Chapter 14

MYCOPLASMA-LIKE DISEASES

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Introduction

Some plant diseases, known as "yellows," were believed to have a viral etiology. However, in 1967, various workers (Doi et al., 1967b; Ishiie et al., 1967), through the use of electron microscopy and antibiotics, have demonstrated that "yellows" are actually caused by mycoplasma-like microorganisms (MLOs). Many diseases have since been associated with MLOs. Symptoms are characterized by plant chlorosis, stunting, excessive proliferation of branches (witches' broom), bud proliferation (Derrick and Newsom, 1984), and disorders of floral organs (phyllody and virescence) (Davis, 1974; Davis and Whitcomb, 1970; de Lourds, 1975; Kitajima and Costa, 1972; Maramorosch, 1974; Maramorosch et al., 1974; Whitcomb, 1973). Many of the causal agents are transmitted by leafhoppers (Homoptera) to various hosts, including cultivated crops of the Leguminosae family (Bowyer and Atherton, 1970 and 1971; Bowyer et al., 1969; Derrick and Newsom, 1984; Granada, 1976 and 1979b; Iwaki, 1975; Kaloostian et al., 1976; Murayama, 1966; Nielson, 1968; Shinkai, 1965).

Mycoplasma organisms, including MLOs and spiroplasmas, are prokaryotes, lack a cell wall but possess a membrane, are highly pleomorphic, measure 0.2-1.0 μm in diameter, and contain ribosomes, RNA, and DNA (Murayama, 1966). Using electron microscopy, MLOs can be seen normally within plant sieve elements, but also within phloem parenchyma. MLOs are very difficult to multiply in vitro. However, Sugiura et al. (1977) maintained, and apparently multiplied, MLOs associated with Peach-X-disease by placing them in the dead cells of salivary glands of its leafhopper

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vector, *Colladonus montanus* (van Duzee). MLOs are resistant to penicillin but are susceptible to other antibiotics such as tetracycline.

Spiroplasmas infect various hosts but have not been detected in beans. Spiroplasmas are motile, have a definitive helicoid morphology, and measure 0.25 by 3.25 μm. Spiroplasmas have been cultured in vitro (Chen and Liao, 1975; Fudl-Allah et al., 1972; Saglio et al., 1971; Williamson and Whitcomb, 1975). They are transmitted by leafhoppers (Chen and Liao, 1975; Markham et al., 1974; Williamson and Whitcomb, 1975). Corn stunt (Davis et al., 1972) and stubborn disease of citrus (Fudl-Allah et al., 1972) are caused by spiroplasma organisms.

Pathogenic MLOs Associated with Legumes

Various MLOs infect beans and other leguminous crops. They cause diseases such as legume little-leaf, witches' broom, phyllody, and virescence.

Legume little-leaf. Hutton and Grylls (1956) described the legume little-leaf disease associated with forage legumes in Australia as being transmitted by the leafhopper *Orosius argentatus* (Evans) which is also a vector of tomato big bud. Electron microscopic studies have revealed the presence of MLOs in the sieve tubes and phloem parenchyma of naturally infected siratro (*Macroptilium atropurpureum* (DC.) Urb.), alfalfa (*Medicago sativa* L.), tomato (*Lycopersicon esculentum* Mill.), and cowpea (*Vigna unguiculata* (L.) Walpers ssp. *unguiculata*). They also appeared in experimentally infected plants of *Nicotiana glutinosa* L., *Datura stramonium* L., periwinkle (*Vinca rosea* L.), and common bean (*Phaseolus vulgaris* L.). MLOs were also detected in the sieve tubes of dodder (*Cuscuta australis* R. Br.) that was experimentally used for little-leaf transmission and in the salivary glands of those leafhoppers (*O. argentatus*) that had fed on the infected plants (Bowyer and Atherton, 1970 and 1971; Bowyer et al., 1969).

Trials showed that tetracycline, applied as spray (100 μg/ml) every two or three days for four to eight weeks, caused remission of little-leaf symptoms on the new growth of *N. glutinosa*, *Callistephus*

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chinensis (L.) Nees, and *Lycopersicon esculentum*. However, the symptoms reappeared when treatment was suspended. Electron microscopic examinations revealed that there were no pleomorphic corpuscles present in the phloem of plants exhibiting a decreased symptom severity. Moreover, leafhoppers were unable to transmit the pathogen from these plants (Bowyer and Atherton, 1972).

