CITRUS GREENING DISEASE

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INTRODUCTION

Citrus greening disease is a major cause of crop and tree loss in many parts of Asia and Africa. Before it was identified as one disease, it became known by various names: yellow shoot (huanglungbin) in China; likubin (decline) in Taiwan; dieback in India; leaf mottle in the Philippines; vein phloem degeneration in Indonesia; and yellow branch, blotchy-mottle, or greening in South Africa. As it became clear that all these were similar diseases the name "greening" was widely adopted.

Many reviews of citrus greening have appeared, but with few exceptions (9,186), are either brief, restricted to one country, or by now out of date. This review aims to present an overview of greening worldwide. There is no shortage of literature; Ōtake (186) lists 556 papers in his 1990 bibliography, and the present author found a further 86, although many do not advance our understanding of the disease.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Although citrus dieback was documented in India in the eighteenth century (192), this disease may not have been greening-induced decline. Indian dieback was first accurately described in 1929 and attributed to poor drainage (190). Yellow shoot disease was well known in south China in the 1890s. 
(278), and likubin was identified in Taiwan 60 years ago as a nematode-associated problem (186). The first mention of it in English may have been in 1919 when Reinking (196) described a yellowing and a leaf mottle of citrus in southern China; by 1935 it had become a serious problem there (132). In 1921 Lee (129) described mottle leaf disease in the Philippines, which he likened to a disease in California, probably stubborn, and attributed the former to zinc deficiency. It was not a serious problem there until 1957 (143). In Indonesia the disease was noticed in the 1940s (16).

When the disease was first described in South Africa in 1937 (254), it was presumed to be a mineral toxicity. It was known as yellow branch in the western Transvaal province, where it had been observed in 1928/1929 (185), and greening in the eastern Transvaal.

Greening has now also been confirmed in the following countries: Bangladesh (53), Burundi (15), Cameroun (15), Central African Republic (125), Comoros (220), Ethiopia (247), Hong Kong (48), Japan (163), Kenya (230), Madagascar (125), Malawi (15), Malaysia (120), Mauritius (175), Nepal (119), Pakistan (118), Réunion (175), Rwanda (15), Saudi Arabia (25, 28), Somalia (J. M. Bové, personal communication), Swaziland (52), Tanzania (241), Thailand (223), Yemen (25, 28) and Zimbabwe (229). A greening-like disease occurs in Australia (29, 30), but the known insect vectors of greening are absent (30). The disease probably exists in other countries that border on the above, although surveys failed to confirm its presence in Gabon, where evidence of the insect vector was found, and Zambia and Namibia, where no signs were encountered (15).

No evidence of greening has been found in Brazil (10), despite the occurrence of one of the vectors. No reports of greening have been confirmed from Mediterranean areas of Europe, North Africa or the Middle East, or California, all areas where the symptomatically similar citrus stubborn disease occurs. The two diseases have so far not been found to occur together in one area.

**ECONOMIC IMPORTANCE**

Losses due to greening are not easy to assess. Sometimes only sectors of a tree are affected and losses are small, but in other cases the entire tree is infected and crop loss is total (155). No detailed loss studies have been published, but the severity of the disease is substantiated in the literature. In India Fraser et al (76) commented on catastrophic losses. In the Philippines greening affected an estimated 7 million trees in 1962 (146), was largely responsible for reducing the area planted to citrus by over 60% between 1961 and 1970 (3), and in 1971, killed over 1 million trees in one province (194). In Thailand up to 95% of the trees in the northern and eastern provinces are severely affected (23).
In Taiwan the disease has caused widespread citrus destruction (236), and in Indonesia not less than 3 million trees were destroyed between 1960 and 1970 (244), with groves in most regions of Java and Sumatra being abandoned by 1983 (201). Practically all sweet orange and mandarin trees in south-western Saudi Arabia have declined and disappeared, leaving only limes (25).

In South Africa the incidence was severe from 1932 to 1936, 1939 to 1946, and again after 1958 (185). Crop losses of 30–100% were recorded in some areas (212). Incidence in the late 1960s and early 1970s declined slightly (226), but its overall effect is still marked, with citrus production eliminated in three major production areas (170).

**SYMPTOMOLOGY**

Symptoms can occur throughout the tree, especially if the infection occurs at or soon after propagation (151). If infection occurs later the symptoms and the causal organism are often partially confined. Earlier reports suggesting that the organism’s movement is restricted to new growth with little downward movement are challenged by recent findings that it can move 30–50 cm downwards in 12 months (S. P. van Vuuren, personal communication). Infected trees or branches suffer heavy leaf drop followed by out-of-season flushing and blossoming, with dieback occurring in severe cases (141).

In general leaf symptoms are of two types: Primary symptoms are characterized by yellowing of normal-sized leaves along the veins and sometimes by the development of a blotchy-mottle (209). With secondary symptoms the leaves are small, upright, and show a variety of chlorotic patterns resembling those induced by zinc and iron deficiencies. Analysis of symptomatic leaves shows a higher potassium content and lower calcium, magnesium, and zinc concentrations (7, 121).

Infected fruit are small, lopsided, and have a bitter taste (155), probably because of higher acidity and lower sugars (114). Many fall prematurely, while those that remain on the tree do not color properly, remaining green on the shaded side (155), hence the name of the disease. Any seeds in severely affected fruit are often abortive.

