boletín Técnico, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Manuscript in preparation, 24 pp.

This manuscript is now being advanced for submission to the CIAT graphics arts section for publishing. It features two sections. The first section summarizes the status of information on the bioecology and management of spittlebugs in the following subsections: Introduction, Diversity and Distribution, Biology, Recognition of the Life Stages, Behavior, Ecology, Natural Enemies, Management Tactics and Management Strategies.

The second section summarizes our knowledge of the spittlebug complex in the varying ecoregions of Colombia where they are economically important: Caribbean Coast, Orinoquian Piedmont and Eastern Llanos, Amazonian Piedmont, Cauca River Valley, and Pacific Coast

4.5.6 Bibliographic database on spittlebug bioecology available on webpage

Contributors: Daniel Peck, Mariano Mejía, Carlos Saa, Edith Hesse

Rationale

A major limitation to advances in the management of spittlebugs in forage grasses and sugar cane is difficult access to information. There are no published reviews of the insect family Cercopidae despite its economic significance in forage grasses and sugar cane. Such material exists for other groups of economically important Homoptera such as the leafhoppers, planthoppers, aphids, scales and whiteflies, but students of the spittlebugs and froghoppers must turn to articles and gray literature to acquire an understanding of this group of insects. Second, reviews of the biology and management of spittlebugs are inadequate. The few that exist are not widely disseminated, are outdated, and contain overgeneralizations and erroneous information, particularly regarding taxonomy. Third, much of the available information is in gray literature sources that are difficult to access. The quality of research from small and isolated universities or research teams is challenged by not being able to acquire the information necessary to support studies on this pest group.

Methods

To start to overcome some of the limitations in information dissemination, we are strengthening our reference collection on the Cercopoidea. References have been gathered over the last 11 years. In 2001 we began working with the CIAT Information Services to make this information source available on-line and here report advances in that endeavor.

Results and Discussion

We currently have physical copies of 688 references related to the superfamily Cercopoidea. Of these, 478 are directly related to spittlebugs in graminoids, 325 related to forage grasses, 146 related to sugar cane and 26 related to other graminoids such as rice and turfgrass. At present, all references are housed alphabetically in filing cabinets of the Spittlebug Bioecology and IPM Research Group and by the end of the year will be housed in the CIAT library.

All citations have been entered into an electronic database (EndNote) and most have now been edited and corrected for consistency and accurateness. Key words have been assigned to each citation to facilitate searching from within the program software (CIAT 2001). Categorical labels were also assigned to facilitate subgrouping of references in the initial on-line database (CIAT 2001). The initial version of the on-line database is a rigid (non-searchable) database divisible into categories with relevant references listed alphabetically. This version will probably be available on the CIAT web page by the end of the year and will include information on how to order copies of references from the CIAT library.

4.5.7 Symposium on spittlebug to communicate research results to Pronatta

Contributors: Daniel Peck, Anuar Morales, Jairo Rodríguez, Ulises Castro

Two symposia were carried out to transmit the results of two Pronatta projects to a Pronatta review board and the public. The period of execution of each project was 9 February 2000 to 6 August 2002. Project titles were "Establecimiento de hongos entomopatógenos como alternativa para manejar el salivazo de los pastos" and "Avances en el manejo del mión de los pastos (Homoptera: Cercopidae) mediante el pronóstico e interpretación de la primera generación."

The first symposium was 5 August 2002 at CIAT with a review panel of three Pronatta staff. This featured presentations of research results, discussion of impact and a review of the final technical reports.

The second symposium was carried out 26 September 2002 at the Universidad Nacional in Palmira as the 47th Foro Entomológico "Taxonomía, distribución y manejo del salivazo de los pastos (Homoptera: Cercopidae)." A total of 27 professionals and 77 students attended this event that featured presentations and a round table discussion.

