ASIAN CITRUS PSYLLIDS (STERNORRHYNCHA: PSYLLIDAE) AND GREENING DISEASE OF CITRUS: A LITERATURE REVIEW AND ASSESSMENT OF RISK IN FLORIDA

Authors: Susan E. Halbert, and Keremane L. Manjunath
Source: Florida Entomologist, 87(3) : 330-353
Published By: Florida Entomological Society
ASIAN CITRUS PSYLLIDS (STERNORRHYNCHA: PSYLLIDAE)
AND GREENING DISEASE OF CITRUS: A LITERATURE REVIEW
AND ASSESSMENT OF RISK IN FLORIDA

SUSAN E. HALBERT1 AND KEREMANE L. MANJUNATH2
1Division of Plant Industry, Florida Department of Agriculture and Consumer Services
P.O. Box 147100, Gainesville, FL 32614-7100

2University of Florida Department of Plant Pathology, Gainesville, FL 32611

ABSTRACT

The Asian citrus psyllid, Diaphorina citri Kuwayama, was discovered in Florida in 1998. It can be one of the most serious pests of citrus if the pathogens that cause citrus greening disease (huanglongbing) are present. Citrus greening recently has been reported in Brazil by Fundecitrus, Brazil. The establishment of D. citri in Florida increases the possibility that the disease may become established. Diaphorina citri can be separated from about 13 other species of psyllids reported on citrus. The biology of D. citri makes it ideally suited to the Florida climate. Only two species, D. citri and Trioza erytreae (del Guercio), have been implicated in spread of citrus greening, a disease caused by highly fastidious phloem-inhabiting bacteria. The disease is characterized by blotchy mottle on the leaves, and misshapen, poorly colored off-tasting fruit. In areas where the disease is endemic, citrus trees may live for only 5-8 years and never bear usable fruit. The disease occurs throughout much of Asia and Africa south of the Sahara Desert, on several small islands in the Indian Ocean, and in the Saudi Arabian Peninsula. Transmission of citrus greening occurs primarily via infective citrus psyllids and grafting. It is transmissible experimentally through dodder and might be transmitted by seed from infected plants and transovarially in psyllid vectors. Citrus greening disease is restricted to Citrus and close citrus relatives because of the narrow host range of the psyllid vectors. Management of citrus greening disease is difficult and requires an integrated approach including use of clean stock, elimination of inoculum via voluntary and regulatory means, use of pesticides to control psyllid vectors in the citrus crop, and biological control of psyllid vectors in non-crop reservoirs. There is no place in the world where citrus greening disease occurs that it is under completely successful management. Eradication of citrus greening disease may be possible if it is detected early. Research is needed on rapid and robust diagnosis, disease epidemiology, and psyllid vector control.

Key Words: Diaphorina citri Kuwayama, Asian citrus psyllid, citrus greening disease, huanglongbing, citrus psyllids, Citrus.

RESUMEN

El psílido Asiático de cítricos, Diaphorina citri Kuwayama, fue descubierto en Florida en 1998. Esta puede ser una de las plagas de cítricos más serias si los patógenos que causan la enfermedad “greening” de los cítricos (huanglongbing) están presentes. Recientemente, la enfermedad “greening” de los cítricos ha sido reportada en Brasil por Fundecitrus (Brasil). El establecimiento de D. citri en Florida aumenta la posibilidad que la enfermedad pueda establecerse. Diaphorina citri puede ser separado de aproximadamente 13 otras especies de psílidos reportadas en cítricos. La biología de D. citri lo hace idealmente adaptable al clima de Florida. Solamente dos especies, D. citri y Trioza erytreae (del Guercio), han sido implicadas en la dispersión del “greening” de cítricos, una enfermedad causada por una bacteria altamente fastidiosa que habita el floema. La enfermedad se caracteriza por causar áreas moteadas en las hojas, y frutas mal formadas, mal coloradas y con sabor anormal. En áreas donde la enfermedad es endémica, los árboles de cítricos pueden vivir por solamente 5-8 años y nunca dar fruta provechosa. La enfermedad ocurre en la mayor parte de Asia, en África al sur del Desierto Sahara, en varias islas pequeñas del Océano Índico, y en la Península de Arabia Saudí. La transmisión de “greening” de cítricos ocurre principalmente por medio de psílidos infectados y por injertar las plantas. Puede ser transmisible experimentalmente a través de cuscuta, posiblemente transmitida por la semilla de plantas infectadas y transversalmente en los vectores psílidos. La enfermedad de “greening” de cítricos es restringida al Citrus y sus relativos cercanos debido al rango estrecho de hospederos de los vectores psílidos. El manejo de la enfermedad de “greening” de cítricos es difícil y requiere una estrategia integrada incluyendo el uso de plantas no contaminadas, la eliminación de inóculo por medios voluntarios y regulatorios, el uso de pesticidas para controlar los vectores psílidos en los huertos de cítricos, y el control biológico de los vectores psílidos en depósitos de plantas.
Asian citrus psyllid (Diaphorina citri Kuwayama, Sternorrhyncha: Psyllidae) may be the most serious pest of citrus in the world if any of the pathogens that cause citrus greening also are present. If none of the pathogens are present, the psyllids usually are minor pests. Citrus greening was reported in Brazil in July 2004 by Fundecitrus. This is the first report of the disease in the Western Hemisphere (Anon. 2004).

The Asian citrus psyllid causes damage to the crop primarily by transmission of the pathogen that causes greening, or “huanglongbing” (yellow dragon disease) in Chinese. Huanglongbing has been translated loosely as yellow shoot disease in English language publications because of characteristic yellow shoots caused by the disease. In addition to yellow shoots, the disease also causes mottling, chlorosis resembling zinc deficiency, twig dieback and reduced fruit size and quality. Fruit do not color properly, leading to the name greening. Fruit from diseased trees have a bitter taste. Other names include citrus vein phloem degeneration, and likubin, which means immediate withering disease. Australian citrus dieback, a disease of unknown etiology, is suspected to be caused by a similar psyllid-transmitted pathogen (Broadbent 2000). Although the official name of the disease is huanglongbing (anom. 1996), we use the name “citrus greening disease” throughout this review because it is the name commonly used in the United States, and by our audience for this paper.

Citrus greening probably is the worst disease of citrus caused by a vectored pathogen. The dynamics, epidemiology, and molecular characteristics of the complex are poorly understood. Trioza erytreae (del Guercio) (Sternorrhyncha: Trioziidae) in Africa and Diaphorina citri Kuwayama (Sternorrhyncha: Psyllidae) in Asia are the only known vectors of the two forms of the disease, namely African and Asian citrus greening, respectively. Two species of fastidious phloem-limited bacteria (Candidatus Liberibacter africanus and asiaticus), are thought to be the causal organisms, but Koch’s postulates have not been fulfilled because the bacteria have not been cultured yet. (The term Candidatus is used for bacteria species that cannot be cultured. If Candidatus is used, the actual genus and species are not italicized.) The pathogens prevalently transmitted by the two psyllids are different, but both psyllid species will transmit either pathogen under experimental conditions (Lallemand et al. 1986). The pathogens at present are extremely difficult to detect and characterize, although great strides have been made in recent years with development of detection methods based on polymerase chain reaction (PCR) and DNA hybridization.

Excellent reviews of citrus greening disease complex have been published (da Graça 1991; Garnier & Bové 1993, 2000a; Mead 1977; Roistacher 1991; Viraktamath & Bhumannavar 2002). We do not intend to duplicate these earlier efforts. The recent introduction of D. citri into Florida (Halbert 1998) has greatly increased the threat of citrus greening disease for North American citrus. The intent of this paper is to provide a convenient summary of information that is relevant to risk assessment for the Florida citrus industry and possible eradication of citrus greening disease. The emphasis will be on recognition of citrus psyllids, host range information, detection, epidemiology, and management.

BIOLOGY OF ASIAN CITRUS PSYLLID

Systematics and Recognition of Psyllids Reported on Citrus

Diaphorina citri (Euphalarus citri (Kuwayama 1908)) was described from citrus in Shinchiku, Taiwan in 1907 and published in a double volume in 1908. Species of Diaphorina usually are separated based on the pattern of maculation in the forewings and the shape of the genital cones. Diaphorina citri has a distinct pattern on the forewings and can be separated easily from most of the other species reported on citrus and its relatives.

There are six other obscure species of Diaphorina also reported from citrus and other closely related plants: Diaphorina amoena Capener 1970b (reported on citrus by da Graça 1991), Diaphorina auberti Hollis 1987a (reported on citrus in original description), Diaphorina communis Mather 1975 (reported on citrus in original description) and Auber (1987), Diaphorina murrayi Kandasamy 1986 (reported on citrus in original description), Diaphorina punctulata (Pettey 1924) (reported on citrus in original description), and Diaphorina zebrana Capener 1970b (reported on citrus by Catling & Atkinson 1974). Diaphorina auberti was described (Hollis 1987a) from the Comoro Islands. The host is citrus, on which nymphs concentrate on the upper surfaces of young leaves near the midribs. This causes the lateral margins of the leaves to curl upwards and inwards, sometimes forming an en-
closed leaf-roll (Dr. B. Aubert, pers. comm., in Hollis 1987a). Hollis (1987a) placed D. auberti in the D. amoena species group. The wing patterns of D. auberti are very similar to those of D. amoena (see Fig. 10 and Fig. 11 (amoena) compared with Fig. 22 and Fig. 23 (auberti) in Hollis (1987a)); however, the genae of auberti are much shorter than those of amoena (see Fig. 1 and Fig. 7 of Hollis (1987a)). The host for D. amoena is Strynchos innocua Delile (Loganiaceae (Strychnaceae)). It is not reported from citrus or citrus relatives either in Hollis (1987a) or in the original description (Capener 1970b). Thus, reports of D. amoena on citrus can probably be attributed to D. auberti or possibly D. citri. Both D. amoena and D. auberti can be separated from D. citri by the pattern on the forewings.