These descriptions are of greening in Africa. Other investigators described very similar symptoms in the Philippines (146), India (201), and China (278), but their papers contain descriptions of more extensive yellowing, dieback, and decline than are found in Africa. In China the disease was reported to kill young trees in 1–2 years (133).

Root systems of both forms are poorly developed with relatively few fibrous roots, possibly because of root starvation (9, 199). New root growth is suppressed and the roots often start decaying from the rootlets (278).

The causes of the symptoms are unknown. Amino acid concentrations are
generally lower (70, 94, 240, 266) except for L-proline, which is higher in the leaves and flower buds (70). There are also increases in amylase activity and glucose and fructose production (149), increased respiration early in infection (265), and the appearance of gentisoyl-β-D-glucose (74, 211), which seems to inhibit peroxidase activity (257). These changes do not, however, appear to be involved in symptom production.

Cytopathic studies have revealed pockets of necrotic phloem, excessive phloem formation, abnormal cambial activity and accumulation of starch in plasmids (209, 242, 246). Cytoplasmic membranes formed from invaginations of the plasmalemma, aberration of the chloroplast thylakoids and mitochondrial collapse have also been observed (272). These changes indicate significant metabolic disturbances, possibly via toxins and/or hormones.

Often citrus trees are infected by more than one pathogen, with consequent influence on symptom expression. In the Philippines (141), India (21), and Taiwan (107), plants infected with both greening and seedling yellows (tristeza) develop more severe leaf symptoms, stunting, and increased plant collapse. Such dual infection is common as tristeza is widespread in Africa and Asia, and was previously thought to cause yellow shoot in China (65). Furthermore, it has been noted that *Fusarium* spp. (136, 232), *Colletotrichum gloeosporioides*, and *Diplodia natalensis* (136) augment the symptoms of greening.

**TYPES AND STRAINS**

Although Asian greening symptoms are more severe than African, they can also be clearly distinguished on the basis of temperature tolerance. With African greening, severe symptoms were obtained under cool glasshouse conditions (22°C for 8 h nights, 24°C for 16 h days), whereas no symptoms appeared at 27–30°C (27). The symptoms of the Asian form are as pronounced at both temperatures. The higher temperature for an extended period appears to completely inactivate the African form (124).

This difference relates to their areas of natural occurrence. In South Africa, leaf symptoms are more pronounced in the cool areas than in the low-lying hot areas, and are more pronounced in winter (215). The most strongly developed symptoms are found at an altitude of 900 m, whereas very poor symptoms occur at or below 360 m (221). In Kenya greening is found only above 700 m, and in the Arabian peninsula the African form occurs in the higher areas of Yemen, and the Asian form in the lower altitudes of neighboring Saudi Arabia (28). Réunion and Mauritius possess both forms, similarly occurring at different altitudes (19).

Within the African type some strain differences are discernible. Isolates from the central Transvaal in South Africa have a higher graft-transmission
rate than those from the eastern Transvaal (219). An isolate from one area induced a blotchy-mottle whereas two others caused zinclike deficiencies (S. P. van Vuuren, personal communication). Strains of the Asian form have also been reported. In India different isolates showed varying degrees of virulence when grafted onto a common host: These isolates were grouped as mild, severe, and very severe (176). The mild strains offered only partial cross protection against the severe. Monoclonal antibodies raised against greening from Poona, India, failed to detect greening from China, Thailand, Malaysia, and other parts of India (86), but did react with an African greening sample (85).

VARIETAL SUSCEPTIBILITY AND HOST RANGE

Greening in South Africa is primarily a disease of sweet orange (Citrus sinensis), with valencias showing more pronounced leaf symptoms than navels (185). It is also particularly severe on mandarins (C. reticulata) and tangelos (C. sinensis × C. reticulata), but less so on lemon (C. limon). The least affected is the acid lime (C. aurantifolia). In Taiwan (162), India (181), and the Philippines (93) sweet orange and mandarin are the most susceptible, with lime, lemon, sour orange (C. aurantium), and grapefruit (C. paradisi) more tolerant. In India the rough lemon (C. jambhiri), sweet lime (C. limettoides) and pomelo (C. grandis) are tolerant and the trifoliate orange (Poncirus trifoliata) is fairly tolerant (181). As with African greening, some citrus selections are more affected by Asian greening than others—Blood Red sweet orange is more susceptible than Hamlin orange (72).

However, some differences have been reported. In India the widespread use of Kagzi lime (C. aurantifolia) as an indicator for greening (180) may have been effective because of co-infection with tristeza. In Australia the greening-like disease is most severe on grapefruit and sour orange (75) and in Taiwan the Wentan pomelo, once regarded as tolerant, has been suffering from a decline attributable to the likubin agent (106, 239).

Manicom & van Vuuren (140) have grouped the common citrus cultivars according to their general reaction to greening as follows: Severe (sweet orange, tangelo, mandarin), Moderate (grapefruit, lemon, sour orange) and Tolerant (lime, pomelo, trifoliate orange). That a particular type is not severely affected by greening does not necessarily reduce its significance—lemons probably serve as important reservoirs of infection because their more frequent flushes of new growth make them attractive to the insect vector (100).

