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Mycoplasmas and Fastidious Vascular Prokaryotes Associated with Tree Diseases in China

James H. Tsai, Zuo-yi Chen, Chu-ying Shen, and Kai-xuan Jin

Introduction

The reports of the presence of mycoplasmalike organisms (MLO) in diseased plants (Doi et al. 1967, Ishiie et al. 1967) set off a chain reaction in the field of plant pathology. Many yellows plant diseases previously thought to be virus diseases have since been associated with prokaryotelike organisms (PLO). The requirements of Koch's postulates have not been fulfilled for the estimated 150 plant diseases of mycoplasmal etiology, except citrus stubborn and Rio Grande corn stunt. These claims were based on observations of MLO in diseased phloem cells but not in healthy tissue, and the remission of symptoms following treatment with tetracycline but not penicillin. In some cases, PLO transmission by insect vectors was provided as additional evidence (Tsai 1979).

PLO have been implicated as agents in 25 tree diseases, and their impact is extensive. Five of these diseases occur in the People's Republic of China and threaten to eliminate the production of their hosts. They are mulberry dwarf, paulownia witches'-broom, jujube witches'-broom, citrus huanglungbin, and melia witches'-broom. These diseases are of major importance internationally because of their widespread distribution. Until now, most of the information on these diseases was available only in Chinese, although a few recent publications have included English summaries. This chapter will provide a brief review of past work on various aspects of disease investigation and highlight current research.

Mulberry Dwarf

Historical Background

Sericulture and the silk industry, which depend on mulberry, date back to China's Period of Agriculture (ca. 5000 to 2500 B.C.) and are among the earliest evidences of Chinese civilization. Sericulture was first introduced into Korea as early as the 12th Century B.C. Its introduction into Japan occurred as late as A.D. 200 because of geographical barriers (Tsai 1982). Mulberry dwarf (MD) was first recorded in the early 1600s in the *Book of Agriculture by Shen.* Zhu (1981) suggested that the causal agent was transmitted by the cutting knife and that removal of infected trees was the most effective measure of disease control. Numerous research reports on MD have been published in Japan since the beginning of the Meiji era in 1868 (Ishijima and Ishiie 1981). Following the discovery of MLO in MD (Doi et al. 1967, Ishiie et al. 1967), researchers at the Shanghai Institute of Biochemistry presented a series of reports on MD in 1974.

Symptomatology

Two distinct MD syndromes have been recognized in China: one, yellow dwarf (Fig. 1), is most prevalent in Jiangsu province; the other, common dwarf (Fig. 2), is most prevalent in Zhejiang province (Fig. 3).

Trees infected with the yellow dwarf agent first show a reduction in leaf size; the accessory buds and lateral buds sprout early on the top. As the disease progresses, the leaves become smaller and turn a golden yellow. The proliferated branches become rather slender and the internodes shorten. Dieback often follows infection. No syncarps appear on the affected branches. Infected trees always die within 2 to 3 years.

The symptoms of common dwarf are somewhat different. Early symptoms include a reduced foliar size, with no serration and lobing of the leaf, and a crinkled leaf surface. Infected branches grow more slowly, and thus plants are stunted. As the disease progresses, axillary and secondary buds appear early in the season, forming groups of small branches that give rise to the characteristic witches'-broom appearance. The leaves are small and yellowish. Since the floral tissue is converted into vegetative shoots, infected trees produce no flowers or syncarps. Infected trees usually have a poor root system; the main root is greatly reduced in size and is often killed by the disease. The reduction of rootlets is also a distinguishing mark of the disease. Infected trees decline over a period of several years; they rarely die within 2 or 3 years (Anonymous 1974*b*).

Fig. 1. Mulberry yellow dwarf is characterizec
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Fig. 2. Mulberry trees infected with commo
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Fig. 1. Mulberry yellow dwarf is characterized by a reduction in leaf size and a proliferation of slender branches.

Fig. 2. Mulberry trees infected with common dwarf develop crinkled leaves that have no serrations or lobings.

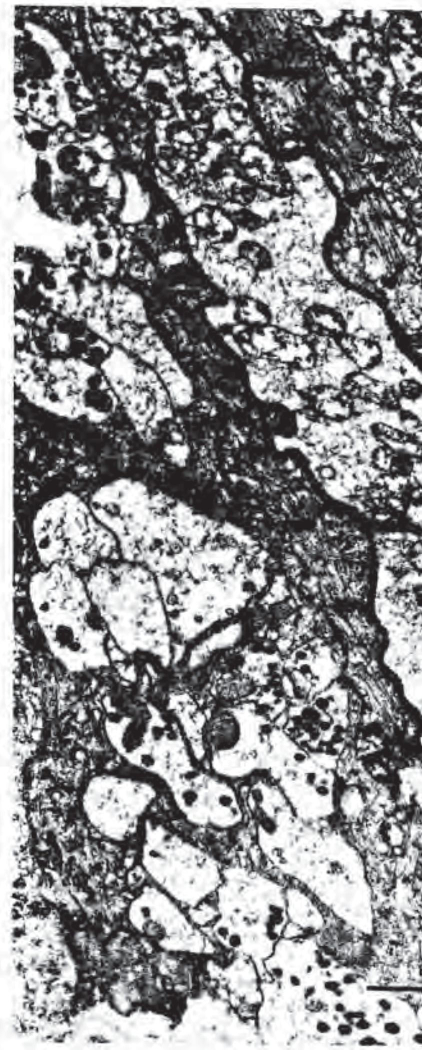
Fig. 3. Map of China.

Etiology

MD is one of the four yellows diseases in which Doi et al. (1967) demonstrated the presence of MLO in infected phloem but not in healthy trees. Ishiie et al. (1967) provided additional evidence of symptom remission after tetracycline treatment. Similar research began in China in the 1970s. Leaf veins and petioles, as well as the bark of new shoots of yellow dwarf and common dwarf trees, were examined in the electron microscope. Pleomorphic MLO, mostly having spherical to ellipsoidal shapes similar to those described by Doi et al. (1967), were found in the ultrathin sections of phloem cells (Anonymous 1974*a*, 1974*b*).

MLO similar to those found in infected plants were also found in ultrathin sections of the salivary glands of infectious leafhoppers, *Hishimonoides sellatiformis* Ishihara. Many MLO were seen in the cytoplasmic membranes at the periphery of glandular cells, and in some cases in the salivary tubes (Fig. 4). Of 12 infective leafhoppers examined, 7 were found to have MLO in their salivary glands (Anonymous 1977*b*).