Diaphorina communis was described from a long series of specimens collected in Uttar Pradesh, India (Mather 1975). It is reported to be common on Murraya koenigii and occurs occasionally on citrus. It is mentioned as a possible species on citrus by Burckhardt (1994a). The forewings of D. communis are almost totally dark, making it easily separable from D. citri.

Diaphorina murrayi was described by Kandasamy (1986) from 11 specimens taken on Murraya exotica L. in Madras (now Chennai, Tamilnadu), India. According to the original description, it is closely related to D. citri but differs in having a slightly different wing pattern and slightly different tarsal spine formula. So far, it is reported only from M. exotica. Further study is needed to determine whether it differs sufficiently from D. citri to be considered a separate species.

Diaphorina punctulata (=Euphalorius punctulatus Pettey 1924) was described from a female specimen collected in southern Africa. The host was Sclerocarya caffra Sond. (Anacardiaceae) Capener (1970a). The original description says that the species also has been found on Chorda [sic] caffra Sond. (Boraginaceae) and Clausena inaequalis (DC.) Benth. (=anisata (Willd.) Hook, S. ex Benth.) (Rutaceae). The description is very brief (barely 13 lines long) and includes no figures or designation of paratypes. Capener (1970a), apparently with access to Pettey’s notes, said that the species was described from seven specimens - 1 male, 6 females. Seven specimens (evidently not entirely from the type series because sexes do not match, but determined by Pettey) still exist in the National Collection of Insects, Pretoria. There is some doubt as to whether all of this material is the same species. Catling & Atkinson (1974) mention that D. punctulata was found on citrus in Swaziland (Mead 1977) but was a non-vector of citrus greening. If the specimens reported from Clausena (above) are in fact D. punctulata, it is plausible that this species could infest citrus occasionally. Pettey (1924) (original description), Capener (1970a), and Catling & Atkinson (1974) do not include a thorough description or figure of D. punctulata, so it is not possible to explain from the descriptions how to separate it from D. citri. Photos of identified specimens of D. punctulata, including a paratype, kindly provided by Ian Millar, ARC-Plant Protection Research Institute, South African National Collection of Insects, Queenswood, Pretoria, South Africa, and by Dr. G. J. Begemann, Transvaal Sugar Ltd, Komati-poort, South Africa, show that D. punctulata looks very similar to D. citri. The two species can be separated by a difference in the maculation pattern on the wings. Both species have a dark band around the edge of the wings, with a clear area in the middle containing irregular spots. The dark outer band on D. citri has a definite break near the terminus of the Rs vein. The band on D. punctulata lacks that break. In addition to the wing pattern, the wing shape is more angular in D. punctulata, and the genal processes of D. punctulata are more massive and irregularly tapered than those of D. citri (Daniel H. Burckhardt, Naturnhistorisches Museum, Basel, Switzerland, pers. comm.).

Diaphorina zebrana was described from Ozoroa paniculosa (Sond.) R. & A. (Anacardiaceae). Additional specimens that varied slightly in the intensity of wing banding were collected from Ozoroa reticulata (Bak. f.) R. & A. Rernandes. Diaphorina zebrana, like D. punctulata, was mentioned as a citrus infesting species and a non-vector of greening in Swaziland (Mead 1977) by Catling & Atkinson (1974). There is no mention of infestations on citrus or related plants in the original description. Diaphorina zebrana, as its name suggests, has striped forewings and thus is readily separable from D. citri.

In addition to the seven species of Diaphorina reported on citrus, there are six other psyllid species reported to occur on citrus: Mesohomotoma lutheri (Enderlein 1918) (=Udastigmatia lutheri Enderlein), Psylla citricola Yang & Li 1984, Psylla citrisuga Yang & Li 1984, Psylla murrayi Mathur 1975, Trioza citrumpoora Yang & Li 1984, Trioza erytreae (del Guercio 1918) (=Aleurodes erytreae del Guercio, =Trioza citri Laing, =Trioza merwei Pettey, =Spaniozara merwei (Pettey), =Spaniozara erytreae (del Guercio) (Hollis 1984), and Trioza litseae Bordage 1898 (=Trioza easteperi Orian 1972).

Mesohomotoma lutheri was described from Peradeniya, Ceylon (Sri Lanka) from collections made in 1910 by Dr. Luther (Enderlein 1918). It was re-described carefully by Mathur (1975). The host was Urena lobata L. (Malvaceae), and it also may infest Hibiscus (also Malvaceae). There is some doubt about the synonymy for this species. Hollis (1987b) said that there is confusion about the validity of the species currently placed in Mesohomotoma because of variation in size and color among collections of the same species. The varia-
tion does not correlate well with host plant and distribution data. Hollis (1987b) suspects that several of the species, including *M. lutheri*, may in fact all be synonyms of *Mesohomotoma hibisci* (Frogaegg). Aubert & Quilici (1984) reported that adults of *M. lutheri* were seen on citrus leaves for short feeding periods, but that this was extremely rare, and no eggs were laid. They reported that the preferred host of *M. lutheri* was *Hibiscus*.

*Psylla citricola* and *P. citrisuga* were described from *Citrus grandis* (L.) Osbeck (now *Citrus maxima* (Burm.) Merr.) and *Citrus medica* L. in Yunnan Province in China (Yang & Li 1984). The two species are very similar and apparently occur together in mixed colonies on the hosts. Yang & Li (1984) suggest that a report of *Psylla alni* on citrus in Sichuan Province may actually be *P. citrisuga*. There is a report by Cen et al. (1999) that *P. citricola* occurs in Guangzhou. Further study is needed to determine whether these are distinct species.

*Psylla murrayi* normally feeds and reproduces on leaves of *Murraya koenigii* (L.) Sprengel (Mather 1975), but adults have been observed on citrus in Malaysia (Lim et al. 1990). Based on illustrations in the descriptions, the male genitalia appear to be distinct from those of the two Chinese *Psylla* spp.

*Trioza citroimpyra* also was described from Yunnan Province in China. The host was *Citrus reticulata* Blanco. Both male and female genitalia appear distinct from those of *T. erytreae*, based on figures given in the description.

*Trioza erytreae* is the well-known African citrus psyllid. It was described as a whitefly, *Aleurodes erytreae* del Guercio 1918; however, the drawing of the nymph is clearly a psyllid, and the photograph of the damage is that of *T. erytreae*. It is part of a species group that includes at least ten species that are very difficult to separate morphologically but have different host plant preferences (Hollis 1984). Hosts of *T. erytreae* are listed as *Clausena anisata* (Willd.) Oliv. (=*Clausena inaequalis* (DC.) Benth.), *Citrus* spp., *Vepris undulata* (Thunb.) Verdoorn & C.A. Smith (=*Todalia lanceolata* Lam.) and *Fagara* spp. There is extensive literature about this species, but particularly about its relationship to African citrus greening disease. *Trioza erytreae* can be found throughout much of Africa south of the Sahara, in Saudi Arabia and Yemen in the Saudi Peninsula, in Mauritius and Réunion, and in Madeira (Toorawa 1998). A key to African *Trioza*, along with additional characters useful in separating species in the *T. erytreae* group can be found in Hollis (1984). It is easily separated from *D. citri* because it has clear forewings that are pointed at the tips. Nymphs of *T. erytreae* live in individual depressions on the undersides of citrus leaves, whereas nymphs of *D. citri* tend to colonize the stems of new growth and never produce individual pits on the leaves.

*Psylla loranthi* Capener 1973 is not reported to feed on citrus or citrus relatives, but it feeds on *Loranthus zeyheri* Harv., a parasitic plant that sometimes attacks citrus. There is a small possibility that *P. loranthi* might potentially transmit citrus greening bacteria via the parasitic plant, so the species is included in this list. However, we note that no phloem connection may exist between the parasitic plant and its host (Sallé 1983). It can be distinguished from other *Psylla* species associated with citrus by the long slender genitalia, especially of the female (Capener 1973), and by the presence of immatures on its parasitic host plant. *Psylla loranthi* would not be found where its host does not occur.
Finally, Leuronota fagarae Burckhardt 1988, the wild lime psyllid, showed up in Florida in July 2001. Its only known host is Zanthoxylum fagara (L.) Sarg., a citrus relative. The psyllid is native to South America. Damage consists of rolled leaf edges that enclose the nymphs. We have surveyed citrus growing near infested Z. fagara and have not found any L. fagarae on citrus. Leuronota fagarae is very slender and has dark wings. It is not likely to be confused with Diaphorina citri or any other citrus psyllids.

Life Cycle

Mead (1977) has an excellent annotated summary of the life cycle of D. citri. Eggs are laid on “feather flush” and hatch in 2-4 days (Chavan & Summanwar 1993). There are five nymphal instars (Aubert 1987), which are completed in 11-15 days (Chavan & Summanwar 1993). The total life cycle takes 15-47 days, depending upon the temperature. Adults may live several months and the females lay as many as 800 eggs in a lifetime (Mead 1977). Catling (1970) provided further information on life cycle and biology. Life table parameters at different temperatures have been studied for Florida D. citri (Liu & Tsaï 2000). Time for completion of the life cycle was the same as Mead (1977) reported. The optimum development temperature range was found to be 25-28°C. Liu & Tsaï (2000) found that the maximum average number (748.3) of eggs produced per female occurred at 28°C.