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Awards

Hernán Alcaráz Viecco Award
"Desarrollo de híbridos de Brachiaria resistentes a cuatro especies de salivazo (Homoptera:Cercopidae)"
Guillermo Sotelo, Cesar Cardona y John W. Miles

Francisco Luis Gallego Award
Caracterización de la resistencia a tres especies de salivazo (Homoptera:Cercopidae) en un híbrido de Brachiaria sp. resistente a *Aeneolamia varia* (F.)
Paola Fory, Guillermo Sotelo y Cesar Cardona

Coconut Island Commemorative Award
Granted by Hainan Provincial Government- Gold Medal, for collaboration on participatory evaluation of forages: Ralph Roothaert

List of Donors

	Project duration
Australia – **ACIAR**	1998-2001
Anthracnose disease in *Stylosanthes*	
Development of a knowledge system for the selection of forages for farming systems in the tropics (co-financed with BMZ)	2002-2005
CURTIN UNIVERSITY	2001-2003
Incorporating socio-economic data and expert knowledge in representations of complex spatial decision-making (stipend PhD Rachel O'Brien)	
Centroamérica – **Common Fund for Commodities (CFC)**	2003-2006
Enhancing beef productivity, quality, safety, and trade in Central America (Guatemala, Nicaragua, Honduras)	
Colombia – **COLOMBIAN GOVERNMENT**	1999-2003
Grasses and legumes with high nutritive quality	
Grasses and legumes adapted to low fertility acid soils	
Grasses and legumes with adaptation to drought, poor soil drainage and resistant to pests and diseases	
Integration of improved grasses and legumes in production systems in savannas	
PRONATTA	
Fungal entomopathogens as an alternative for spittlebug management	2000-2002
Intepretation and prediction of spittlebug phenology	2000-2002
Utilidad de la leguminosa semi-arbustiva *Cratylia argentea* en sistemas de ganado de doble propósito del piedemonte llanero: Validación y Difusión	2001-2003
ECOFONDO (through FIDAR)	2000-2002
Alternativas para la recuperacion de suelos degradados en zona ladera del departamento del Valle del Cauca	
COLCIENCIAS (through FIDAR)	2000-2003
Evaluación biofísica y económica de especies arbustivas y arbóreas del ciclo corto para el manejo de suelos erosionados en ladera	
Banco de la República	2001-2002
Caracterización del salivazo de los pastos *Prosapia simulans*	
Germany – **BMZ**	
Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America	2000-2003
Utilising multipurpose legume diversity to improve soil and feed quality including application in a watershed in the Central American hillsides – PostDoc program A. Schmidt	2000-2003
An integrated approach for genetic improvement of aluminium resistance of crops on low-fertility acid soils	2001-2003
Development of a knowledge system for the selection of forages for farming systems in the tropics (co-financed with ACIAR)	2002-2005
DAAD (German Academic Exchange Service, University of Hohenheim)	2001-2003
Genetic diversity and core collection approaches in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*	

List of Donors

Donor / Project	Project duration
Haiti - **USAID-HGRP-** Haiti Hillsides Agricultural Project Germplasm Improvement Project	2001-2005
Italy - **Food and Agriculture Organization of the United Nations** (FAO) Update of Grassland Index	2000-2002
Japan – **The Ministry of Foreign Affairs-**JIRCAS The role of endophytes in tropical grasses	1995-2002
Mexico - **SEMILLAS PAPALOTLA, S.A. de C.V.** Mejoramiento de *Brachiaria*	2001-2005
Nicaragua – **FONDEAGRO** Introducción participativa de forrajes mejorados en sistemas de producción de leche de pequeños productores en Matagalpa, Nicaragua	2002-2003
Nigeria – **IITA/CIEPCA** Mucuna G*E trial L-Dopa	2001-2002
Philippines – **Asian Development Bank** RETA 5866: Fourth Agricultural and Natural Resources Research at CGIAR Centres: Developing Sustainable Forage Technologies for Resource-Poor Upland Farmers in Asia	2000-2002
Switzerland **- ETH, Zurich** Strategies to increase feed utilization and to limit methane emission of tropical smallholder livestock using the potential of native plants	2000-2003

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