Tetracycline antibiotics effectively suppressed symptoms of both yellow and common dwarf (Anonymous 1972*a*, 1972*b*, 1972*c*). The injection of



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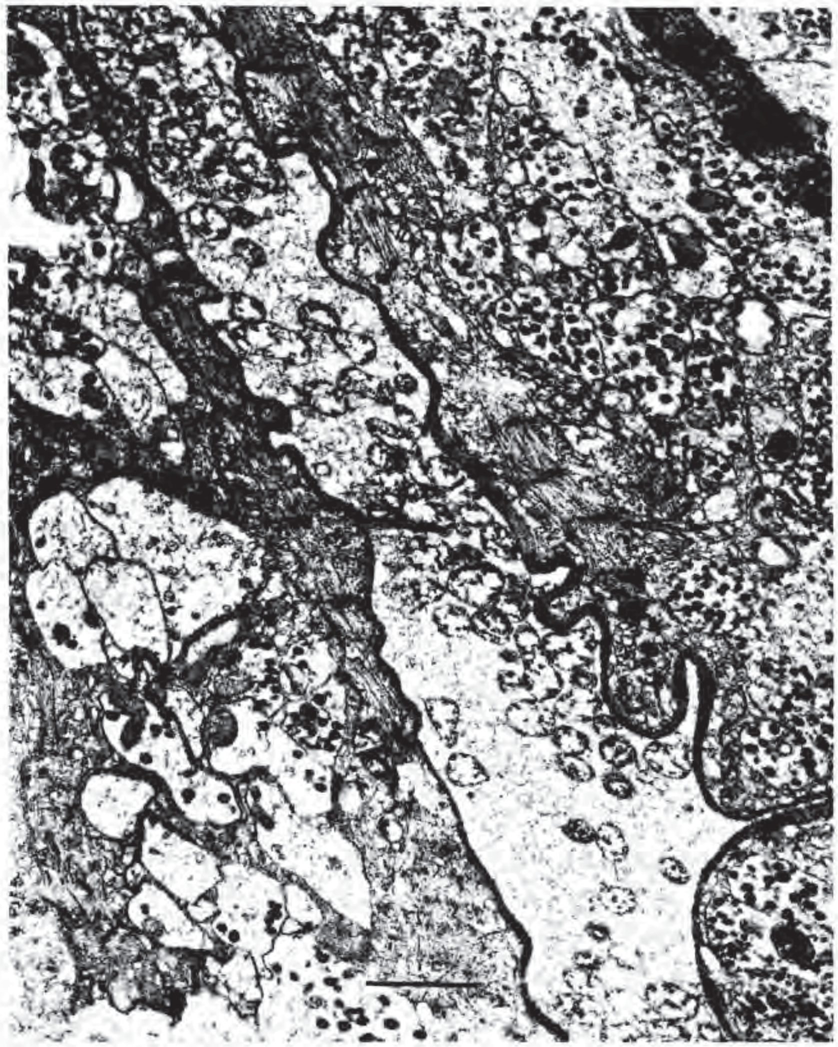

Fig. 4. Mycoplasmalike organisms in the salivary tube of an infective leafhopper, *Hishimonoides sellatiformis* Ishihara. (Bar = 1 μm)

a 20,000 ppm Terramycin solution in the stem at a rate of 20 to 40 ml per tree, and the immersion of mulberry seedling roots in a Terramycin solution (1,000 to 2,000 ppm) for 3 hr before transplanting, was recommended (Zhu 1981).

Seasonal Abundance of MLO in Common Dwarf Trees

An electron microscope study was conducted to measure the fluctuation of MLO populations in an infected tree throughout the seasons. From February 1974 to January 1975, monthly samples were taken from the leaf veins or stem bark of new shoots and the cortex of the main root. MLO were seen in the above-ground tissue only from April to November, before the abscission of leaves, and in the root tissue only from February to April. Very high MLO populations measuring 50 to 60 nm were seen in the infected tissue during July and August, which coincided with the appearance of severe symptoms on the foliage (Anonymous 1977*a*). Graft transmission was much less successful when diseased scions were collected before April instead of in the summer months (Anonymous 1977*a*, Zhu 1981).

Host Range

Morus spp. are the only known MD hosts in China. Several cultivars reacted differently to infection by yellow and common dwarf agents; the cultivars Hoyiehpai and Chienchih were most susceptible to yellow dwarf, and Muchow Green and Chienchih were most susceptible to common dwarf. Other cultivars, such as Round-leaf Hoyiehpai, were resistant to yellow dwarf, and cultivars Hoyiehpai, Tunghsiang Green, and Round-leaf Hoyiehpai were resistant to common dwarf (Anonymous 1974*b*).

Transmission

Transmission of the MD agent can be achieved either by grafting or by leafhopper vectors. Leafhopper vectors play the most important role in MD epidemics. Two species of leafhopper are known to transmit MLO: *Hishimonoides sellatiformis* (Fig. 5) and *Hishimonus sellatus* Uhler (Anonymous 1977*b*). The former is a more efficient vector than the latter; the average transmission efficiency for *H. sellatiformis* was 49.7% after a 7-day acquisition access period, compared with 10.5% for *H. sellatus*. The mean inoculation efficiency for *H. sellatiformis* was 43.9%. The minimum incubation period in the insects was 24 to 28 days.

Dual Infection of Mulberry Trees

In the course of studying MD, filamentous virus particles 11 to 13 nm in diameter and 600 to 1,000 nm long were isolated, by purification using

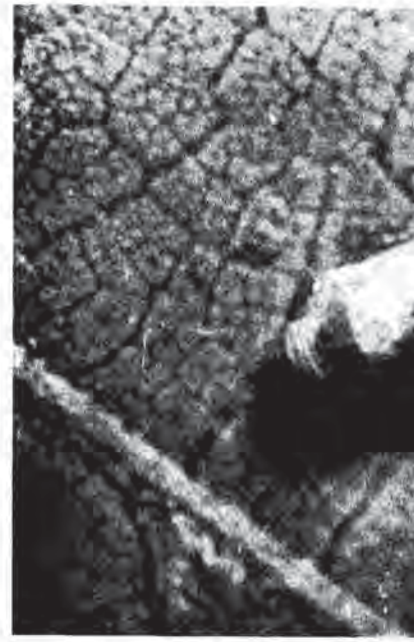

Fig. 5. *Hishimonoides sellatiformis*, a vecto

polyethylene glycol, from the tiss
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Paulownia Witches'-Broom

Introduction

The paulownia tree (empress tree;
mentioned in the *Book of Poems*,
(Anonymous 1982). It is now fou
Shanxi in the north, Sichuan and
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Common Dwarf Trees