Witches' broom and phyllody. Witches' broom has been known to occur on sweet potato (*Ipomoea batatas* (L.) Lamk.), soybean (*Glycine max* (L.) Merrill), peanut (*Arachis hypogaea* L.), pea (*Pisum sativum* L.), bean, and cowpea for many decades in Japan (Murayama, 1966; Shinkai, 1965). Shinkai (1972) found that the leafhopper vector of sweet potato witches' broom differed from that transmitting the pathogen to legumes. However, both vector species belonged to the genus *Nesophrosyne* (later reclassified as *Orosius*). The sweet potato vector transmitted the pathogen only to species in the Convolvulaceae family and to *Vinca rosea*. The legume vector transmitted the pathogen to members of the Leguminosae and several species of Compositae, Amaranthaceae, Cruciferae, and Chenopodiaceae (Murayama, 1966; Shinkai, 1965). The vectors of MLOs causing witches' broom in legumes and sweet potato are now classified as *Orosius orientalis* and *O. ryukyuensis*, respectively (Shinkai, 1972).

The latent period of the causal agent in the legume vector is about one month. This can be shortened by raising the temperature, for example, 17 days at 30 ^{0}C. Diseased bean plants exhibit typical symptoms of witches' broom such as yellowing, reduced leaflets, shoot proliferation, and phylloid-like disorders of floral organs (Murayama, 1966; Shinkai, 1965). Mycoplasma-like corpuscles are found in the phloem of diseased legume plants (Doi et al., 1967a) in different parts of the world.

Although *Phaseolus vulgaris* was not included in the list presented by Iwaki (1975), the occurrence of witches' broom and phyllody in Indonesia was reported in several legume crops, including soybean, peanut, urd bean (*Vigna mungo* (L.) Hepper), cowpea, and *Crotalaria* sp. The MLO has a latent period of nearly three weeks in the vector *Orosius argentatus*. Transmission trials have shown that the causal agent of witches' broom in legumes can infect other plant species. Histological examination using the electron microscope confirmed the presence of MLOs in plant tissues.

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Witches' broom and phyllody have caused economic damage to cowpea in the Philippines (Benigno, 1977) and Thailand (Deema, 1977). Electron microscopy revealed the presence of MLOs in the phloem of infected plants. However, no additional information exists concerning the transmission and vectors of these diseases. In a revision of virus and plant problems associated with MLOs, Mishra (1977) described witches' broom in *Vigna radiata* (L.) Wilczek var. *radiata* and *V. mungo* in India but gave no information concerning the pathogen.

Kitajima and co-workers (Kitajima and Costa, 1972 and 1979; Kitajima et al., 1974) reported the occurrence of witches' broom in several legumes such as *Crotalaria juncea* L., *C. paulina*, *Desmodium* sp., soybean, and siratro. Electron microscopic observations demonstrated that there was a consistent association between the presence of MLOs and the disease. No work has yet been conducted on its transmission or the identification of its vector.

A low (1%-3%) incidence of witches' broom and phyllody has been observed in the green belt of the Federal District in Brazil. The infectious nature of this disease was demonstrated by grafting. Mycoplasma-like corpuscles were found in sieve tubes of the vascular region of naturally or experimentally infected plants (Figures 96 and 97). The vector remains unknown.

Maramorosch et al. (1974) detected MLOs in sieve tubes of pigeonpea (*Cajanus cajan* (L.) Millsp.) exhibiting witches' broom symptoms. However, no details were given for its pathology or transmission.

Virescence. In Zagora and Morocco, Cousin et al. (1970) identified mycoplasma-like corpuscles in the cortical parenchyma of beans exhibiting symptoms of virescence. However, they did not furnish economical or pathological data concerning the disease or its pathogen.

Unfortunately, little data are available which identify the MLOs associated with virescence or witches' broom of legumes in different parts of the world. In the three cases studied in most detail—Australia, Japan, and Indonesia—the similarity of host range and vector (Hutton and Grylls, 1956; Iwaki, 1975; Shinkai; 1965) suggests that the etiological agent may be similar. There is not

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enough information to conclude that virescence and witches' broom are caused by the same or different mycoplasma species. Host and vector specialization may explain why certain MLOs are associated with diseases that have restricted host ranges.

Machismo. A mycoplasma-like disease was first detected in 1968 in infected soybean plants growing in the Cauca Valley of Colombia (Baeza, 1970; Granada, 1976). Since then it has increased in cultivated soybean crops and its incidence in individual fields varied from 0.4%-80%, with corresponding yield losses of 8-1600 kg/ha (Granada, 1979b). After 1980, a disease with similar symptoms was observed in commercial bean fields grown in the Cauca Valley with a disease incidence of 8%-15% (Granada, 1978b). During 1981-1985, incidence of the disease in both beans and soybeans has been less than 1% (Granada, 1984).