Climatic Requirements

Aubert (1987) states that Diaphorina citri does not tolerate frost very well; however, we have observed that populations have overwintered in Gainesville, FL, where temperatures dropped to at least -5°C on several nights. Populations of D. citri in the Florida panhandle have been limited so far to Murraya and Citrus plants for sale at discount outlets, so it is not known whether D. citri can overwinter north of Gainesville. Aubert (1987) also states that populations of D. citri do not tolerate humidity close to the saturation point because it promotes fungal epizootics, to which the nymphs are very susceptible; however, high humidity in Florida has not prevented extremely high summer populations of D. citri in local groves and backyards. Similarly, few D. citri regulatory samples sent to Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) have cadavers resulting from fungal infection. Diaphorina citri was not found above 1300-1500 m in elevation in various places searched in Asia, presumably because of occasional frosts (Aubert 1987). Populations of D. citri moved north in China in the 1980s as a result of more citrus plantings and higher winter temperatures (Qiu et al. 1996).
fication rather than an indication that *D. citri* is established in Western Africa. Several interceptions reported from the Caribbean Basin indicate that *D. citri* already is moving in cargo within five years of known establishment. If citrus greening disease ever became established anywhere in the Caribbean Basin, the potential for movement is high.

Direct Plant Damage

Direct plant damage occurs as a result of high populations of psyllids. Copious amounts of honeydew and moderate leaf distortion have been observed on infested plants (Aubert 1987). In Florida, after the initial invasion of *D. citri*, new growth on some citrus plantings was severely damaged. Feeding by Asian citrus psyllid caused leaves to be curled and notched. In cases of severe infestation, newly emerged sprouts were killed. Lateral leaf notching is particularly characteristic of *D. citri* damage. In dry weather, we have observed curled waxy secretions from nymphs. Heavy oviposition or larval activity sometimes will kill developing terminals or cause abscission of leaves or entire terminals (Michaud 2004).

Populations can reach extremely high levels. A survey technique reported by Ahmad (1961) consisted of spraying citrus trees in West Pakistan with insecticide and collecting the psyllids on a white sheet beneath the tree. This method yielded an average of 41,561 adults per tree! On *Murraya paniculata* hedges in Réunion, catches of 200 adults per m² were obtained with a D-VAC machine (Aubert 1987).

**Biology of the Greening Pathogens**

Nature and Classification of the Pathogens

The greening pathogens are thought to be highly fastidious phloem-inhabiting bacteria in the genus *Candidatus* Liberibacter. Although the bacteria have not been cultured for completion of Koch’s postulates, circumstantial evidence points strongly to a bacterial disease agent because citrus greening symptoms abate temporarily when trees are injected with antibiotics (Buitendag & von Broembsen 1993; Lim et al. 1990; Su et al. 1986). The isolate from South Africa has been named *Candidatus* Liberibacter africanus, and the isolate from Asia has been named *Candidatus* Liberibacter asiaticus (Garnier et al. 2000). A subspecies of *Candidatus* L. africanus, *Candidatus* L. africanus subsp. capensis, has been described from the Western Cape Region of South Africa from *Calodendrum capensis* Thunb., a native South African plant. This subspecies also infects citrus (Garnier et al. 2000). Garnier et al. (2000) changed the generic name from Liberobacter to Liberibacter, following the International Code of Nomenclature of Bacteria, which states that since “bacter” is of masculine gender and “Liber” is of Latin origin, the connecting vowel should be an “i.”

It is widely accepted that both species of bacteria multiply in both of the psyllid vectors, but this has not been demonstrated with molecular evidence. However, Moll & Martin (1973) noticed marked increases in the number of citrus greening bacteria in *T. erytreae* vectors over 9 days, and concluded that the bacteria were multiplying in the vectors. Neither species of citrus greening bacteria has been cultured on artificial media. Molecular analysis indicates genetic differences between the two species, and specific DNA probes have been developed for each (Bové et al. 1993, 1996; Garnier & Bové 1996; Harakava et al. 2000; Tian et al. 1996).

African greening manifests symptoms primarily under cool conditions (below 25°C), whereas Asian greening does well under hot conditions (Garnier & Bové 1993). African greening does not show symptoms above 27°C under glasshouse conditions. In South Africa, the greening symptoms are more pronounced in winter than in summer. Similarly, the African citrus greening symptoms are severe in elevations above 700 m, whereas they are absent in low-lying hot areas. Indian greening does well in hot conditions, above 25°C. Asian citrus greening symptoms are less pronounced and disappear above 1500 m, possibly because the vector is absent (Aubert 1987). In a laboratory study, Bové et al. (1974) showed that symptoms of African citrus greening were moderate to severe at 22° to 24°C and disappeared at 27° to 32°C, whereas symptoms of Asian citrus greening from India and Philippines were expressed strongly at both temperature regimes.

*Candidatus* Liberibacter asiaticus is presumed to be Asian and may have developed with citrus, while *Candidatus* L. africanus probably came from native African rutaceous plants, since citrus is an introduced species in Africa. A native plant, *Todalia lanceolata*, was found to be a good host of both *Candidatus* L. africanus and its natural vector, *T. erytreae* (Garnier & Bové 1996). Lin & Lin (1990) postulate that Asian citrus greening originated in the northeastern part of Guangdong Province in China. It is also possible that the Asian greening pathogen has a geographical origin similar to that of its primary vector, *D. citri*, which probably evolved with similar species of *Diaphorina* in the Indian subcontinent.

**Distribution**

It is important to keep an updated file on the known distribution of citrus greening disease because rutaceous plants or citrus psyllids from those locations may harbor the pathogens. Toorawa (1998) compiled a summary of countries known to have citrus greening in his Table 3. Each
entry in his table is referenced by literature citation, and he notes the laboratory that did the molecular confirmation. Locations listed below are from Toorawa (1998) unless noted otherwise. Asian countries include: China (including Hong Kong), Indonesia, southern islands of Japan, Malasia, Philippines, Taiwan, Thailand, and Vietnam. Evidently citrus greening disease is spreading in Japan. Subandiyah et al. (2000) have confirmed citrus greening using molecular diagnosis in four places in Okinawa. Prior to this survey, citrus greening was known only from the southernmost island of Iriomote. Countries with citrus greening in the Indian subcontinent include Bangladesh, Bhutan, India, Nepal, and Pakistan. In the Indian Ocean, citrus greening disease is found in Sri Lanka, the Comoros Islands, Madagascar, Mauritius, and Réunion. All of these places have established populations of *D. citri* and Asian citrus greening. Mauritius and Réunion also have African citrus greening and *T. erytreae*. Similarly, in the Saudi Arabian peninsula, Saudi Arabia and Yemen have both species of vectors and both pathogens. In Africa, Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Malawi, Rwanda, Somalia, South Africa, Swaziland, Tanzania, and Zimbabwe all have African citrus greening and *Triozia erytreae*. *Diaphorina citri* is not known to be established in the African mainland. Although *D. citri* has been observed in Iran (Danet, pers. comm., ex Toorawa 1998), it is not known if citrus greening disease occurs there. Additionally, Varma & Atiri (1993) reported that over 50% of plants in some areas of Nigeria show symptoms of citrus greening. Symptoms have been observed all over Nigeria, but presence of the pathogen has not been confirmed by molecular analysis. The CABI map 766 (1998) additionally lists Laos and Myanmar as positive for *Candidatus L. asiaticus*. It states that there is an unconfirmed report for Syria. Garnier & Bové (2000b) added Cambodia to the list of countries where citrus greening is present. Citrus greening disease was found in Papua New Guinea in 1999 (Lee 2002). The status of citrus greening in Afghanistan, Brunei, and Singapore is unknown. In July 2004, as this paper was in press, citrus greening disease was reported in Brazil by Fundecitrus (Anon. 2004).

Plant Damage

Citrus greening is a very destructive disease. A survey conducted over an 8-year period in Réunion Island indicated that 65% of the trees were badly damaged and rendered unproductive within 7 years after planting (Aubert et al. 1996). In Thailand, citrus trees generally decline within 5-8 years after planting due to citrus greening (Roistacher 1996). Roistacher (1996) showed that groves must live for a minimum of about 10 years in order to make a profit. Infected trees are stunted and sparsely foliated. The symptoms can resemble nutritional stress, especially zinc deficiency symptoms on recent growth; however, a more diagnostic mottle usually occurs on slightly older leaves that resembles symptoms of luteoviruses in dicots (e.g., *Potato leafroll luteovirus*). The mottle differs from nutrition-related mottle in that greening induced mottling usually crosses leaf veins, whereas nutrition-related mottling usually occurs between or along leaf veins. Off-season bloom, fruit drop, and twig dieback are other symptoms. Fruit are small, lopsided, hard, and have a bitter flavor. Seed abortion is common (Capoor et al. 1974). Citrus greening disease may predispose plants to other pest problems such as the citrus longhorned beetle, *Anoplophora chinensis* Forster (Aubert 1990b). A combination of citrus greening, citrus longhorned beetle, and associated *Phytophthora* fungi are common in advanced citrus greening epidemics (Aubert 1990b).

Toorawa (1998) attempted to compile global infection statistics. He estimated 50 million trees infected in south and southeast Asia, three million trees infected in Indonesia, and ten million trees infected in Africa. In India and Saudi Arabia, there has been a marked decline in citrus industries as a result of citrus greening disease.