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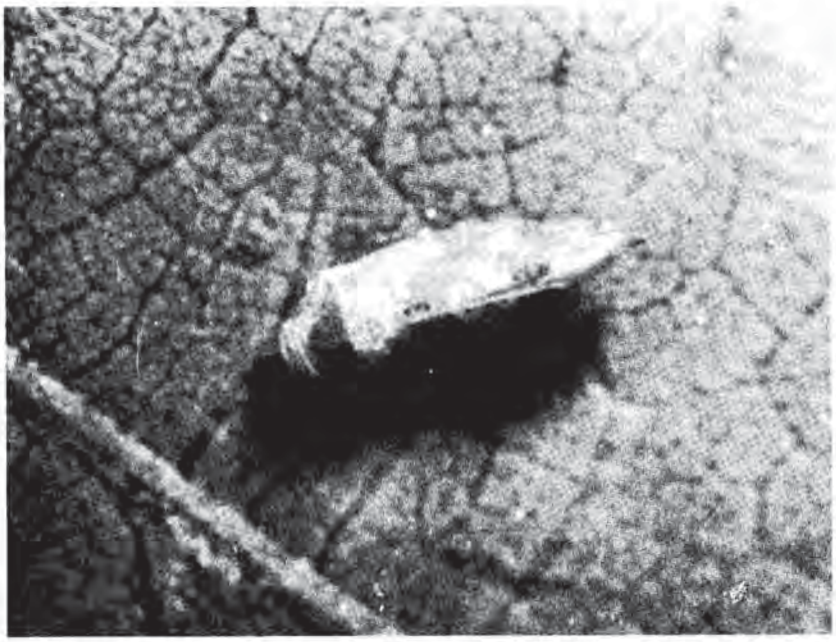

Fig. 5. *Hishimonoides sellatiformis*, a vector of the mulberry dwarf agent.

polyethylene glycol, from the tissue of leaf veins, stem bark, and the cortexes of roots affected by yellow or common dwarf. The virus particles had a hollow central core and a subunit fine structure. They were observed by the leaf-dipping method. The morphology of virus particles resembles that of partially purified preparations. In ultrathin sections of phloem cells, both MLO and paracrystalline aggregates of virus particles were observed (Anonymous 1974*a*, 1974*b*). No such particles were found in healthy controls.

Paulownia Witches'-Broom

Introduction

The paulownia tree (empress tree; princess tree) originated in China; it is mentioned in the *Book of Poems,* which appeared over 2,600 years ago (Anonymous 1982). It is now found in the provinces of Liaoning and Shanxi in the north, Sichuan and Yunnan in the west, Guangdong and Guangxi in the south, and Fujian in the east (Fig. 3). Eight species grow throughout China: *Paulownia fortunei* (Seem) Hemsl, *P. albiphloea* Z. H. Zhu, *P. catalpifolia* Gong Tong, *P. elongata* S. Y. Hu, *P. fargesii* Franch, *P. kawakamii* Ito, *P. tomentosa* (Thunb) Steud, and *P. quiwaniana* Hu et Chang. *P. taiwaniana* Hu et Chang and *P. kawakamii* grow in Taiwan (Ying 1979).

Paulownia, one of the best timber species in the world, belongs to the Bignonia family. It grows rapidly, reaching a 30 to 40 cm diameter in less than 10 years. Its superior qualities include durability, softness, light weight, and resistance to heat, moisture, decay, and insect borers. The wood is mostly used for fine furniture, boats, craft goods, and stringed instruments. Paulownia leaves and flowers, known for high contents of nitrogen, phosphorus, and potassium, are used as fertilizers and animal feed. The dense hairs and sticky exudate on Paulownia's large leaves help clean the air in urban areas. Its deep roots, which do not interfere with other agricultural crops, are especially suitable in intercropping systems. In 1978 it was estimated that in Henan province alone the areas planted with a paulownia intercrop covered about 670,000 ha.

Paulownia witches'-broom (PWB) has existed in China for many years. Reports have attributed physiological disorder, bacteria, and fungi as causes of the disease (Anonymous 1982). PWB, one of the first MLO diseases (Doi et al. 1967), has been reported to be of MLO etiology in Korea and Taiwan (La 1968, Chang et al. 1979, Chen 1979, Ying 1979). The PWB agent was transmitted by bark graft, and MLO were found in ultrathin sections of infected leaves (Anonymous 1982, Jin et al. 1978). This disease is most prevalent in Shanxi, Shandong, and Henan provinces. About 30 to 50% of the trees are infected with PWB, and in some areas infection is greater than 80% (Anonymous 1982). Growth is greatly reduced in the infected trees and leads to a gradual decline and premature death. The wood of infected trees is of poor quality.

Symptomatology

PWB is characterized by a proliferation of axillary or lateral buds on new branches in the current season, followed by the production of secondary and tertiary branches (Fig. 6). The proliferated branches are always slender and have short internodes that resemble a broom. In newly infected trees under field conditions, only one or two branches develop PWB (Fig. 7), but later the brooms appear throughout the entire crown. In some cases, stems enlarge and the bark cracks. Infected shoots often show dieback in late fall and early winter. Leaves on infected branches are small and yellowish. Floral symptoms are virescence and phyllody, and the

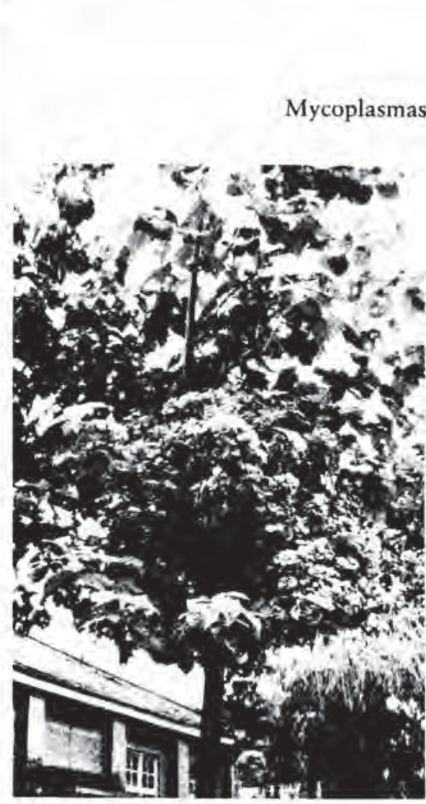

Fig. 6. Paulownia witches'-broom is characterized by tight clusters of branches, which form brooms.

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Etiology

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Transmission

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Fig. 6. Paulownia witches'-broom is characterized by tight clusters of branches, which form brooms.

Fig. 7. An affected paulownia tree, showing only one broomed branch.

flowers are often sterile. Infected roots are underdeveloped and discolored by necrosis, which is readily visible in cross-section. Saplings sprouted from infected roots develop the same symptoms and die within a year. In some instances infection remains localized, and after winter kill the other branches remain healthy in the following years.

Etiology

MLO were found in ultrathin sections of leaf veins collected from bark-grafted and field-infected trees, but not in healthy controls. Symptom remission occurred after tetracycline and Terramycin injections; the former was more effective than the latter (Anonymous 1978*a*, Jin et al. 1978, Shen et al. 1980).