In some cases the rootstock can affect symptom expression. Five out of 23 rough lemon rootstock selections in India induced a degree of tolerance in the sweet orange scion in greenhouse trials (59). In another study 100% of trees
on rough lemon were infected, compared to only 25% of trees on "Blood Red" sweet orange rootstock (113). In South Africa the percentage of greening in valencias was higher on trifoliate orange rootstock than on Empress mandarin and Troyer citrange. Possibly the trifoliate rootstock causes an extension of the flushing period and thus extends the feeding time of the insect vector (263). However, no differences were found in a Chinese study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (133).

Greening has been experimentally transmitted to many Citrus spp., the trifoliate orange (155) and to other rutaceous plants, causing leaf symptoms in kumquat (Fortunella sp.) (170), stunting (257), small leaves, and yellowing in Murraya paniculata (9), and stunting in Atalantia missionis and Swinglea glutinosa (240). These and other species may be natural hosts as the insect vectors of greening feed on them (18, 55, 173, 255). While the vector in Africa prefers citrus possibly because of its softer flush leaves (173), the vector in Asia displays a preference for Murraya spp. (55), which tend to flush all year round (188), but survives on Clausena and Atalantia (277). Moran (172) suggests that Vepris undulata, Clausena anisata, and Zanthoxylum (f. Fagara) capense are the original hosts of the vector in Africa. Efforts to transmit African greening to these plants by the vector were unsuccessful (J. V. da Graça and S. P van Vuuren, unpublished data). Other indigenous hosts may exist since the vector can feed on several nonrutaceous plants (250).

Greening has been experimentally transmitted by dodder to periwinkle (Catharanthus roseus) in which it induced marked yellowing (82, 116). As in its citrus hosts, African greening in periwinkle required temperatures below 27°C for symptom development, whereas the Asian form was more heat-tolerant. The dodder itself is a host for the greening pathogen with titers higher than has been observed in citrus (88, 116). Attempts in China to transmit the disease via dodder and the insect vector to cucumber, cabbage, tobacco and Solanum nigrum were unsuccessful (116).

TRANSMISSION

In 1943, Chen (64) suggested on the basis of graft inoculations that yellow shoot may be a viral disease. Similar opinions were soon expressed in South Africa (101, 150), strengthened by the finding in grafting trials that greening was inconsistently transmitted to healthy plants (184). Meanwhile, a report by Lin (132) in China confirmed that yellow shoot was indeed graft-transmissible. Graft-transmissibility of African greening was confirmed in 1965 by McClean & Oberholzer (154). The pathogen does not readily pass to progeny trees propagated by buds from infected trees (151), possibly because of necrosis of sieve tubes (154) and uneven distribution of the pathogen (105),
but more transmission occurs if stem pieces are used. No infection could be obtained when material from apparently healthy sectors of diseased trees were used. Schwarz (218) reported a higher graft-transmissibility rate in winter.

In 1964 Schwarz (210) reported that seedlings exposed to insects in a greening-infected orchard developed yellowing symptoms similar to greening. McClean & Oberholzer (154) also noted that greening appeared to spread in the field. These investigators then placed insects from diseased trees on healthy seedlings, and found that only adults of the citrus psylla species, *Trioza erytreae*, transmitted greening (153). Schwarz et al (224) later showed a positive correlation between the degree of greening infection, the number of psylla, and the rate of transmission.

Research on Asian greening followed close behind. In 1966 Salibe & Cortez (199) demonstrated graft transmission in the Philippines, and reported on an insect vector, soon identified there (144, 145, 200) and in India (40) as another psylla, *Diaphorina citri*.

The number of adult psylla of either species in a population that carry the disease is relatively small (152, 278), but under experimental conditions, a single adult of either species can transmit greening (52, 192).

The pathogen can be acquired by *D. citri* in 15–30 min (184) with a latent period of 8–12 days (192). One hour or more is required for 100% transmission. *T. erytreae* acquires the organism after one day of feeding and transmits greening 7 days later (S. P. van Vuuren, personal communication), and can infect with an exposure time of less than 1 h (261). Longer feeds can render the psylla more infective. Long overwintering feeding on old leaves makes adults highly infective on young flush in spring (47). Psylla are strongly attracted by yellow green of wavelength 550 nm (202), making diseased trees attractive targets and thereby increasing the proportion of disease-carrying insects.

Although nymphs are reportedly unable to transmit greening (40, 154, 192), the fourth and fifth instars of *D. citri* can acquire the pathogen and adults from such nymphs transmit the disease (41, 278). Nymphs of *T. erytreae* can also acquire the greening agent (S. P. van Vuuren, personal communication).

Analysis of the spread of greening in orchards in Réunion, China, and the Philippines has shown that epidemics appear to follow a sigmoid curve (95–97). Clustering of diseased trees was observed, with a higher aggregation correlated in Réunion to analysis of rows running in the direction of prevailing winds, which presumably aid vector movement. In China, a north-south aggregation corresponded to general orchard traffic and closer tree spacing in that direction (96).