Detection

Vector

In low numbers, *Diaphorina citri* is an inconspicuous pest of citrus. The adults are the most easily observable stage. They are about 3-4 mm long. The wings have distinct bars on the top and bottom, giving the insects a flattened X-pattern when viewed laterally. Characteristically, they sit at a 45° angle to the shoot or leaf on which they feed. Adults jump readily when approached. It is best to collect them either by using an aspirator, or by bagging the entire shoot. Another way to collect specimens in excellent condition is to place an inverted empty test tube above an infested shoot while disturbing the colony. The adults will jump up into the tube and remain there.

Nymphs are difficult to see. They are flat and tend to wrap themselves around the shoot where they feed. Superficially, they look similar to scale insects. Nymphs may be green or orange in color, but, unlike scale insects, they have large wing pads. Eggs, bright yellow or orange and shaped like a pointed football, are attached in the plant tissue at one end. Eggs are deposited on the “feather flush” of the host. It is very difficult to see eggs without a hand lens.

Pathogen

It is only within the last few years that reliable detection of the greening pathogens has been
available. DNA probes now have been used successfully to detect *Candidatus Liberibacter* spp. both in infected plants and in psyllid vectors (Bové et al. 1993; Tian et al. 1996). The bacteria also can be detected with an electron microscope, ELISA (Garnier & Bové 1993), and by biological assay. Roistacher (1991) gives a detailed methodology for preparing specimens for electron microscopy.

Unfortunately, infected trees may be overlooked if symptoms alone are used for detection. Aubert (1990b) estimated that 15% to 20% of the infected plants are overlooked by nursery inspectors who rely only on visual inspection.

Lafleche & Bové (1970) using a transmission electron microscope observed a “mycoplasm-like organism” in citrus phloem tissue infected with citrus greening disease. The organisms were about 2000 nm long and 100-200 nm in diameter. Similar bodies soon were observed in both vectors of the citrus greening disease, *T. erytreae* (Moll & Martin 1973) and *D. citri* (Chen et al. 1973). A further comparison of the greening organism (Saligo et al. 1971) with citrus stubborn, a spiroplasma, showed that the outer membrane of the greening organism was much thicker (25 nm) than that of the spiroplasma (10 nm).

Further studies showed the bacterial nature of the greening organism, and a peptidoglycan-containing outer membrane of gram negative bacterial type was identified (Garnier et al. 1984). Molecular information provided the basis for accurate nomenclature for the two species. The bacterium was recognized as a new *Candidatus* genus Liberibacter, in the alpha subdivision of proteobacteria (Jagoueix et al. 1994).

Monoclonal antibodies raised against proteins purified from infected greening tissue from Africa, China, and India reacted selectively with the source antigens and a few other isolates of citrus greening, demonstrating the existence of several serotypes of greening (Garnier et al. 1987; Gao et al. 1993). These monoclonal antibodies are too isolate-specific to be used for general detection of greening.

Molecular approaches such as PCR and strain-specific DNA probes now have been used successfully to detect and differentiate *Candidatus Liberibacter* spp. both in infected plants and in psyllid vectors (Bové et al. 1993; Jagoueix et al. 1996; Tian et al. 1996). Unfortunately, detection is not always reliable. Sometimes trees with classic greening symptoms test negative with PCR (Toorawa 1998).

Molecular detection methods have been difficult to develop since the greening organism has not yet been cultured. Villechanoux et al. (1992) isolated total DNA from periwinkle plants infected with Indian greening, and digested it with restriction enzyme, HindIII. The digested DNA was cloned and the clones were screened by differential hybridization with DNA from both healthy and infected tissues. They identified three clones with 2.6, 1.9, and 0.6 kb inserts to be specific to the greening bacterium. The two larger clones reacted with all the Asian forms, but not with the African isolates, while the 0.6 kb clone reacted only with the Indian greening. Villechanoux et al. (1993) sequenced and analyzed the three greening specific clones. The larger 2.6 kb clone contained the genes of the nusG-rplK-AJL-rpoBC operon, confirming the eubacterial nature of the greening organism at the molecular level. The 1 kb insert contained sequences for a bacteriophage-type DNA polymerase. The sequences from the 0.6 kb insert did not match anything in the database of known sequences.

Since the bacterial nature of the greening organism was established, Jagoueix et al. (1996) used universal primers for general amplification of prokaryotic 16S rDNA. Based on sequence information, primers have been developed to amplify a 1,160 bp region of ribosomal DNA for detection of greening by PCR. Further differentiation of Asian and African forms of greening can be achieved by restriction enzyme XbaI digestion. The XbaI digestion of an 1160 bp fragment from L. africanus yields three fragments of 520 bp, 506 bp, and 130 bp, while the Asian greening, L. asiaticus, yields only two fragments of 640 bp and 520 bp.

Ribosomal DNA primers have been used widely for detection of both forms of greening. These primers have been shown not to amplify 16S ribosomal sequences of other citrus pathogens (Jagoueix et al. 1996).

Additionally, some citrus species, such as sweet oranges and mandarins, produce a compound (gentisic glucoside) as a result of infection. Gentisic acid glows violet under UV light and can be seen directly in the fruit albedo of sweet oranges. A bark extract procedure (Roistacher 1991) can be used with other commercial citrus species (mandarin and tangelo). Results are not consistent for lemon, lime, pummelo, and grapefruit. Gentisic acid analysis sometimes produces false negatives and false positives, so it cannot be used alone for definitive diagnosis. However, results are fairly consistent for sweet oranges, so the technique can be useful in conjunction with other more reliable (but also more expensive and time-consuming) tests (Hooker et al. 1993).

For many years, biological indexing has been used for citrus greening diagnosis. Miyakawa (1980) found that ponkan (*Citrus reticulata* Blanco) and Orlando tangelo (*Citrus tangelo* J. Ingram & H. Moore) are the best indicators, particularly if severe forms of *Citrus tristeza virus* (CTV) are present and may confound symptom expression.

Detection of citrus greening pathogens from asymptomatic tissue is inconsistent by any known method. Similarly, the molecular assays sometimes are complicated to run, and results are not always believable. Clearly, more accurate, timely, and robust detection methodologies are needed.
EPIDEMIOLOGY

Since it has only been in the last several years that citrus greening pathogens could be detected reasonably reliably by means of molecular methods, some of the basic characteristics of transmission and epidemiology are poorly understood. There are reports of transmission via psyllid vectors, grafting, dodder, and seed.

Psyllid Transmission

Psyllid transmission is the primary means of spread in the field. Acquisition times of 30 min for Asian psyllids (Roistacher 1991) and 24 h for African psyllids (Buitendag & von Broembsen 1993) have been reported. In some experiments, acquisition feeding of 5-7 h was sufficient to transmit citrus greening pathogens, while feeding periods of 1-3 h were not (Xu et al. 1988). The pathogen probably multiplies in the vector (Aubert 1987; Moll & Martin 1973; Xu et al. 1988), but this has not been demonstrated by molecular experiments. It is not known if psyllids can be infected simultaneously by both bacteria species (Garnier et al. 1996), although both psyllid species transmit both pathogens experimentally (Lallemand et al. 1986; Massonie et al. 1969). Adults and fourth and fifth instar Asian citrus psyllids are able to transmit the pathogen after a latent period as short as one day or as long as 25 days (Roistacher 1991; Xu et al. 1988). Fourth and fifth instars were able to retain the pathogen as adults, which were able to transmit the disease immediately after emergence (Xu et al. 1988). First through third instars were unable to transmit citrus greening (Xu et al. 1988). A latent period of 24 h has been reported for African greening (Buitendag & von Broembsen 1993). Transmission is thought to occur via salivary secretions (Aubert 1987). Serial transfer experiments by van den Berg et al. (1992) suggest that young nymphs of T. erytreae can acquire the bacteria even though they do not transmit them.

Candidatus Liberibacter spp. potentially should be considered pathogens of the insect as well as the plant, if in fact they multiply in the psyllid vectors, as suggested by Xu et al. (1988) and Moll & Martin (1973). There are conflicting reports as to whether Candidatus Liberibacter spp. are transmitted transovarially (Buitendag & von Broembsen 1993; Roistacher 1991; van den Berg et al. 1992; Xu et al. 1988). Xu et al. (1988) reported that there is no evidence for transovarial transmission, because D. citri nymphs collected immediately after hatching on diseased plants did not transmit citrus greening disease to indicator plants. The most extensive studies on transovarial transmission of citrus greening pathogens were done with T. erytreae. van den Berg et al. (1992) allowed immature psyllids to develop on heavily infected plants. When adults emerged, they were allowed to feed and mate on infected plants. After 14 days, the mouthparts of 100 of the females were severed. Ten of these females were placed on each of ten healthy indicator plants, where they laid eggs. Adults from those eggs were allowed to feed on the same plants for 30 days after emergence. Plants were later sprayed, kept insect-free, and tested for citrus greening disease after six months. One of the ten plants developed citrus greening disease. In another experiment, oviposition was allowed to occur on the infected plants. Crawlers were removed immediately after hatching and prior to feeding and placed on indicator plants. Five of the 24 plants on which these psyllids completed development became infected with citrus greening disease. The most logical explanation for these infections is transovarial transmission; however, the authors postulate that the plant in the first experiment could have been infected via oviposition, and those in the second experiment could have been infected as a result of absorption of greening bacteria from the infected host by the egg. These experiments should be repeated with D. citri. To our knowledge, there are no studies on sexual transmission of Candidatus Liberibacter spp. in psyllids. Similarly, it is not known if parasites that develop in infected psyllids can transmit the pathogen to the hosts of their offspring. Hoy et al. (2001) has a good review of relevant literature about transmission of plant pathogens via parasites of vectors.