Transmission

PWB is readily transmitted by grafting but not through seeds (Anonymous 1982). Six homopteran and hemipteran insect species were tested, and all but one hemipteran, *Halyomorpha holys* (Stals) (Heteroptera: Pentatomidae) (Fig. 8), transmitted MLO. A total of 107 *Paulownia tomentosa* seed-

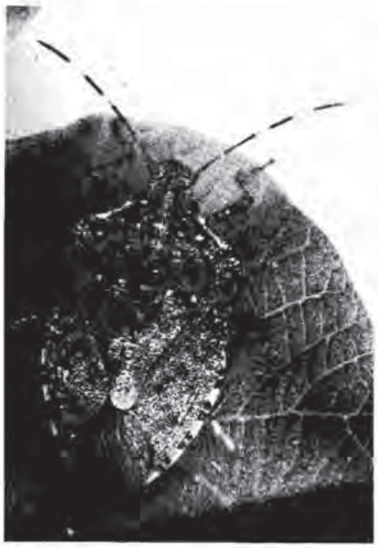

Fig. 8. *Halyomorpha holys* (Stals), the vector of the paulownia witches'-broom agent.

lings were inoculated by test insects (1 to 3 insects per plant) from 1980 to 1981; 33 of these seedlings developed PWB symptoms in 26 to 30 days. The results were verified by electron microscope examination. MLO measuring 150 to 750 nm were seen in ultrathin sections of leaf veins and petioles of the inoculated plants as well as in the salivary glands of *H. holys*. No MLO were found in healthy leaves or in *H. holys* (Shao et al. 1982).

Host Range

In China, paulownias seem to be the only hosts, but susceptibility varies with different species. *Paulownia elongata, P. catalpifolia, P. tomentosa,* and *P. taiwaniana* are the species most susceptible to PWB; *P. kawakamii, P. fortunei,* and *P. fargesii* are moderately susceptible (Anonymous 1982).

Dual Infection of Paulownia

In an attempt to isolate causal agents of PWB, rod-shaped virus particles 15 nm thick and 250 to 300 nm long were observed in the electron microscope. A hollow core structure was visible in most of the virus particles. No such virus particles were observed in control samples taken from healthy seedlings (Hung et al. 1981).

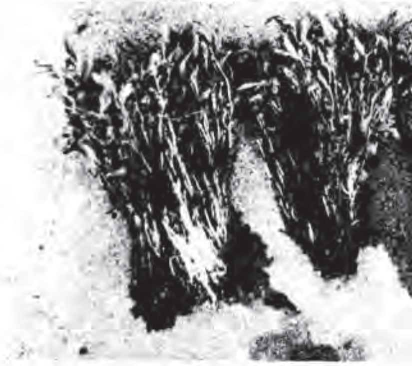

Fig. 10. Clusters of root sprouts from a jujul

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Fig. 9. A jujube tree branch affected with witches'-broom (right), and a healthy branch (left).

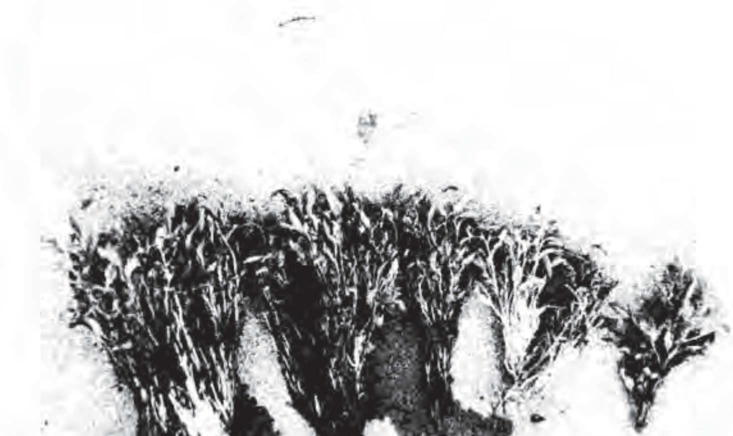

Fig. 10. Clusters of root sprouts from a jujube tree affected with witches'-broom.

Jujube Witches'-Broom

Introduction

The Chinese jujube tree, *Ziziphus jujuba* Mill., has long been cultivated in China for its fruit. It is grown in Shaanxi, Shanxi, Henan, Hebei, Sichuan, Shandong, Guangdong, Guangxi, Hunan, Anhui, Fujian, Jiangsu, and Zhejian provinces (Fig. 3). The variety Jingsi small jujube, grown in Henan and Shandong, is of especially good quality; its sugar content is as high as 70 to 80%. Jujube witches'-broom (JWB), first recognized in 1951 (Chi 1951), is widespread in most jujube-growing areas and is devastating large plantations in Shandong, Hebei, and Henan. It is considered the major limiting factor in jujube production in China (Anonymous 1974*c*) and Korea (La and Chang 1979).

Symptomatology

JWB is characterized by the branches' excessive production of axillary and terminal buds (Fig. 9) and by the clustering of root sprouts (Fig. 10), which produce chlorotic and spindly leaves. Symptoms always develop first on the lower branches of the main stem and then spread throughout the crown. Trees of all ages are susceptible to JWB. When infection takes place before the normal flowering season, phyllody develops on the floral parts in the following year, resulting in very little or no fruit production. Tip dieback occurs on infected branches. In severe cases infected trees die in the year of infection, but usually death occurs within 1 to 2 years for young trees and 3 to 6 years for old trees (Wang et al. 1981*b*, Zhu 1981).

Etiology

The MLO etiology of JWB is again based on symptomatology, electron microscope examination, and sensitivity to antibiotics. MLO are found in ultrathin sections of leaf veins and in the stems of new shoots of broomed trees. Pleomorphic MLO ranged from 80 to 720 nm (Fig. 11), and no MLO were seen in healthy controls (Anonymous 1974*d*, Chen et al. 1978, Shu 1980, Wang et al. 1981*b*). Diseased trees showed a remission of symptoms after being injected with 100 ml tetracycline or Terramycin at a concentration of 1,000 ppm (Wang et al. 1981*b*, Zhu 1981). Therapeutic effects were also achieved after injecting 50 ml Terramycin at a concentration of 5,000 ppm. Higher dosages or concentrations (50 to 500 ml at 10,000 to 50,000 ppm) resulted in the complete cure of infected trees (Wang et al. 1980).

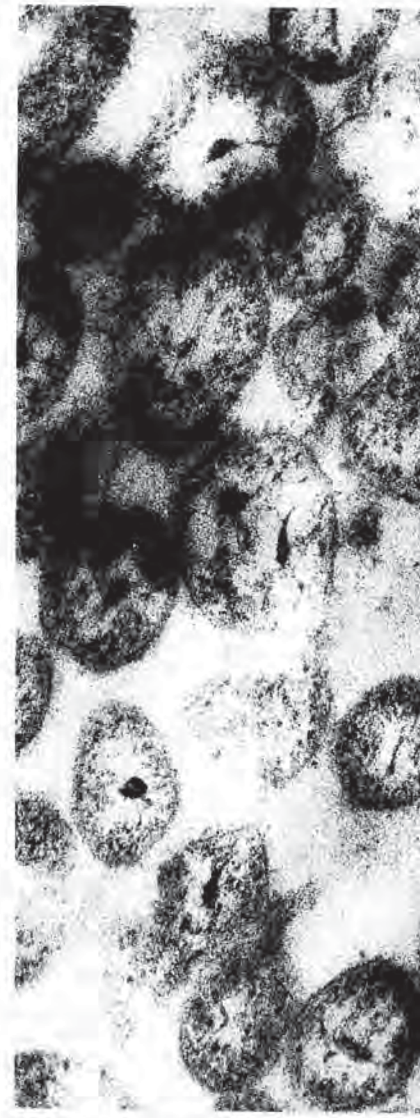

Fig. 11. Mycoplasmalike organisms in the si
witches'-broom. (Bar = 0.5 μm)