*T. erytreae* is sensitive to heat. In laboratory tests, high temperatures (a 32°C plateau) killed all stages, 27°C allowed rapid development of the insect
but with a 52% mortality, whereas at 21°C 91% survived (174). In the field, populations were consistently higher in cool, moist upland areas of southern Africa, and always low in hot, drier lowlands, with eggs and first instars being particularly vulnerable to heat and desiccation (46). The prolonged flushing of trees in the cooler areas is more favorable to the insect (44, 49). The mortality of the developing stages was over 70% when the saturation deficit index (SDI), a value based on regression curves depicting the combined effects of temperature and humidity on egg and first instar mortality, is over 35 mb (98). These values were used to explain the distribution of *T. erytreae* in South Africa, connecting infestations of the insect with outbreaks of greening in past years (52). Since it is adult psylla that mainly transmit greening and are more tolerant to weather extremes, SDI is unsuitable, in Samways’ view (204), for early detection of population upsurges. He recommends instead the use of sticky yellow traps to monitor numbers. Such traps, together with trap seedlings, were used to monitor psylla populations and greening transmission in an orchard for three years (262). High populations in 1976 were associated with high greening transmission, while the hotter, drier years that followed had both low populations and low transmission.

*D. citri* has a similar biology, but it is more resistant to extremes of temperature (49) and more sensitive to high rainfall and humidity (10). Since it prefers hot dry weather to cold wet conditions, high populations are found in early spring and summer, and dramatically lower numbers during high rainfall in spring (195). *D. citri* can withstand low temperatures for short periods, with a 45% survival at −3°C for 24 h under natural conditions and 39% survival at −5°C for 24 h under experimental conditions (274).

In Réunion and Mauritius both vectors occur, *T. erytreae* above 500 m and *D. citri* below 400 m where it is hotter (48, 51). Similarly in the Arabian peninsula, *D. citri* occurs in the low-lying citrus areas of Saudi Arabia and *T. erytreae* above 1000 m in neighboring Yemen (25). However, in Ethiopia only *T. erytreae* has been recorded (1).

There does not appear to be any specificity between the psylla species and the greening type. In Réunion and Mauritius the African form is spread by both vectors (48, 51). Indian greening has been experimentally transmitted by *T. erytreae* (147) and African greening by *D. citri* (127).

In the South African western Cape province greening disease does not spread despite the presence of the psylla (252). In the laboratory, trapped specimens from this area were able to transmit greening and breed with Transvaal specimens. The absence of greening transmission in this area may be influenced by climatic factors, this being a winter rainfall area with hot, dry summers.

In an ecological study, Samways & Manicom (206) found that adult *T. erytreae* invaded an orchard in exponentially increasing numbers; on an area
basis 4% of new trees were infested per day. Mean population density is not significantly different for managed citrus orchards, neglected orchards, and natural bush (202).

Other psylla species have been noted on citrus, namely T. eastopi (T. litseae) and Mesohomatoma lutheri in Réunion (17), D. communis in India (10), T. citroimpura, Psylla citrisuga, and P. citricola in China (268a), D. auberti and D. amoena in Comoros, (10, 16) and D. punctulata and D. zebrana in Swaziland (52), but there is as yet no evidence that any transmit greening.

The only other means of transmission known is by dodder (Cuscuta spp.). C. reflexa was used to transmit the pathogen from citrus to citrus (190), and C. campestris for transmission from citrus to periwinkle (82, 116), but C. japonica failed to transmit (116).

**NATURE OF THE CAUSAL AGENT**

The demonstrations that greening is a graft- and insect-transmissible disease led to the conclusion that a virus was responsible. In China some researchers believed tristeza virus to be the cause (65, 131, 149), but Lin (135) publicly disagreed. In South Africa it was shown that tristeza and greening could readily be distinguished since the aphid Toxoptera citricidus transmitted tristeza but not greening, and psylla vice versa (153).

In 1970 Laflèche & Bové (125, 126) reported observing mycoplasma-like organisms (MLOs) in citrus phloem tissue infected with African and Indian greening. These organisms measured 100–200 nm in diameter, with filamentous forms up to 2 μ long. The phloem restriction of the greening pathogen was supported by the observation that girdling prevented spread within a plant (142).

Apparently identical organisms were soon observed in the hemolymph and salivary glands of T. erytreae (167), the phloem of yellow shoot (278) and likubin-infected citrus (61, 243), and in infective D. citri (63). Their identification as MLOs was soon questioned because whereas MLOs are surrounded by a true unit membrane 10 nm thick, the greening organism has an outer envelope 20 nm thick (198). Comparative electron microscope studies on the structures of the greening organism and several other types of prokaryote clearly showed that it was not an MLO (84, 168). Garnier & Bové (81) suggested that it should be classified as a true bacterium, possibly belonging to the Gracilicute division of the prokaryotes (26). Although no distinct R-layer of peptidoglycan (PG) had been observed (168, 242), the inner layer of the outer membrane was somewhat thicker in places, suggesting the presence of an R-layer (81). Treatment of infected plants with penicillin G, which inhibits a late step in PG synthesis, caused symptom remission and
the disappearance of organisms from the phloem (26). In a comparable cytochemical treatment study with the greening organism in periwinkle, and with gram negative and gram positive bacteria, Escherichia coli, and Staphylococcus aureus, respectively, Garnier et al (83) showed that papain caused a visible R-layer to separate from the outer membrane of both E. coli and the greening organism. The R-layer disappeared after lysozyme treatment. They concluded that the greening organism is gram negative.