Candidatus Liberibacter spp. can be detected in single psyllids (Bové et al. 1993). Experimental data showing detection of citrus greening pathogens in psyllids indicate that percent transmission by psyllids that feed on infected trees may be variable. Thirty-nine percent of psyllids collected in Malaysia in September 1991 tested positive for the pathogen, whereas less than 1% of those collected in February 1992 in India had positive DNA hybridization reactions for citrus greening (Bové et al. 1993). Toorawa (1998) also found a higher percentage of D. citri that had positive DNA hybridization reactions in the fall. The relationship between positive detection of the pathogen in the psyllids and ability to infect indicator plants is not known. Field infectivity experiments, in which adult psyllids are trapped alive and allowed to feed on indicator plants, are badly needed. These kinds of experiments, though labor intensive, would provide valuable information on infectivity and seasonality of transmission.

Graft Transmission

The citrus greening pathogens are graft transmitted (Bové et al. 1996; van Vuuren 1993); however, graft transmission of Candidatus Liberibacter spp. is variable, depending upon the plant part used for grafting, the amount of tissue used, and the...
pathogen isolate. With single buds, graft transmission of African greening varied from 0 to 50%, depending upon the isolate used (van Vuuren 1993). Side grafts with twigs were even more efficient at transmitting the pathogen, whereas fruit stems and bark strips were not effective (van Vuuren 1993).

Lin & Lin (1990) reported some early experiments performed but apparently not previously published by Chen Qi-bao. Seven months after grafting diseased buds on healthy rootstock, 58% of grafts had survived, and of those, 20% showed citrus greening symptoms. In another experiment, 10-16% of grafts with buds from asymptomatic branches on diseased trees developed symptoms, while 40% of grafts from symptomatic branches developed symptoms of citrus greening in 3-9 months.

Seed Transmission

There is little information on seed transmission. Most fruit is lost, and that which remains has a high proportion of aborted seed; however, Tirtawidjaja (1981) collected normal and greening-affected (very small) fruit and harvested normal-looking seeds from each. No symptoms were observed on seedlings from seed taken from normal fruit, even when they were collected from infected plants; however, seeds derived from smaller, greening-affected fruit produced some stunted chlorotic seedlings. Three of the seedlings had the same appearance as insect-infected seedlings. This experiment bears repeating. Miyakawa et al. (1990) reported that greening-infected Troyer citrange trees showed few leaf symptoms and bore a good crop, although there were relatively high numbers of aborted seeds. If seed transmission occurs in cultivars like citrange that are used for citrus rootstocks, spread could occur through liners as well as by budding.

Timing, Patterns, and Rates of Spread

Many of the parameters necessary to develop epidemiological models of citrus greening disease spread are not known. Little is known about how soon after infection by psyllids the pathogens can be detected in the infected plant; however, symptoms of African greening developed 40 cm back towards the trunk of the tree from the vector feeding site on infected shoots within 12 months (van Vuuren 1993). Pathogens became detectable in shoots between 2.5 and 3.5 months after leaves emerged from buds on diseased trees, and symptoms expressed themselves in a similar period of time (Su & Huang 1990). Pathogens could be detected in the root system of Luchen seedlings five months after graft inoculation (Su & Huang 1990). The time interval between the infection of a citrus shoot and the possibility of subsequent acquisition of the pathogen by new vectors is not known.

Percent citrus greening disease transmission by psyllids raised on infected plants is variable. Efficiency may vary from around 1% to 80% for single insects (Xu et al. 1988). Xu et al. (1988) list several conditions that enhance transmission efficiency, including psyllid-inoculated source plants, young (3-4 leaves) indicator plants, psyllids raised on infected plants in the laboratory, and control of shade and temperature in the greenhouse where indicator plants are kept. The genetic makeup of the pathogen and vector also may account for some of the variation in that some populations of citrus psyllids may be inherently better vectors, and some populations of citrus greening bacteria may be inherently more transmissible.

Although patterns of spread in groves have been studied (Gottwald et al. 1991a,b), there has not been an attempt to match disease spread information with a reproducible measurement of vector abundance. It is significant that Gottwald et al. (1991a) found that the source of infection was a small planting near a farmhouse of 24 trees with severe disease in a study in China. The incidence of detectable citrus greening disease rose to 14% in the first 5 years after planting. They estimate that the disease would have reached its asymptote in the next 2-4 years, and thus, the productive life of the grove would be less than 10 years, even with a clean start.

In another Chinese study in Shantou, Guangdong, ingress into densely planted groves showed a typical edge effect. Twenty percent of the plants were lost to greening by the fifth year, and the groves lost their commercial value by the time they were seven to eight years old (Aubert 1990b).

There are robust experimental data for dispersal and infestation patterns for *T. erytreae*, the African citrus psyllid; however, we could not find similar data for *D. citri*. Samways & Manicom (1983) severely pruned a citrus grove and inspected it carefully for initial psyllid infestation in the spring. The initial distribution was random on a tree-to-tree basis, but the side of the grove closest to the neighbor’s infested grove had a higher density of *T. erytreae*, suggesting that the source of the infestation was the neighboring grove. Results showed that even after the initial invasion, there was considerable tree-to-tree movement, which potentially can spread the pathogens further. *Trioza erytreae* readily invaded the whole grove within days.

There is good experimental evidence for flight distance of *T. erytreae*, but not for *D. citri*. van den Berg & Deacon (1988) released clean *T. erytreae* in an area that had no citrus. Yellow traps were placed in a grid at various distances from the release site. From these data, it was determined that *T. erytreae* could fly at least 1.5 km in the absence of their host plants. A similar estimate was made for *D. citri*, but it was not based on experimental evidence (Tolley 1990).
Infected plants were clustered within groves, possibly indicating that most *D. citri* normally do not fly very far (Aubert et al. 1996; Gottwald et al. 1991a,b). Similarly, Toorawa (1998) says that in Mauritius, *D. citri* are not very mobile. This assertion is based on the very low percentage (0.33%) of *D. citri* captured on *M. paniculata* that were contaminated with the citrus greening pathogen. An estimate of maximum flight distance for insect vectors is needed for determining safe isolation distance for quarantine and eradication purposes, and this is not well known. A 30-km separation was sufficient in Nepal (Regmi et al. 1996) but not in Vietnam (Bové et al. 1996).

Although the distance *D. citri* can fly is poorly known, experiments on height of capture and color preference have been done. Aubert & Hua (1990) reported that “brown yellow” traps worked better on sunny days. Maximum catch occurred at cloudy days. However, plain yellow traps worked (1990) reported that “brown yellow” traps worked somewhat differing host ranges. The fact that *Murraya* spp., which are native to the Indian subcontinent (Coile 1995) and are good hosts of *D. citri*, are resistant or at least tolerant to citrus greening disease further supports a South Asia origin for the pathogens.

Several citrus relatives have been shown with modern detection methods to harbor greening pathogens, including *Severinia buxifolia* (Poiré) Ten. (Hung et al. 2000), *Limonia acidissima* L. (Koizumi et al. 1996; Su et al. 1995; Hung et al. 2000), and *Todalia lanceolata* Lam (Korsten et al. 1996). Many other citrus relatives are implicated as hosts of citrus greening pathogens, but symptoms have been the only criteria (Table 2).

### Host Range and Vector Specificity

The host range of *D. citri* includes many of the close citrus relatives (Table 1). DPI’s Citrus Arboretum in Winter Haven, Florida provided a good opportunity to determine which plants serve as field hosts of *D. citri* in Florida. Two species of native *Zanthoxylum* are represented at the facility. No *D. citri* were ever found on *Zanthoxylum clava-hercules* L.; however, *Zanthoxylum fagara* (L.) Sarg. may be an occasional host. *Zanthoxylum fagara* nearly always has suitable flush, sporting a nearly year-around population of *Toxoptera citricida* (Kirkaldy), the brown citrus aphid; however, we found *D. citri* nymphs present only once, and in very low numbers. Similarly, no *D. citri* were found on *Z. fagarae* growing next to an infested lime grove in South Florida. *Zanthoxylum* spp. may be non-hosts, or as in the case of *Z. fagara*, very poor hosts, of *D. citri*. Another apparent non-host, based on our observations at the DPI Citrus Arboretum, is *Casimiroa edulis* Llave & Lex., white sapote. Both *Zanthoxylum* and *Casimiroa* are of Western Hemisphere origin, suggesting a preference by *D. citri* for Old World Rutaceae.

There are many observations about preferred hosts of *D. citri*, but only one comparative laboratory study (Tsai & Liu 2000). In their study, Tsai & Liu (2000) tested *Murraya paniculata* (L.) Jack (orange jasmine), *Citrus jambhiri* Lushington (rough lemon), *Citrus aurantium* L. (sour orange), and *Citrus × paradisi* Macf. (grapefruit). Grapefruit was the best host, followed by the other hosts, among which there was no statistical difference.

There is not much information on the host range of the pathogens because of the difficulty in measuring the presence of the pathogen with certainty (Table 2). Most, if not all, *Citrus* spp. apparently are susceptible to some degree, although some species (*Citrus indica* Tan. and *Citrus macroptera* Montr.) remained symptom-free under heavy inoculum pressure (Bhagabati 1993). *Citrus limetta* remained symptom-free after laboratory inoculation (paper does not specify means of inoculation) (Nariani 1981).