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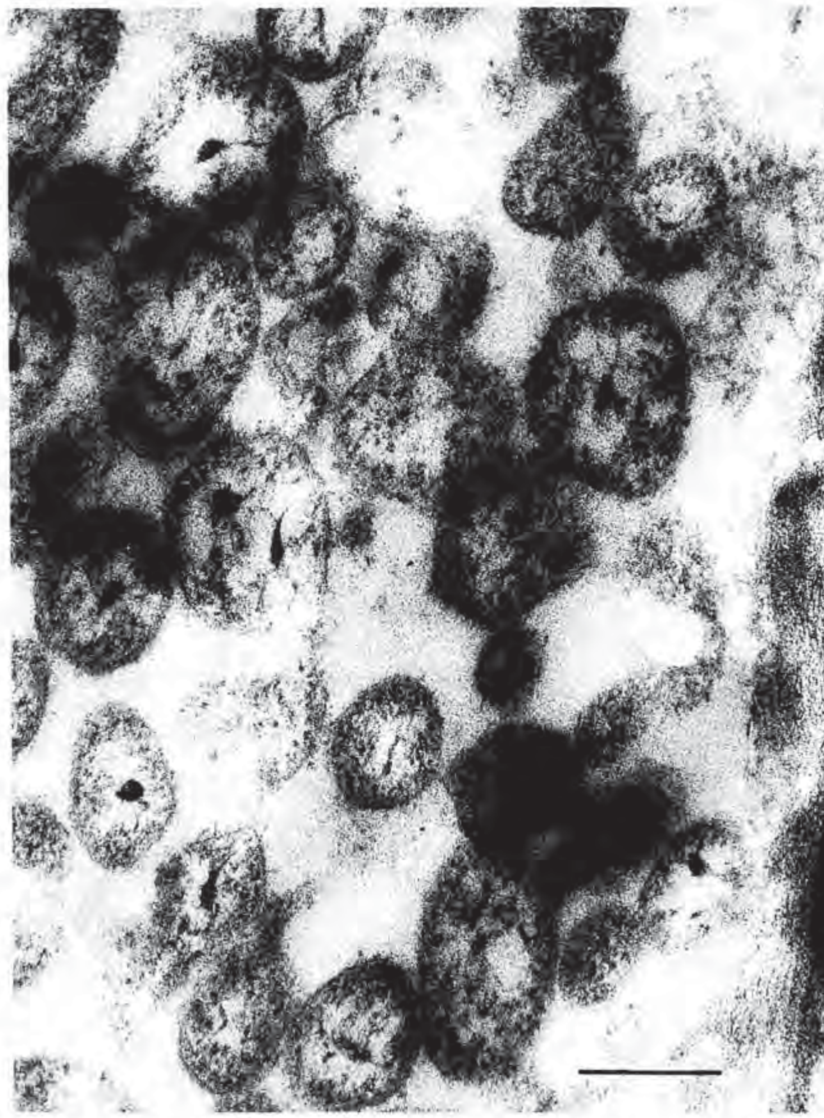

Fig. 11. Mycoplasmalike organisms in the sieve element of a jujube tree affected with witches'-broom. (Bar = 0.5 μm)

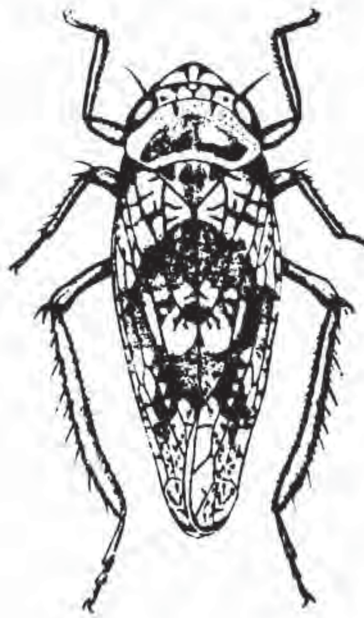

Fig. 12. *Hishimonoides chinensis* Anufriev, the vector of the jujube witches'-broom agent.

Host Range

Z. jujuba Mill. and another *Ziziphus* species are the only known hosts in China (Zhu 1981).

Transmission

JWB is transmitted both by grafting and by insect vector(s). The transmission of JWB by grafting was reported in the early 1960s (Wang et al. 1964, Wen et al. 1962). A series of transmission experiments was conducted using nine insect species, including Homoptera: Fulgoridae: *Lycorma delicatula* White, Homoptera: Cicadellidae: *Psammotettix striatus* (L.), *Chlorita flavescens* (Fab.), *Bythascopus chlorophana* (Melichar), *Hishimonoides chinensis* Anufriev, *Hishimonus sellatus* (Uhler), *Erthyroneura* spp., *Chlorita* sp., and Heteroptera: Miridae: *Pseudoloxops* sp. collected from JWB trees as test insects. The insects were given either a 3- to 9-day acquisition access period or no acquisition access period. The number of insects per inoculated plant was 2 to 105, depending on their availability.

Of the 298 seedlings tested, only
showed typical JWB symptoms. No
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Dual Infection of Jujube Trees

Rodlike virus particles with an av
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Citrus Huanglungbin (Citrus Y

Introduction

Many types of citrus originated in
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grandis (L.) Osbeck, *C. reticulata*
Among these, *C. reticulata* is the m
3 to 8 years old are most susceptibl
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1966), "leaf mottle yellows" in t
1967), and "Likubin" in Taiwan (C
et al. 1961).

Fig. 12. *Hishimonoides chinensis* Anufriev, the vector of the jujube witches'-broom agent.

s species are the only known hosts in

and by insect vector(s). The transmis-
d in the early 1960s (Wang et al. 1964,
smission experiments was conducted
g Homoptera: Fulgoridae: *Lycorma*
dellidae: *Psammotettix striatus* (L.),
opus chlorophana (Melichar), *Hishi-*
monus sellatus (Uhler), *Erthyroneura*
Miridae: *Pseudoloxops* sp. collected
nsects were given either a 3- to 9-day
uisition access period. The number of
o 105, depending on their availability.

Of the 298 seedlings tested, only 7 inoculated by *H. chinensis* (Fig. 12) showed typical JWB symptoms. None of the 34 healthy controls developed symptoms. The average rate of transmission by *H. chinensis* was 21.8%. The incubation period in plants was 10 to 12 months. Ultrathin sections of the salivary gland, excised 32 days after the acquisition access period, revealed pleomorphic MLO measuring 100 to 200 nm and containing high-electron-density materials. Similar MLO were found in the thin sections of the leaf veins of inoculated plants. The MLO varied from 90 to 890 nm in size, with a membrane 10 nm thick. No MLO were observed in either control insects or plants (Wang et al. 1981*a*).