The greening organism's resistance to culture on artificial media has made study of the organism difficult. Some isolates from Indian greening-infected citrus have been reported (89, 90, 179, 235), but all had the characteristics of true mycoplasmas and were therefore probably contaminants. Although some isolates induced foliar symptoms in inoculated citrus (179, 235), various microorganisms are known to cause transient leaf blotches (123, 139). Initial attempts to isolate the African greening organism were unsuccessful (164).

The presence of other bacteria in healthy (78) and greening-infected citrus vascular tissue, and in psylla (24) impedes attempts to isolate the greening organism.

In 1984 Garnett (79) reported the isolation of a long rod-shaped gram negative organism from African greening-infected citrus leaf mid-ribs. The ultrastructure of this organism was described as similar to that of the organisms observed in greening-infected citrus, periwinkle, and insect vectors (4). On a solid medium, it formed small round colonies with predominantly long rod-shaped cells near the edges, but rounder cells in the oldest parts. Antibodies raised against this isolate reportedly gave positive ELISA results only with greening-infected plant material (73). Gold-labeled antibodies reacted only with the cell wall of a bacterium resembling the observed greening organism in infected citrus phloem and extracted sap (5).

Garnett and others have established a collection of greening cultures in plants from South Africa, Réunion, China, Taiwan, India, and the Philippines for comparative studies in quarantine facilities in the USA (130). Isolates from these plants share common serological and protein profiles (80), and a high percentage of DNA homology (103). Inoculated plants develop foliar symptoms, but completion of Koch's postulates has not been reported.

Other research groups in South Africa have not been able to confirm the above results. Manicom (139) isolated several species but none resembled the greening organism. Chippindall (personal communication) has isolated a bacterium that on mechanical inoculation to tangelo induces foliar symptoms. However, this bacterium could not be graft- or psylla-transmitted to other plants, no gentiosyl-β-glucoside was present, and the symptoms later disappeared. This organism has been identified as a Clavibacter sp., and although it is not the greening organism, may, in the opinion of Chippindall & Whitlock (67), be a component of the overall disease syndrome. Labuschagne et al (123) isolated several bacterial species in South Africa, of which one,
identified as *Acinetobacter lwoffii*, induced transient yellowing symptoms (234). Serological tests show it to be present in both healthy and African greening-infected trees, but of higher incidence in the latter, especially when foliar mottling occurs (208). It also reacted positively with infected material from the Philippines. *A. lwoffii* was one of the species isolated from citrus in Florida (78) where greening does not exist. Clearly, *A. lwoffii* is not the greening organism, but it may play a role in the overall greening syndrome.

In an effort to confirm the isolation claim by Garnett (79), Garnier et al (85) found that antibodies against the cultured organism failed to react with greening-infected tissue in their laboratory, and that a chemically fixed culture failed to react with monoclonal antibodies raised against greening-infected phloem (268a). These researchers raised monoclonal antibodies against infected phloem tissues (85) and used them to purify the organism from infected periwinkle plants (86). Filamentous and round cells have been trapped in this way.

DETECTION

The use of indicator seedlings was the first diagnostic test developed. In South Africa either valencia sweet orange or Orlando tangelo are used (213). In Taiwan Ponkan mandarin is preferred (148), while in India the Kagzi lime (see above; 21, 179), has been replaced by the Mosambi sweet orange or the Darjeeling orange, a mandarin type (2). Graft sticks are grafted into the indicator stems, using about 20 seedlings per test: Symptoms appear in 3–4 months in a greenhouse at 21–23°C (213).

A quicker laboratory test is always desirable, and since the causal bacterium had not been isolated, attention turned to biochemical changes in infected plants. Schwarz (214) reported that a fluorescent substance, later identified as gentisoyl-β-glucoside (74), was detectable in ether extracts of greening-infected bark, but not in healthy ones. When separated by paper or thin layer chromatography using n-butanol:acetic acid:water (5:1:1) (214) or chloroform-methanol (223), a bright violet-blue spot fluoresced under ultraviolet light (365 nm). The same fluorescence can be observed in the albedo of greening-affected fruit (214), and was found to be reliable for early detection (217). The test is not absolutely specific for greening, however, as Californian stubborn-infected material also contained such a marker (214). The marker is detectable in all sweet oranges and mandarins, most tangelos and lemons, but only sporadically in grapefruit (111, 216).

In South Africa its concentration was reported to vary with seasons but not enough to affect its diagnostic use (218), whereas in India the concentrations were high all year (231). Flower buds were found to be as suitable as bark for extraction (57).
The test has been modified by using water extraction by centrifugation instead of ether (227), by reducing the volume of water to eliminate the lengthy evaporation step (260), and employing acid hydrolysis to liberate gentisic acid, which produces a clearer fluorescent spot (39). Gentisic acid has since been shown to be present in many healthy citrus species and cultivars, but its concentration increases significantly in some as greening symptoms develop (258).

Diagnostic tests using light microscopy have been developed in China. Sections of infected leaves are examined either by fluorescent microscopy, which reveals a yellow fluorescence in infected phloem that is absent from healthy, virus-infected and nutrient-deficient tissues (270), or by staining sections with safranin, which shows red patches in infected phloem (271).