*Murraya paniculata* is a preferred host of *D. citri*; however, there is not agreement on whether it is a host for the greening pathogens. Careful work using dot hybridization by Hung et al. (2000) indicates that Asian greening pathogens from Taiwan will not multiply in *M. paniculata* or *M. koenigii*. Toorawa (1998), who worked in Mauritius, concurs. On the other hand, Tirtawidjaja (1981) was able to observe consistent external and internal symptoms on 25-33% of inoculated *M. paniculata* plants. Asian greening may be caused by a population of bacterial strains with somewhat differing host ranges. The fact that *Murraya* spp., which are native to the Indian subcontinent (Coile 1995) and are good hosts of *D. citri*, are resistant or at least tolerant to citrus greening disease further supports a South Asia origin for the pathogens.
<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeglopsis chevalieri Swingle</td>
<td>Koizumi et al. 1996</td>
<td>Koizumi et al. 1996</td>
</tr>
<tr>
<td>Afraegle gabonensis Engl.</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Afraegle paniculata (Schaum.) Engl.</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Artocarpus heterophyllus Lamarek (Moraceae)</td>
<td>Shivankar et al 2000</td>
<td>Shivankar et al 2000</td>
</tr>
<tr>
<td>Atalantia missionis Oliver</td>
<td>Tirtawidjaja 1981</td>
<td>Tirtawidjaja 1981</td>
</tr>
<tr>
<td>Atalantia monophylla (L.) Corr.</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Atalantia sp.</td>
<td>Koizumi et al. 1996; Aubert 1990a adult feeding only (Aubert)</td>
<td>Koizumi et al. 1996; Aubert 1990a adult feeding only (Aubert)</td>
</tr>
<tr>
<td>Balsamocitrus dawei Stapf</td>
<td>Koizumi et al. 1996</td>
<td>Koizumi et al. 1996</td>
</tr>
<tr>
<td>Citropsis gilletiana Swingle &amp; M. Kellerman</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Citropsis schweinfurthii (Engl.) Swingle &amp; Kellerm.</td>
<td>Chavan &amp; Summanwar 1993</td>
<td>Chavan &amp; Summanwar 1993</td>
</tr>
<tr>
<td>Citrus aurantifolia (Christm.) Swingle</td>
<td>Aubert 1987, 1990a; Florida surveys preferred host</td>
<td>Aubert 1987, 1990a; Florida surveys preferred host</td>
</tr>
<tr>
<td>Citrus aurantium L.</td>
<td>Aubert 1987</td>
<td>Aubert 1987</td>
</tr>
<tr>
<td>Citrus deliciosa Tenore</td>
<td>Aubert 1987</td>
<td>Aubert 1987</td>
</tr>
<tr>
<td>Citrus grandis (L.) Osbeck</td>
<td>Aubert 1987; frequent host (Aubert 1990)</td>
<td>Aubert 1987; frequent host (Aubert 1990)</td>
</tr>
<tr>
<td>Citrus hystrix DC.</td>
<td>Aubert 1987; Lim et al. 1990</td>
<td>Aubert 1987; Lim et al. 1990</td>
</tr>
<tr>
<td>Citrus ×jambhiri Lushington</td>
<td>Florida surveys</td>
<td>Florida surveys</td>
</tr>
<tr>
<td>Citrus limon (L.) Burm. f.</td>
<td>Aubert 1987, 1990a</td>
<td>Aubert 1987, 1990a</td>
</tr>
<tr>
<td>Citrus madurensis Lour.</td>
<td>Aubert 1990a</td>
<td>Aubert 1990a</td>
</tr>
<tr>
<td>Citrus maxima (Burm.) Merr.</td>
<td>Aubert 1990a; occasional development, nymphal stage (Aubert 1990)</td>
<td>Aubert 1990a; occasional development, nymphal stage (Aubert 1990)</td>
</tr>
<tr>
<td>Citrus medica L.</td>
<td>Aubert 1987, 1990a</td>
<td>Aubert 1987, 1990a</td>
</tr>
<tr>
<td>Citrus meyeri Tan</td>
<td>Florida surveys</td>
<td>Florida surveys</td>
</tr>
<tr>
<td>Citrus ×nobilis Lour.</td>
<td>Aubert 1987; Florida surveys common</td>
<td>Aubert 1987; Florida surveys common</td>
</tr>
<tr>
<td>Citrus × obvovida Hort. ex Tanaka cv ‘Kinkoji’</td>
<td>Florida surveys</td>
<td>Florida surveys</td>
</tr>
<tr>
<td>Citrus × paradisi Macfad.</td>
<td>Aubert 1987; Florida surveys; Tsai &amp; Liu 2000</td>
<td>Aubert 1987; Florida surveys; Tsai &amp; Liu 2000</td>
</tr>
<tr>
<td>Citrus reticulata Blanco</td>
<td>Aubert 1987, 1990a, Koizumi et al. 1996; Florida surveys common</td>
<td>Aubert 1987, 1990a, Koizumi et al. 1996; Florida surveys common</td>
</tr>
<tr>
<td>Citrus × sinensis (L.) Osbeck</td>
<td>Aubert 1987, 1990a; Florida surveys common</td>
<td>Aubert 1987, 1990a; Florida surveys common</td>
</tr>
<tr>
<td>Citrus spp.</td>
<td>Aubert 1990a; Florida surveys common</td>
<td>Aubert 1990a; Florida surveys common</td>
</tr>
<tr>
<td>Clausena anisum-olens Merrill</td>
<td>Aubert 1990a; occasional host, observed nymphal development</td>
<td>Aubert 1990a; occasional host, observed nymphal development</td>
</tr>
<tr>
<td>Clausena excavata Burm. f.</td>
<td>Aubert 1990a; Lim et al. 1990</td>
<td>Aubert 1990a; Lim et al. 1990</td>
</tr>
<tr>
<td>Clausena indica Oliver</td>
<td>Aubert 1990a</td>
<td>Aubert 1990a</td>
</tr>
<tr>
<td>Clausena lansium (Lour.) Skeels</td>
<td>Koizumi et al. 1996; Aubert 1990; Florida surveys</td>
<td>Koizumi et al. 1996; Aubert 1990; Florida surveys</td>
</tr>
<tr>
<td>Eremocitrus glauca (Lindley) Swingle</td>
<td>Koizumi et al. 1996</td>
<td>Koizumi et al. 1996</td>
</tr>
<tr>
<td>Eremocitrus hybrid</td>
<td>DPI Citrus Arboretum Survey</td>
<td>DPI Citrus Arboretum Survey</td>
</tr>
<tr>
<td>Fortunella crassifolia Swingle</td>
<td>DPI Citrus Arboretum Survey</td>
<td>DPI Citrus Arboretum Survey</td>
</tr>
<tr>
<td>Fortunella margarita (Lour.) Swingle</td>
<td>DPI Citrus Arboretum Survey</td>
<td>DPI Citrus Arboretum Survey</td>
</tr>
<tr>
<td>Fortunella polyandra (Ridley) Tanaka</td>
<td>Aubert 1987, 1990a</td>
<td>Aubert 1987, 1990a</td>
</tr>
<tr>
<td>Fortunella spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonia acidissima L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merrillia caloxylon (Ridley) Swingle</td>
<td>Koizumi et al. 1996</td>
<td>Koizumi et al. 1996</td>
</tr>
<tr>
<td>Microcitrus australasica (F.J. Muell.) Swingle</td>
<td>Koizumi et al. 1996; Aubert 1987, 1990a; DPI Citrus Arboretum survey</td>
<td>Koizumi et al. 1996; Aubert 1987, 1990a; DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Microcitrus australis (Planch.) Swingle</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
<tr>
<td>Microcitrus papuana H.F. Winters</td>
<td>DPI Citrus Arboretum survey</td>
<td>DPI Citrus Arboretum survey</td>
</tr>
</tbody>
</table>
The potentially wide physiological host range of the pathogens, combined with low vector-pathogen specificity, has potential implications for the epidemiology of the disease. A naturally-occurring native _Candidatus Liberibacter_ sp., normally spread by native psyllids in plants unrelated to citrus, could be inoculated into citrus in a rare event. This scenario has been postulated for Australian citrus dieback, a disease of unknown etiology (Miyakawa & Yuan 1990; Broadbent 2000; Broadbent et al. 1976). Once in citrus, the citrus psyllids might spread the pathogen further within the crop, causing major damage.

**MANAGEMENT**

Control of _D. citri_ and citrus greening disease will involve all aspects of an integrated pest management program. The following is a summary, based on a standard IPM approach.

**Chemical Control**

If both the insect and the pathogen are present, the world literature is basically in agreement that it is necessary to control psyllids with pesticides (Tolley 1990). It is important to control psyllids, even on apparently disease-free plants (Aubert 1990b). Aubert (1987) recommended protecting spring flush. Populations are considered high when they reach three nymphs and five adults per twig. A Chinese program to rehabilitate citrus production in an area affected with citrus greening disease requires 10-13 sprays per year during flush periods (Roistacher 1996). In Thailand, procedures to monitor for psyllids and spray when necessary are recommended (Roistacher 1996). Su et al. (1986) recommended spraying at 10-20 day intervals during critical infection periods. Gonzales & Viñas (1981) recommend spraying young trees at weekly intervals during the rainy season and every ten days during the dry season. Aubert (1990b) indicates that it is very important for farmers in a citrus production area to synchronize chemical applications.