Dual Infection of Jujube Trees

Rodlike virus particles with an average diameter of 12 nm and a mean length of 300 to 400 nm were isolated from JWB-infected leaves. A hollow central core and subunit fine structure were visible at high magnifications. No rodlike particles were present in healthy controls. (Anonymous 1974*c*).

Citrus Huanglungbin (Citrus Yellow Shoot)

Introduction

Many types of citrus originated in China, and they have been cultivated there for many centuries. Over 40 species and varieties grow in Guangdong, Guangzi, Fujian, Sichuan, Zhejiang, Jiangsu, and Jiangxi provinces in south China (Fig. 3). Citrus huanglungbin was recognized in the 1930s (Chen 1943) and has since spread throughout China's citrus-growing areas. It affects most species and varieties of *Citrus sinensis* (L.) Osbeck, *C. grandis* (L.) Osbeck, *C. reticulata* Blanco, and *C. limon* (L.) Burm. f. Among these, *C. reticulata* is the most susceptible to huanglungbin. Trees 3 to 8 years old are most susceptible to this disease. Although tens of thousands of citrus trees are killed by this disease annually and it threatens China's citrus industry, no data are available to assess the financial losses due to this disease. A similar disease was recognized as "citrus greening" in South Africa (McClean and Oberholzer 1965) and India (Fraser et al. 1966), "leaf mottle yellows" in the Philippines (Martinez and Wallace 1967), and "Likubin" in Taiwan (Chen 1979, Lo et al. 1952, Matsumoto et al. 1961).

Symptomatology

Citrus huanglungbin is characterized by foliar chlorosis, excessive leaf and fruit drops, root decay, and twig dieback. Young leaves show mild chlorosis, and mature leaves show the interveinal chlorosis or mottling chlorotic patches (Fig. 13) characteristic of zinc or iron deficiency. Infected leaves often become leathery, deformed, and abscised. Most infected trees bloom profusely early in the spring, but most flowers drop prematurely. Fruits on infected trees are few, small, and acidic.

Etiology

For many years, citrus huanglungbin in China was thought to be associated with physiological disorders, including zinc or iron deficiency and virus infections. It was confused with citrus tristeza infection as early as 1956 (Anonymous 1978*c*, Chen and Mei 1965, Lin 1956). Following the first demonstration of the association of MLO with citrus greening disease (Lafleche and Bové 1970, Saglio et al. 1971), PLO were found in ultrathin sections of the mid and lateral veins of symptomatic leaves of huanglungbin-infected trees. The sizes of these PLO ranged from 60 to 700 nm, with a double complex membrane measuring 20 nm. The ribosome and fibrillar nuclear materials were visible in these organisms (Fig. 14) (Chen et al. 1979, Ke et al. 1979, J. H. Tsai unpublished), which resembled the citrus greening organisms described elsewhere (Moll and Martin 1974).

Like other yellows diseases, citrus huanglungbin responds to tetracycline and heat therapy. The injection of tetracycline-HCl concentrations of 1,000 ppm into tree trunks proved to be the most effective therapy. Dosages varied with the age of tree; generally 1,350 ml was administered to each tree with a trunk 3 cm in diameter. Other antibiotics, such as Terramycin-HCl, Aureomycin-HCl, and penicillin, were less effective (Chen et al. 1981). The diseased scions treated with tetracycline-HCl at 500 ppm before grafting were free of symptoms for 4 years (Chen et al. 1980, Zhu 1981). Heating diseased scions by using hot air (46 to 60C) for various lengths of time also proved to be an effective measure (Lin 1956, Lin and Cheng 1964).

Host Range

Most species and varieties of *Citrus reticulata*, *C. sinensis*, *C. grandis*, and *C. limon* are susceptible to huanglungbin in China.

Fig. 13. Interveinal chlorosis is typical of

Fig. 14. The citrus huanglungbin agent, wh
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Fig. 13. Interveinal chlorosis is typical of citrus huanglungbin disease.

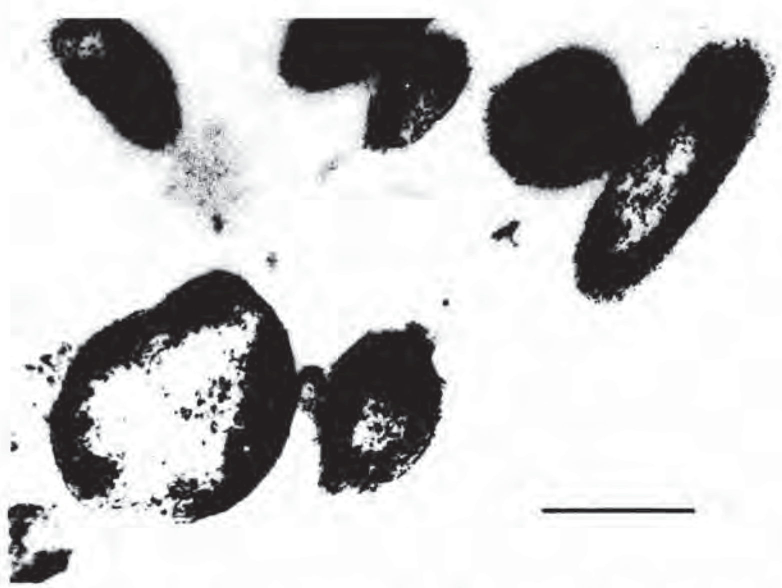

Fig. 14. The citrus huanglungbin agent, which has a double complex membrane measuring 20 nm. (Bar = 0.5 μm)

Transmission

The huanglungbin agent has been transmitted by grafting diseased scions, leaf pieces, and bark to healthy trees (Kao et al. 1963, Zhao et al. 1982). In a 3-year graft transmission study of 177 plants, the part of the plant used as a tissue source was found to affect graft transmission results. The highest rate of transmission (84.8%) was by a stem containing a single bud, and the second-highest rate was by a stem without a bud (52.4%). Grafting a leaf piece was more effective (22.7%) than grafting stem bark (3.0%). Budwoods taken from May to July had a much lower mean transmission rate (3.9%) than those taken from August to April (67.6%) (Zhao et al. 1982).

Early reports implicated the citrus psyllid as a vector of the huanglungbin agent (Anonymous 1978*b,* Luo et al. 1977, Zhao et al. 1979). *Diaphorina citri* Kuw. (Fig. 15) transmitted the huanglungbin agent to healthy citrus plants. In a recent test in which insects were reared on infected plants until mature, three inoculation periods (1, 5, and 20 days) were used in the test and 10 of the 59 inoculated plants developed symptoms. All healthy controls remained free of symptoms. The minimum incubation period in the plants was 3 months. Test results were verified by electron microscope examination; PLO ranging from 160 to 440 nm in size were seen in ultrathin sections of the salivary glands of test insects. The organisms in the thin sections of phloem cells were longer, measuring 360 to 830 nm, and some were up to 1,800 nm long. All organisms had a double membrane 20 nm thick (Dai et al. 1982).