Serological tests are being developed. Using infected plant tissues, French researchers have raised monoclonal antibodies against Indian and African greening that recognized greening isolates from India, the Philippines, and Réunion (85), but not ones from China (77), Thailand, Malaysia, or other parts of India (86), while monoclonals against a Chinese strain did not react with Indian greening (86). This group has now raised monoclonals against several Asian and African isolates (J. M. Bové, personal communication), which in combination may have potential universal use. In South Africa, monoclonals have also been prepared using extracts of greening-transmitting psylla that react positively with infected citrus (122), as does a polyclonal against infected citrus tissue (66).

Another diagnostic technique in France is the use of DNA probes prepared from cloned DNA from infected periwinkle (86), and from greening organism cells trapped by immunoaffinity chromatography (267). Research into developing probes from the cultured putative greening organism has begun in Australia (103).

CONTROL

Control of the Greening Pathogen

THERMOTHERAPY In 1964 Lin (134) reported eliminating yellow shoot disease by water-saturated hot air treatment of graftwood at 48–58°C with no loss of tissue viability. Other attempts to eliminate Asian greening with heat have had varying degrees of success. In India treatment of budwood at 47°C for 2 h reduced disease incidence (56) and longer treatments eliminated the pathogen (178). Treatment of infected young plants or seedlings budded with infected tissue at 38–40°C for three to four weeks also killed the pathogen (104, 178).

In South Africa budwood from infected trees heated over a hot water bath at
51°C for 1 h, 49°C for 2 h and 47°C for 4 h eliminated the disease although some tissue viability was lost at the higher temperatures (222). Infected trees covered for 2–5 months with polyethylene-covered fiberglass sheets showed a dramatic decrease in the number of diseased fruit. However this method is impractical for large-scale use.

Heat treatments have some application in the elimination of greening from horticulturally desirable trees, possibly in conjunction with shoot tip grafting (238).

CHEMOTHERAPY The association of prokaryotic organisms with greening prompted investigations into the use of antibiotics. Martinez et al (143) in the Philippines suppressed leaf symptoms with a foliar spray of tetracycline hydrochloride and apparently eliminated greening in budwood by immersion in the solution. In India immersion in a penicillin-carbendazin dip reportedly gave complete control (60). In South Africa Schwarz & van Vuuren (228) injected various tetracyclines into the trunks of greening-infected sweet orange trees and found that tetracycline hydrochloride gave the best results, reducing fruit symptoms in the next crop from 60 to 20%. The incidence of infection remained unchanged for three years when a severe psylla outbreak and a slight increase in greening occurred (169). The trees received a second treatment that reduced fruit symptom incidence to below 10% (171). Better results were achieved when two injections were given one month apart (259), while a 97% reduction of fruit symptoms was achieved when trees were injected continuously under pressure for 7 days (264). The best time for injection is spring (226), and distribution in the tree can be enhanced by the use of hyaluronidase (233). Trunk injections of tetracycline hydrochloride have also been successful in Taiwan (68), China (278), Réunion (13), and the Philippines (143).

Other antibiotics have been tested. Penicillin had a less suppressive effect than tetracycline hydrochloride in Asia and Réunion (13, 14, 161, 278), but was totally ineffective in South Africa (225). In India partial to total remission occurred following injection with a compound called BP-101 (Hindustan Antibiotics Ltd.) (42, 43, 190), ledermycin (demeclocycline hydrochloride) and a streptomycin-chlortetracycline mixture (178), while complete remission using foliar sprays of agrimycin and carbendazin has also been reported (58, 112).

However, tetracycline hydrochloride is phytotoxic. Buds immersed in concentrations of over 250 ppm did not survive after grafting (161). Van Vuuren (259) observed leaf narrowing, lamina yellowing, veinal browning, and occasional defoliation of individual twigs. A brown discoloration was found in the trunk in a zone extending some 300 mm from the hole, decreasing in intensity with distance. Leaf narrowing has also been recorded in
Taiwan with oxytetracycline, but not with tetracycline hydrochloride or chlor-tetracycline (68).

A derivative of tetracycline hydrochloride, N-pyrrolidinomethyl tetracycline (PMT) is more soluble in water, giving slightly better control of greening, and causing no foliar phytotoxicity and minimal wood discoloration (35). Midsummer treatments gave the best results (37). The solubility of PMT in water is 1250 mg/ml, compared to 10.8 mg/ml for tetracycline hydrochloride, so that smaller volumes can be injected from syringes without employing high pressures (99).

Moll (165) was unable to detect by fluorimetry any antibiotic residues in the juice of injected trees one month after treatment. Other studies using growth inhibition of Bacillus megaterium (36, 37), B. cereus var. mycoides (68) or B. subtilis (13), have confirmed that antibiotic residues drop rapidly.

**BREEDING FOR RESISTANCE** In South Africa various hybrids of sweet oranges and Tahiti lime have been produced, and are being tested for possible resistance (71). The use of callus tissue and protoplasts is also being evaluated. Peroxidase activity, which is higher in more tolerant varieties, may serve as a marker for tolerance to greening in screening new hybrids (256).

**Control of the Vectors**

**USE OF INSECTICIDES** Because psylla are sap feeders, systemic insecticides are the most effective. Those most commonly used in southern Africa are endosulfan sprays (187); monocrotophos applied as sprays (31), aerial sprays (109), trunk applications (38), and trunk injections (225); and dimethoate applied to the soil (158, 159, 269). Weekly sprays of monocrotophos did not prevent infection (32), while high volume and mist blower applications destroy natural enemies of other citrus pests (33). A single application of dimethoate by microject and drip irrigation gave control for over 7 weeks, and two applications gave season-long protection (159). Simultaneous injection of dimethoate and tetracycline hydrochloride controlled psylla without reducing antibiotic efficacy (160).