Timing of pesticides is critical. Aubert (1988) suggests using yellow sticky cards for monitoring _D. citri_ in order to time control action. In Florida, this method may be too slow. Based on our trapping collections in Florida, the peak spring flights...
TABLE 2. HOST LIST FOR *Candidatus Liberibacter* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeglopsis chevalieri</em> Swingle</td>
<td>Koizumi et al. 1996</td>
<td>questionable symptoms</td>
</tr>
<tr>
<td><em>Atalantia missionis</em> Oliver</td>
<td>Tirtawidjaja 1981</td>
<td>symptoms only, vector transmission</td>
</tr>
<tr>
<td><em>Balsamocitrus dawei</em> Stapf.</td>
<td>Koizumi et al. 1996</td>
<td>symptoms only; vector transmission</td>
</tr>
<tr>
<td><em>Calodendrum capensis</em> Thunb.</td>
<td>Garnier et al. 2000</td>
<td>molecular characterization</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em> (L.) G. Don</td>
<td>Tirtawidjaja 1981</td>
<td>symptoms, electron microscopy; (dodder transmission only)</td>
</tr>
<tr>
<td><em>Citrus amblycarpa</em> Ochse</td>
<td>Miyakawa &amp; Yuan 1990; Nariani 1981</td>
<td>symptoms only, vector transmission</td>
</tr>
<tr>
<td><em>Citrus aurantifolia</em> (Christm.) Swingle</td>
<td>Miyakawa &amp; Yuan 1990; Tirtawidjaja 1981</td>
<td>mild symptoms</td>
</tr>
<tr>
<td><em>Citrus aurantium</em> L.</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus depressa</em> Hayata</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus grandis</em> (L.) Osbeck</td>
<td>Miyakawa &amp; Yuan 1990; Su &amp; Huang 1990</td>
<td>symptoms; presence of putative pathogen in tissue; plant reported tolerant to disease, but source of vectors (Lee 1996)</td>
</tr>
<tr>
<td><em>Citrus hystrix</em> DC.</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus ichangensis</em> Swingle</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus jambhiri</em> Lushington</td>
<td>Tirtawidjaja 1981</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus junos</em> Sieb. ex Tanaka</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus kabuchi</em> Hort. ex Tanaka</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms, vector transmission</td>
</tr>
<tr>
<td><em>Citrus limon</em> (L.) Burm. f.</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> × <em>limonia</em> Osbeck</td>
<td>Miyakawa &amp; Yuan 1990; Tirtawidjaja 1981</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> × <em>nobilis</em> Lour. ‘Ortanique’</td>
<td>Koizumi et al. 1996</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> × <em>nobilis</em> Lour.</td>
<td>Koizumi et al. 1996</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> oto Hort. ex Tanaka</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> × <em>paradisi</em> Macfad.</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus reticulata</em> Blanco</td>
<td>Miyakawa &amp; Yuan 199; Tirtawidjaja 1981</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus sinensis</em> (L.) Osbeck</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms, presence of putative pathogen in tissue</td>
</tr>
<tr>
<td><em>Citrus sunki</em> Hort. ex Tanaka</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus unshiu</em> (Mack.) Marc</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> sp. (mandarins)</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Citrus</em> sp. (pomelo/shaddock)</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Clausena indica</em> Oliver</td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms (stunting)</td>
</tr>
<tr>
<td><em>Clausena lansium</em> (Lour.) Skeels</td>
<td>Tirtawidjaja 1981; Koizumi et al. 1996</td>
<td>symptoms only, vector transmission</td>
</tr>
<tr>
<td><em>Cuscuta australis</em> R. Br. (Convolvulaceae (Cuscutaceae))</td>
<td>Su &amp; Huang 1990</td>
<td>observed to multiply in stems, haustoria and flower stalks</td>
</tr>
<tr>
<td><em>Fortunella spp.</em></td>
<td>Miyakawa &amp; Yuan 1990</td>
<td>symptoms</td>
</tr>
<tr>
<td><em>Limonia acidissima</em> L.</td>
<td>Koizumi et al. 1996; Su et al. 1995; Hung et al. 2000</td>
<td>symptoms only; vector transmission; DNA hybridization (Su et al.); infection apparently temporary (Hung et al.)</td>
</tr>
<tr>
<td><em>Microcitrus australasica</em> (F. J. Muell.) Swingle</td>
<td>Koizumi et al. 1996</td>
<td>symptoms (stunting)</td>
</tr>
<tr>
<td><em>Murraya koenigii</em> (L.) Sprengel</td>
<td>Hung et al. 2000</td>
<td>no detection by dot hybridization after attempted graft transmission; no symptoms (Hung et al.)</td>
</tr>
<tr>
<td><em>Murraya paniculata</em> (L.) Jack</td>
<td>Tirtawidjaja 1981; Aubert et al. 1985; Miyakawa 1980; Hung et al. 2000, Koizumi et al. 1996; Toorawa 1998</td>
<td>Mixed results: symptoms only (external and internal), vector transmission (Tirtawidjaja); can harbor greening organism (Aubert et al.). EM negative (Miyakawa); No detection by dot hybridization after attempted graft transmission (Hung et al.); no symptoms (Koizumi et al.; not a host (Toorawa)</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em> L. ‘Xanthii’ (Solanaceae)</td>
<td>Garnier &amp; Bové 1993</td>
<td>symptoms, dodder transmission only</td>
</tr>
</tbody>
</table>
of *D. citri* occur very suddenly, and without prior incremental increase in numbers of adults collected. In Florida, it would be better to monitor buildup of nymphs on shoots, or to sample overwintered adults and observe when they become gravid. (Their abdomens turn orange when egg-laying is imminent.) In our opinion, scouting in the spring should focus on nymphs, because by the time adults emerge, the disease is already spreading. This is true particularly in the case of adults that emerge from nymphs that fed on infected plants, because they can transmit citrus greening bacteria immediately after emergence (Xu et al. 1988).

As is the case with other phloem-sucking Sternorrhyncha, systemic pesticides are particularly efficacious against *D. citri*. Trunk applications have proven useful (Aubert 1988; Buitendag & von Broembsen 1993). Two patent applicators are available in South Africa that calibrate the dose based on the diameter of the tree (Buitendag & von Broembsen 1993). Supriyanto & Whittle (1991) and Shivankar et al. (2000) also had success with trunk applications. The best time to apply was just prior to spring flush.

A computer model indicated that even with careful attention to inoculum reduction, at least 70% reduction in transmission is needed to delay the epidemic significantly (Supriyanto & Whittle 1991). Unfortunately, there was no standard measurement of psyllid abundance, so it is unclear what constitutes 70% reduction; however, it is clear that a pesticide with high efficacy is essential.

There has been little research with “soft” (environmentally friendly) pesticides, but Deacon et al. (1989) have found that certain chitin synthesis inhibitors work for eggs and first instars. Shivankar et al. (2000) report about 90% control for several botanicals, including neem formulations. Use of these materials alone may not be wise in an eradication effort, but they might be used effectively against spring populations if timing were just right.

Antibiotics injected into infected citrus trees provide temporary remission of symptoms (Buitendag & von Broembsen 1993; Lim et al. 1990; Su et al. 1986). Injection with antibiotics is recommended as part of an integrated management program in India (Nariani 1981). It is not known whether the titre of citrus greening bacteria is reduced sufficiently to impact transmission by insects or grafting. Symptoms reappear 1-1.5 years after injection (Zhou 1981). Tetracycline also can be used to treat budwood. The budwood is immersed in 1,000 µg/ml tetracycline hydrochloride for two h, or 500 µg/ml for three h (Zhou 1981).

### Biological Control

Aubert (1987) indicated that pathogenic fungi may be the most important mortality factor for *D. citri*. Nymphal mortality of 60-70% could be expected where minimum daily relative humidity exceeded about 87.9% in Réunion Island (Aubert 1987). Two fungal pathogens were reported, including *Cladosporium* sp. nr. *oxysporum* Berk. & M.A. Curtis and *Capnodium citri* Mont. (Aubert 1987). Étienne et al. (2001) said that the fungus *Hirsutella citriformis* Speare was common during periods when humidity was greater than 80%. Use of insect pathogenic fungal sprays has not been reported. In Florida, fungal cadavers of *D. citri* have not been common in the hundreds of submitted regulatory samples of *D. citri* that we have seen in the past five years, in spite of the high relative humidity that characterizes our subtropical climate.

There are two well-known primary parasites of *D. citri*. One is a eulophid ectoparasite, *Tamarixia* (=*Tetrasticus*) *radiata* (Waterston). The other is...
an encyrtid endoparasite, Diaphorencyrtus aligarhensis (Shaffee et al.). Tamarixia radiata apparently is more efficient at parasitizing D. citri than D. aligarhensis (Tang 1989). In surveys conducted in Réunion, T. radiata attacked 60-70% of D. citri nymphs, whereas, D. aligarhensis parasitism did not exceed 20% (Aubert 1987). Both parasites can be subject to high mortality due to hyperparasitism (Aubert 1987; Garnier & Bové 1993). There is considerable Asian literature about the parasite complex attacking D. citri, including life cycle studies (Tang & Wu 1991; Xu & Tang 1993), identification guides (Tang 1990; Tang & Aubert 1990), and survey information. Introduction of T. radiata into Réunion has improved citrus production significantly on the island (Aubert et al. 1996). While the success on Réunion is spectacular, it has occurred in the peculiar circumstances of an island environment in the absence of hyperparasites. Experience in Southeast Asia has shown that the same parasites are not able to similarly reduce transmission (Supriyanto & Whittle 1991).