In another series of experiments, *D. citri* were collected from infected trees and caged on citrus seedlings grown from an embryo culture. Each test plant was inoculated by 10 or 20 to 130 insects. Of the 243 seedlings inoculated by *D. citri,* only 22 developed huanglungbin symptoms, whereas all 107 control plants remained healthy (Hwang et al. 1980).

Dual Infection of Citrus Trees

Like the other trees mentioned above, huanglungbin-infected trees are often also infected with citrus tristeza virus (CTV) (Anonymous 1973, Ke et al. 1979). This virus is widespread throughout citrus-growing areas in China and has been implicated as the causal agent of huanglungbin (Anonymous 1973, 1978*c,* Chen and Mei 1965, Zhao et al. 1979). In a recent survey using the ELISA (enzyme-linked immunosorbent assay) technique, Ke et al. (1984) reported that 232 of 382 trees tested in Fujian province were infected with CTV, 198 of 250 trees tested in Guangdong province showed positive reactions to CTV antiserum, and 11 of 20 trees tested in

Jiangxi province carried CTV. In
selected trees exhibiting typical I
also infected with CTV (Ke et al. 1
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Melia Witches'-Broom

Introduction

The chinaberry, *Melia azedarach* L
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Fig. 15. *Diaphorina citri* Kuw., the vector of the citrus huanglungbin agent.

Jiangxi province carried CTV. In these three provinces, of 93 randomly selected trees exhibiting typical huanglungbin symptoms, 86 trees were also infected with CTV (Ke et al. 1984). This virus could be partially isolated from huanglungbin-infected leaves by polyethylene glycol precipitation. Threadlike virus particles 12 nm in diameter and 2,500 to 3,000 nm long were seen by using an electron microscope (Anonymous 1973).

Melia Witches'-Broom

Introduction

The chinaberry, *Melia azedarach* L. of the *Meliasceae,* is a rapidly growing deciduous tree. In China, natural stands of melia occur primarily in provinces near the Chang Jiang (Yangtze) River, including Shanxi, Henan, Hebei, Shangdong, Shaanxi, Gansu, Fujian, Guangdong, and Guangxi (Fig. 3). Melia lumber, which is light, soft, and durable, is an important material in buildings, boats, furniture, farm tools, and musical instruments. Melia fruits are used for brewing wine and as herbal medicines and animal feed. Oil from the seeds is used in lubricants and soaps. A bark extract is used to kill the internal parasites of domestic animals.

Melia witches'-broom (MWB) was first noted in the 1950s. In recent years the production of melia trees in China has diminished, mainly due to the widespread development of this disease. In some areas the infection rate was 100%, and more than 30% of the infected trees were killed by this disease (Zhang 1982).

Symptomatology

The symptoms of a new MWB infection occur in three stages. In the early stage the growth of a new stem or new branch is greatly reduced, the internodes shorten, and the terminal and axillary buds sprout early in the season. Chlorosis along the veins or vein clearing becomes evident. In the middle stage secondary buds and tertiary shoots continue to proliferate, while some terminal shoots start to die. The proliferated shoots are slender and weak and produce small and deformed leaves. In the late stage the broomed branches die. When systemic infection occurs in one year, broomed branches will appear on the entire crown the following season. Branches start to die from the top down (Fig. 16). In severe cases, affected trees die in 1 to 2 years. The lateral roots of affected trees are greatly reduced, and necrosis and discoloration often appear on diseased roots (Jin et al. 1982, Zhang 1982).

Etiology

MLO were found in the phloem cells of ultrathin sections of petioles and young stems. They ranged in size from 100 to 420 nm and had a double-layered unit membrane of ca. 8 nm. More MLO were present in leaf tissues than in young stems. No MLO were seen in thin sections of root tissue (Zhang 1982). A recent electron microscope study of infected leaf veins revealed not only that MLO were present in the mature sieve elements of phloem cells, but also that bacterialike organisms (BLO) were present in the tracheary elements of the xylem and in some parenchyma cells (Fig. 17). The BLO appeared to have a gram-positive type of wall structure. No MLO or BLO were seen in ultrathin sections of healthy leaf veins (Jin et al. 1982). An injection of either tetracycline (100,000 ppm) or penicillin (400,000 ppm) in the tree trunk appeared to suppress symptom development (Anonymous 1979). Antiobiotic treatment was especially successful when it was given in the same season as the one in which infection occurred.

Host Range

The MWB host range is limited to
et Zucc.

Transmission

MWB can be transmitted by bud a
in grafted plants is 45 to 60 days
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than adults. Of 15 seedlings inoc
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Discussion and Conclusions

Following the leads of (Doi et al.
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Fig. 16. Systemic symptoms of witches'-broom on *Melia azedarach* L.

Host Range

The MWB host range is limited to *Melia azedarach* and *M. toosendan* Sieb et Zucc.

Transmission

MWB can be transmitted by bud and bark grafting. The incubation period in grafted plants is 45 to 60 days (Zhang 1982). A leafhopper, *Empoasca* sp., was shown to be the vector of MWB; the nymphs were more efficient than adults. Of 15 seedlings inoculated by nymphs, 14 developed symptoms, whereas of 32 seedlings exposed to adults, only 15 seedlings became infected (Zhang 1982).

Discussion and Conclusions

Following the leads of (Doi et al. (1967) and Ishiie et al. (1967), researchers at the various research institutes in China reexamined several economically important yellows diseases of woody plants that had previously been