In India, *D. citri* has been controlled for up to 4 weeks with sprays of several insecticides, including dimethoate and monocrotophos (22, 117) and soil applications of dimethoate (193). In China field control with soil applications of O-methoate has been reported (272).

However insecticides can interfere with biological control of other pests. While dimethoate can reduce the efficacy of the biological of citrus red scale (102), at 0.01% a.i. it does not seriously affect the activity of psylla parasites (47). Another problem for small farmers is cost; one Indonesian farmer reportedly spent $2500 p.a. to control psylla (268).
BIOLOGICAL CONTROL The major parasite of *T. erytreae* in South Africa is a species of parasitoid wasp *Tetrastichus* that oviposits in the psylla nymphs (255), and appears to limit psylla populations (46).

A survey in Réunion in 1973 found no parasitized mummies of psylla (51), and it was suggested that parasitic wasps should be introduced (6). Three species, *Tetrastichus radiatus* (now called *Tamarixia radiata*) from India, and *Tetrastichus dryi* and *Psyllaephagus pulvinatus* from South Africa, were collected and mass reared (14). *T. dryi* was released in 1976, and had dramatically reduced the *T. erytreae* population by 1978 (17, 18), with complete eradication by 1982 (19). *P. pulvinatus* failed to establish itself. *T. radiata*, which is specific for *D. citri*, was introduced in 1978 (17) and 1982 had virtually eliminated psylla from commercial orchards, surviving only on *M. paniculata* hedges (189). The incidence of greening has consequently been reduced (18).

Both species of wasp were subsequently introduced into Mauritius where they reduced psylla populations (13), but more slowly than in Réunion, possibly because they were not mass reared before release (199). *T. radiata* has also been introduced into Taiwan (237), the Philippines (87), Nepal (128), and Indonesia (183), but without much apparent success.

Other parasitic wasps of *D. citri* are *Diaphorencyrtus aligarhensis* (16, 69), *Psyllaephagus* sp. (16, 137), *Chartocerus walkeri*, and *Encarsia* (243a), but none appears to be a potential biological control agent.

The efficacy of parasitic wasps is limited by the activity of hyperparasitic wasps. In South Africa the effect of *Aphidicyrtus cassatus* varies according to its ability to maintain synchrony with its host (45). Another species is *Cheiloneurus cyanonotus* (157). In Taiwan *D. aligarhensis* has at least 10 hyperparasites, of which one, *Pachyneuron*, can also oviposit in *T. radiata* (69). In China a species of *Tetrastichus* attacks both *T. radiata* and *D. aligarhensis* (243a). No hyperparasites occur in Réunion (16), which may in part explain the success of biological control there.

A survey of predators of psylla in South Africa showed that none of them reduce densities low enough for greening control (251).

Fungi provide another means of biological control. *T. erytreae* is attacked by *Cladosporium oxysporium* (205) and *Capnodium citri* (10), but both are sensitive to desiccation and are density-dependent. *D. citri* is parasitized by *Beauveria* (87) and *Cephalosporium lacanii* (275), the latter somewhat effective at high density.

OTHER CONTROL MEASURES The production of greening-free trees is vital. The use of shoot-tip grafted material can ensure this goal (110, 237). Production can be facilitated by using sticky yellow traps to identify nursery and orchard sites that are less susceptible to psylla attack (20, 91). Such sites
should also be distant from indigenous psylla hosts (20). Although psylla were considered not to have strong dispersal powers (50), recent investigation established that adult *T. erytreae* can be wind-dispersed in the absence of host plants 1.5 km or more, and can survive approximately 85 hr without food (249). Strong winds such as those associated with typhoons have transported *D. cirri* over medium to long distances (8).

The removal of infected branches or trees (20, 34) and neglected trees (253) may also reduce inoculum sources (24, 38) although mean population densities in the latter are not always significantly higher (203). If winter irrigation is withheld from lemon trees to suppress new flush, such infection reservoirs (105) can be reduced (248). In China hedges of *M. paniculata* which serve as hosts for *D. citri*, are being removed (274).

Systems of internal quarantine in South Africa prohibit the movement of trees from the greening-affected Transvaal and Natal provinces to the Cape province (154). Similar restrictions apply in China (188).

In Shantou, China, the practice of high-density plantings aimed at generating faster economic returns through early bearing serves to keep greening in check provided that the trees are from a disease-free nursery, that tree canopies remain uniform, and that strict psylla control is enforced (12).

The use of SDI (98) to predict psylla population outbreaks or sticky yellow traps to detect a population threshold value (two or more per set of three traps during each of two consecutive weeks) (207) can assist insecticide application programs. If insecticides are applied from late winter to midsummer the natural enemies of psylla are not seriously affected. Because *D. citri* does not cause foliar damage traps must be used. They are now being employed in China (8, 274) and the Philippines (87). By themselves traps do not appreciably reduce populations (202), but trap trees, preferably heavily pruned valencia sweet orange, could help manage psylla populations (206).