This mycoplasma-like organism can infect the following hosts: soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), *Vigna angularis* (Willd.) Ohwi *et Ohasi, V. umbellata* (Thunb.) Ohwi *et* Ohasi, lima bean (*P. lunatus* L.), *Crotalaria spectabilis* Roth., *C. juncea*, *Desmodium* sp., periwinkle (*Vinca rosea*), pigeonpea (*Cajanus cajan*), *Rhynchosia minima* (L.) DC., and *Galactia glaucescens* Kunth. (Granada, 1978a). Common names frequently used for bean mycoplasma in Latin America are "machismo" and "amachamiento."

Electron microscopic evaluation of infected bean or soybean (Fletcher et al., 1984) tissue revealed the presence of mycoplasma-like corpuscles in phloem cells. The mycoplasma-like etiology also has been confirmed by symptom expression and Dienes' staining with tetracycline (Fletcher et al., 1984; Granada, 1979c).

The mycoplasma-like organism is transmitted by the brown leafhopper *Scaphytopius fuliginosus* Osborn (Figure 98) (Granada, 1976 and 1979b). High population levels of this insect have been detected in infected soybean fields in Colombia (García et al., 1975). This vector has been shown to transmit the mycoplasma-like organism to bean plants grown under controlled conditions (Granada, 1979a). The same vector has been recently reported in association with the machismo-like disease of soybeans in southwestern Mexico (Fletcher et al., 1984).

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When one- to six-day-old bean seedlings were exposed to infective adults of *S. fuliginosus* for five days, the average incubation time of the pathogen was 37 days (range of 31-43 days) (Granada, 1979a). This is similar to the 39-day incubation period obtained in soybeans tested under the same conditions (Granada, n.d.). The organism is not transmitted mechanically or by seed, but can be by grafting (Granada, 1979a). Legume little-leaf disease has an incubation period of only 19-23 days (Bowyer and Atherton, 1971).

Symptoms of mycoplasma infection usually become apparent during flowering and pod development when reproductive structures are converted into vegetative structures. Early infection turns flower petals a light to dark green (virescence) and flowers are smaller but have longer sepals than normal. A corrugated structure emerges from the unopened floral apex which is filiform at the upper end and resembles a rolled leaf when dissected (phyllody) (Figure 99). Later infections may cause pods to be rigid, thin, erect, twisted, corrugated, oriented upward, and shaped like a half-moon (Figure 100). These pods form few, if any, seeds. Severe symptoms are characterized by flowers being reduced to small buds and supported on a large petiole from which additional small leaves and petioles may proliferate (Figure 101). The plant as a whole resembles a typical witches' broom (Figure 102). Late infection of plants bearing healthy appearing pods may stimulate premature germination of seeds still in the pod (Figure 103). Germinated seeds can be transplanted and develop into normal plants free of MLOs (G.A. Granada, unpublished data).

This MLO induces similar symptoms during flowering in other hosts such as lima bean (*P. lunatus*), soybean (Figure 104), *Vigna angularis*, *V. umbellata*, *Galactia glaucescens*, and *Desmodium* sp. (Granada, 1978a). Infected *Crotalaria spectabilis* plants demonstrate abundant vegetative ramification before flowering, which does not occur in *C. juncea* (Figure 105) (G.A. Granada, unpublished data). The pumpkin (*Cucurbita maxima* Duchesne) has recently been found to also be a host of machismo (Varón de Agudelo, 1984).

Control measures are the observation of normal planting dates, maintenance of adequate crop rotation, and not planting continuous or simultaneous cycles of susceptible crops such as beans and

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soybeans. This will reduce the buildup and the continued survival of insect vector populations and sources of inoculum from infected plants. Ideally, when it is economically feasible, infected plants are removed from the field and destroyed. In addition, weed hosts are also eradicated from fields and surrounding borders or irrigation canals. When dealing with a relatively high incidence (5%-10%) of machismo and the vector, insecticides such as those used to control the green leafhopper (*Empoasca kraemeri* Ross *et* Moore), may also reduce brown leafhopper populations.

Under greenhouse conditions the vector has shown sensitivity to all insecticides used on beans. Spraying of oxytetracycline at 100 ppm, every five days, starting 20-30 days before flowering, is recommended in Mexico for plant mycoplasma control (de la Rosa-García, 1981). However, this measure is not considered practical for machismo of either beans or soybeans in Colombia.

Although plant resistance would provide an ideal control measure, the screening of bush type materials from both the Instituto Colombiano Agropecuario (ICA) and the Centro Internacional de Agricultura Tropical (CIAT) bean programs to date has not detected a resistance level that is commercially acceptable to Colombian markets (G.A. Granada, unpublished data).

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