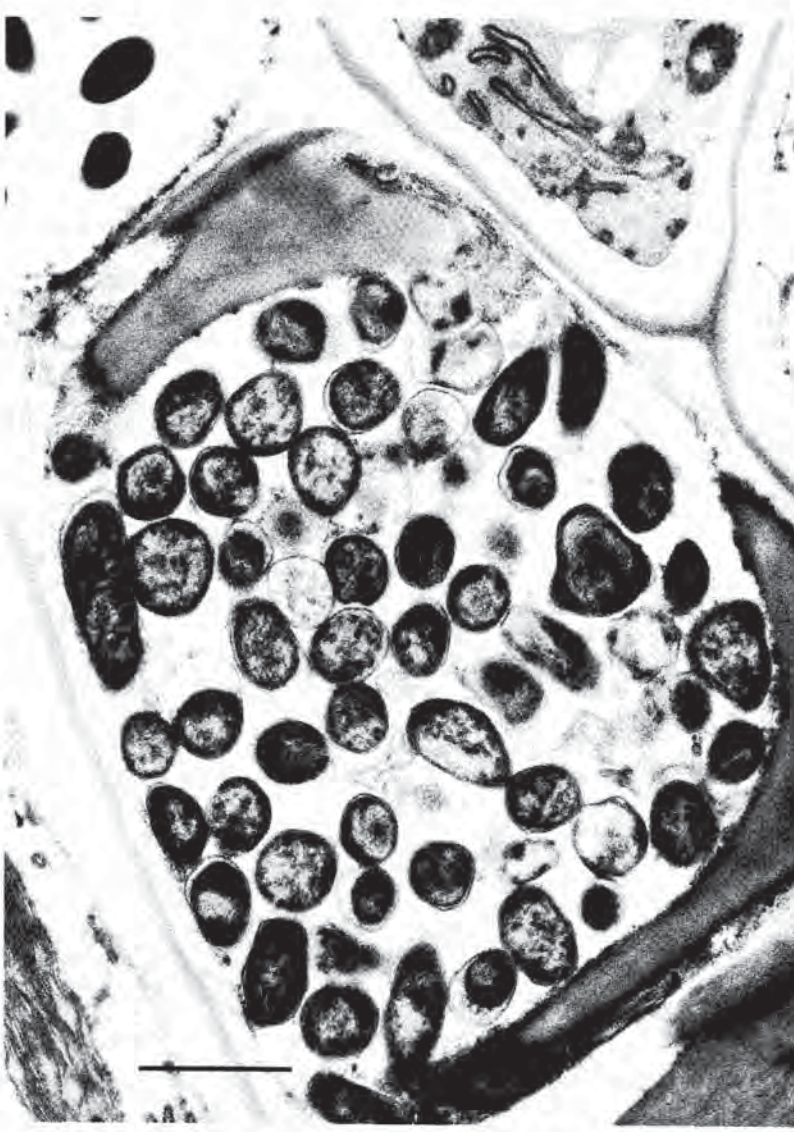

Fig. 17. Bacterialike organisms in tracheary elements of the xylem of a doubly infected *Melia azedarach*. (Bar = 1 μm)

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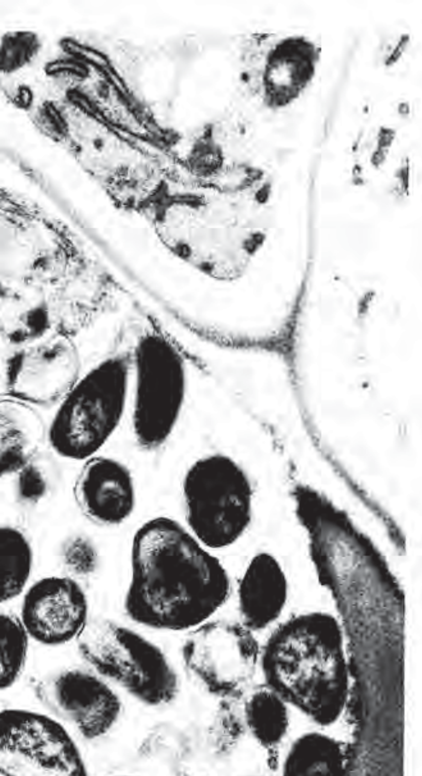

ents of the xylem of a doubly infected *Melia*

considered to be virus diseases. Using an electron microscope, they found a constant association of PLO with mulberry dwarf, paulownia witches'-broom, jujube witches'-broom, citrus huanglungbin, and melia witches'-broom. They also noticed a remission of symptoms following the treatment of diseased trees with tetracycline antiobotics. Various laboratories provided additional evidence for the transmission of phloem-inhabiting PLO by insect vectors. However, the true identity of these yellows disease agents, like that of other nonhelical mycoplasmas, has not been determined by cultural, biochemical, and serological methods; these fastidious organisms have not yet been cultured in synthetic media.

Although all these agents are phloem-limited prokaryotes, electron microscope studies demonstrated that the organisms responsible for mulberry dwarf and the witches'-broom of paulownia, jujube, and melia are different from the organism responsible for citrus huanglungbin. The former organisms are bounded by a unit trilaminar membrane measuring 10 nm, whereas the latter is bounded by double-unit membranes about 20 nm thick. The huanglungbin organism should be grouped with the organisms responsible for citrus greening, leaf mottling, and likubin as a "gracilicutelike bacterium" because of its sensitivity to penicillin, because the outer membrane of the envelope surrounding it resembles a cell wall structure, and because of its heat tolerance (Bové and Saglio 1974, Bové et al. 1974, 1980, Garnier et al. 1976). In addition, these tree diseases share some symptoms, including the yellowing of leaves, the virescence and phyllody of floral organs, the breaking of dormancy, the proliferation of terminal and axillary buds, the dieback of branches, and the sterility of seeds. They are often lethal and cause a gradual decline and reduction in the quality and quantity of fruit, and they are dependent on phloem-feeding insects for transmission (Tsai 1979).

Several antibiotics have been tested against the yellows diseases of woody plants in China. Generally, tetracycline antibiotics have been most effective in obtaining a remission of symptoms, but the treatment has been reported to provide a complete cure for the diseases only once (Wang et al. 1980). Nonetheless, tree injection either by gravity or by pressure is still a simple and economically feasible technique to save valuable trees such as citrus, jujube, and mulberry until a new chemical is found that will kill prokaryotes with a single application. It is imperative to learn more about an antibiotic's pattern of translocation and its persistence in treated plants, and it is important to develop a quick assay technique for detecting the translocation and the metabolites of an antibiotic in various plant tissues. Furthermore, the possibility that the wide use of antibiotics in the environment might induce resistance in many kinds of disease-causing microorganisms should not be overlooked.

The disappearance of mulberry dwarf MLO in the above-ground portions of diseased trees in winter is probably due to the normal inactivation of sieve tube elements by low winter temperatures. This information is important to mulberry growers. Scions taken during the winter and dipped in an antibiotic solution could greatly reduce the spread of MD. This theory has been supported by MD researchers in Japan (Ishijima and Ishiie 1981) and jujube witches'-broom researchers in Korea (Kim 1965).

Since the vectors of these five diseases are known, information on their ecology and biology should facilitate vector control and enable researchers to evaluate various plant species and cultivars for their resistance to disease.

Researchers have noted the frequent dual infections of virus and prokaryote in trees affected by mulberry dwarf, jujube and paulownia witches'-broom, and citrus huanglungbin. The morphology of the virus particles indicates that the viruses associated with paulownia and jujube witches'-broom belong to the tobamovirus group. Attempts should be made to study their host ranges and their serological relations to other members of the group. The particle morphology of the virus associated with mulberry dwarf is similar to potyviruses. Future study should be directed at identifying its vectors and determining the host range of the virus as well as serological relations between it and other potyviruses. One can never rule out the possibility that these viruses might be mycoplasma viruses. In the case of melia witches'-broom, evidence that two PLO affect the same tree suggests a complex relationship between pathogens and hosts. Accordingly, the effects of individual pathogens and a complex pathogen on the host plants should be studied. The behavior of prokaryotes within various host tissues at different stages of infection needs to be elucidated. These studies cannot be effectively conducted until the pathogens are isolated and transmitted by the insect vectors. Thus, the isolation and culture of prokaryotic organisms remains the top priority when studying the pathogen-vector-host relationship.

All five diseases described above are very destructive to trees and urgently require control. Until disease-resistant varieties of trees are developed, the most effective tactic is an integrated pest-management scheme that includes the roguing of diseased trees and other hosts of pathogens and vectors, vector control through chemical and nonchemical means, and chemotherapy.

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