The prevalence of greening in some of the cooler citrus-growing areas of South Africa has caused more citrus cultivation in the hotter areas where citrus blight is prevalent (166). This shift has therefore been problematical.

**CONCLUDING REMARKS**

The conclusive isolation of the causal organism of citrus greening remains the primary goal for many researchers. International cooperation between all laboratories currently involved would substantially increase the chance of success. Serious consideration should also be given to the possibility that the full greening syndrome may be caused by a combination of microorganisms such as the putative greening organism (79), *A. lwofii* (234), *Clavibacter* (67) and possibly others, and/or environmental conditions. The so-called greening organism may not on its own produce all the greening symptoms.
For citrus industries control is paramount. Clearly, integrated control will be the most effective. In Réunion a combination of introducing parasites, treating trees with antibiotics, and raising new trees free of infection has had success (14), and in India the use of insecticides, injections of tetracycline hydrochloride, thermotherapy of budwood and the use of tolerant rootstocks has reduced losses (177). In China, where the use of antibiotics has not yet been widely adopted (278), a combination of quarantine, propagation of healthy plants, thermotherapy and shoot-tip grafting of infected budwood, removal of diseased trees, especially in areas of low psylla incidence (108), and the use of insecticides is recommended (110, 115). In foundation blocks in the Philippines a combination of tolerant varieties, eradication of diseased trees, replanting with healthy plants, and spraying against psylla showed that citrus production is still feasible in greening areas (92a). Shoot-tip grafting and the maintenance of disease-free trees in screenhouses is recommended in Taiwan (92). Experience in the Philippines where the country’s most valuable budwood sources are to a large degree greening-infected (138) underscores the importance of disease-free stock.

Currently, vector control is emphasized in South Africa, combined with removal of infected trees and branches (34). Trees with 50–75% greening fruit symptoms should be removed, while removal of branches is recommended for lower infection levels. Injection with PMT is recommended only for trees over 10 years old with more than 40% greening. Alternative chemothrapeutic agents are needed, however, together with long-term breeding programs. The subject of integrated management of greening has recently been reviewed (11).

A concern for those countries that have so far escaped the ravages of greening is how to avoid it. For Brazil the danger is probably greatest because the vector, D. citri, is already present (10). If it should enter, the rest of South America and subsequently North America would be threatened. The northward migration of the citrus tristeza aphid vector Toxoptera citricidus (197) illustrates the danger. The presence of the vectors and the disease in Pakistan and the Arabian peninsula poses a similar danger for Mediterranean countries. The danger is real that the Asian vector, D. citri, may move either from Arabia or from Réunion and Mauritius, via Madagascar, to the African mainland, and become established in conjunction with the existing African form. Citrus in the hotter, drier areas would then also be threatened.

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Literature Cited


18. Aubert, B., Quilici, S. 1988. Results of the monitoring of citrus psylla on yellow traps in Reunion Island. See Ref. 11, pp. 249–54


48. Catling, H. D. 1970. Distribution of the psylla vectors on greening disease, with notes on the biology and bionomics of...
Da Graça, 128

Diaphorina citri. FAO Plant Prot. Bull. 18:8–15


69. Chiu, S-C., Aubert, B., Chin, C-C. 1988. Attempts to establish Tetrastichus radiatus Waterson (Hymenoptera, Chalcidoidea), a primary parasite of Diaphorina citri Kuwayama, in Taiwan. See Ref. 11, pp. 265–68


The occurrence of a gentisic glucoside in the bark and albedo of virus-infected citrus trees. *Phytopathology* 59:603–6
83. Garnier, M., Danel, N., Bové, J. M. 1984. The greening organism is a gram negative bacterium. See Ref. 17, pp. 115–24
psiylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae), a vector of greening virus, in some citrus producing areas of southern Africa. *Agric. Meteorol.* 8:305–17
108. Huang, S. 1988. The relation between the distribution of *C. citri* and greening disease in Guangxi Zhuang Autonomous Region. See Ref. 77, pp. 78–79


139. Manicom, B. Q. 1984. Attempts at culture, purification and fluorescent antibody tracking of the greening organism. See Ref. 24, pp. 66–69


147. Massonie, G., Garnier, M., Bové, J. M. 1976. Transmission of Indian citrus decline by Trioza erytreae (Del Guercio), the vector of South African greening. See Ref. 3, pp. 18–20


186. Otake, A. 1990. *Assessment of the fungus* *oxysporum* *as a biological control agent* *by means of monoclonal antibodies.* See Ref. 20. 72:374-82


209. Schneider, H. 1968. Anatomy of green-
ing diseased sweet orange shoots. *Phytopathology* 58:1155–60


217. Schwarz, R. E. 1970. Comparative indexing of the annual and seasonal incidence of greening in sweet orange fruits by external symptoms and by the albedo fluorescence test. *Phytophylactica* 2:1–16


technique of citrus shoot tip grafting and rapid propagation method to obtain citrus budwood free of citrus viruses and likubin organism. 


265. Verma, A. K., Singh, B. P. 1977. In-
fluence of tristeza, greening and their complex on respiration in Kagzi lime leaves. *Indian Phytopathol.* 31:79


273. Xia, Y., Xu, C. 1988. First results of monitoring *Diaphorina citri* (Homoptera:Chermidae), the vector of huanglungbin, with yellow traps. See Ref. 77, pp. 32–34