In Mauritius, biological control of T. erytreae was much more effective than biological control of D. citri (Toorawa 1998). Toorawa (1998) postulates several reasons for this. First, the initial population of T. erytreae was much lower than that of D. citri. Trioza erytreae reproduces principally on citrus, which is regularly treated with pesticides, whereas D. citri utilizes M. paniculata, which is unsprayed and is ubiquitous as an ornamental throughout the island. The climate on much of the island also is more suitable to D. citri than to T. erytreae. Second, the parasite of T. erytreae (Tamarixia dryi (Waterston)) has an alternate host in the common psyllid T. litseae, whereas T. radiata, the parasite of D. citri, has no alternate host (Toorawa 1998).

Tamarixia radiata also was introduced into Guadeloupe in January of 1999. The parasites were imported from Réunion and released immediately on arrival. The parasite apparently has been quite successful (Étienne et al. 2001), although release of any insects without prior quarantine is not recommended, particularly when vectored pathogens may be present in the host population.

Both T. radiata and D. aligarhensis were released into Florida (McFarland & Hoy 2001), but with mixed results. Apparently only T. radiata has established in Florida (Michaud 2002). Field data reported by Michaud (2004) indicate that parasitism by T. radiata contributed 1.3%, 0.2%, and 1.0% mortality of psyllid nymphs in three cohorts, respectively, observed in central Florida. The low rate of parasitism was due at least partially to intraguild predation by coccinellids. Exclusion of large predators from some of the observed citrus terminals of cohort 3 increased mummy formation about 20-fold (Michaud 2004).

Viraktamath & Bhumannavar (2002) list several more parasites of D. citri in their Table 1 and in the text on the preceding page. Psylloephagus diaphorinae Lin & Tao probably is a primary parasite. Syrphophagus taiwanus Hayat & Lin, Syrphophagus (= Aphidencyrtus) diaphorinae Myartseva & Tryapitsyn, and Marietta sp. nr. exitosa Compere probably are hyperparasites. Diaphorencyrtus diaphorinae Lin & Tao is listed (Viraktamath & Bhumannavar 2002) as a hyperparasite, but it may be primary.

PREDATORS OF D. CITRI are known from wherever the psyllid occurs. One species of Scymnus (Coccinellidae) has been reported in Brazil (Gravena et al. 1996). Syrphids in the genus Allograpta have been found in Réunion and Nepal (Aubert 1987) and in Florida (Michaud 2002). Several coccinellids and chrysopids also have been reported (Aubert 1987), but there is no information about how much they actually reduce psyllid populations. In Florida, the most abundant predators are Harmonia axyridis Pallas and Olla v-nigrum Mulsant (Michaud 2001; Michaud 2002; Michaud 2004). Olla v-nigrum was a relatively rare species prior to the arrival of D. citri, but it exhibited a marked functional response to the establishment of D. citri (Michaud 2001). Coccinellid predators are by far the most important sources of biological control for D. citri in Florida (Michaud 2002; Michaud 2004). Both Michaud (2002, 2004) in Florida and Al-Ghamdi (2000) in Saudi Arabia have observed that spiders may be important predators for D. citri. In Saudi Arabia, spiders accounted for 33.6% of total predators (Al-Ghamdi 2000). Several other predators, including a histerid beetle, Sapsalinus chalcites Illiger and the predaceous carabid, Egapola crenulata Dejean, were important in Saudi Arabia (Al-Ghamdi 2000). A similar complex of predators, including Coccinellidae, Chrysopidae, and Syrphidae exists in Cuba (González et al. 2003).

Biological control of vectors of pathogens may have limited value in some circumstances, particularly in the case of a perennial tree crop like citrus. Population fluctuations are inherent in any predator-prey relationship. Some years the natural enemies predominate, and sometimes the pest is more abundant. If citrus greening is present, in years when citrus psyllid predominate, the entire grove may be infected and subsequently destroyed by citrus greening disease. Similarly, if pesticides are needed for some other pest problem such as whiteflies, mealybugs, scales, etc., natural enemies could be killed, and psyllid populations would increase dramatically. Once again, if citrus greening disease were present, the entire grove could be lost.

Biological control of the pathogen (cross-protection) has not been well-studied. It is known that plants can become infected with both Asian and African greening bacteria (Garnier et al.
found that a population of several strains of CTV was able to cross-protect citrus from African greening. The isolate and its aphid transmitted sub-isolates are under further study for potential use in cross protection. Naidu & Govindu (1981) did a greenhouse study on cross protection. Mild isolates did not provide complete cross protection when graft-inoculated sweet orange plants were challenged with severe isolates. Moreover, isolates that were mild in sweet orange were severe in grapefruit in a subsequent host range test.

### Host Plant Resistance

Although there is no real resistance in *Citrus* spp. to citrus greening disease, some species and cultivars are somewhat tolerant. Koizumi et al. (1993) did extensive field surveys showing that some cultivars were less susceptible to decline than others. Most of the sweet orange trees became infected with the pathogen and subsequently declined, while grapefruit was more tolerant. In general, sweet oranges, mandarins and tangelos are most susceptible, grapefruit and lemon are more resistant, and limes, *Poncirus trifoliata* and citranges are the most tolerant (Lee 1996).

### Cultural Control

Management of citrus greening in areas where the disease is endemic depends largely upon cultural control. Infected limbs and trees should be removed as symptoms appear. The pathogen apparently moves fairly slowly within the plant after infection, so severe pruning can be helpful. For African greening, Buitendag & von Broembsen (1993) make the following recommendations: If the infected tree is 5 years old or less, remove the tree. If it is between 6 and 10 years, remove it if it is 75% infected; otherwise remove branches. If it is more than 10 years old, remove affected branches up to 40% of the tree. Do not plant young resets in old groves affected by greening. The tendency for suckers that sprout after pruning to be infected with greening depends upon the diameter of the branches. Branches 10-19 mm in diameter grew no suckers. Among branches 20 mm in diameter or more, the smallest ones were most likely to produce infected suckers (86% for branches 20-29 mm, as compared with 29% for those that were 40-60 mm) (van Vuuren 1993).

Roistacher (1996) cited a Chinese program for rehabilitation of citrus in Fujian Province in which cultural control played a major part. Windbreaks were established to protect plants from psyllid vectors (although the efficacy of barriers for protection from a persistently transmitted pathogen is questionable). Trees were examined regularly for citrus greening disease, and all infected trees were immediately removed and replaced with healthy trees from a certified citrus stock program at the Fujian Academy of Agricultural Sciences (Ke & Xu 1990). In another Chinese program, part of the control program involved hand-removal of summer flush in high density citrus plantings following rice cultivation (Aubert 1990b).

### Regulatory Measures

If no citrus greening pathogen is present, *D. citri* is not a major pest, and regulatory measures are unnecessary; however, regulation of host material is an essential part of managing citrus greening disease. Initially, all citrus budwood and liners should be tested and certified free of the greening pathogens. Propagation material must be kept isolated from psyllids and potential sources of *Candidatus Liberibacter* spp. Lin & Lin (1990) recommend eliminating all citrus and citroids within 5-8 km of propagating nurseries. In the Chinese program in Fujian discussed above (Ke & Xu 1990), it was necessary to eradicate all backyard citrus trees, as well as *Murraya* and *Clausena* plants. The introduction of any citrus or other Rutaceous plants into the area was strictly forbidden. Additionally, all newly planted trees were supplied by the Fujian Academy of Sciences and certified free of greening.

### Integrated Management

There is no place in the world where citrus greening disease occurs that it is under completely successful management. In every place where the disease occurs, life expectancy of citrus trees is vastly reduced, and production losses are significant to total. That having been said, in many areas of the world, citrus production has had to adapt to the presence of citrus greening. The most successful management efforts combine production of clean stock with psyllid control, both within the grove and on alternative host plants, and inoculum suppression after groves are established. Taiwan is a case in point, where Hung et al. (2000) and Su et al. (1986) state that there are three main aspects to managing citrus greening disease: Propagation of clean nursery stock, psyllid control, and removal of potential inoculum sources. Many aspects of citrus greening management are costly both in cash outlay, and in lost production. The economic viability of citrus production in a greening-endemic environment, even with the best current management practices, certainly is not assured.

**Can citrus greening be eradicated?**

The success of any eradication program depends upon the extent of the problem. Thus, early detection is essential to the success of an eradica-
tion effort. Once the disease becomes widespread, there is little hope of eradication, particularly if the pathogen has become established in an unknown number of native or ornamental non-citrus hosts. Given an ideal situation with early detection and very limited spread of the disease, it may be possible to eradicate citrus greening disease successfully, especially in an island situation, but the outcome depends on a number of factors.

Detection Capabilities

The success of any eradication program depends on rapid and accurate diagnosis, preferably in the field. It is unknown whether reliance on symptoms, even by personnel with extensive training in citrus greening symptom recognition, will be sufficient to contain the problem. Certainty of diagnosis is vital, given today’s legal climate (Gottwald et al. 2002; Schubert et al. 2001). It is not known if vectors are able to transmit greening pathogens from infected, but asymptomatic plants. If so, rapid diagnostic methods are needed that can detect the presence of pathogens prior to symptom expression in infected plants. In any case, reliable, robust diagnostic methods are needed to confirm infection with certainty if control action (i.e., tree removal) is necessary. Research is needed in the area of rapid, reliable diagnostics for citrus greening disease.

Detection of citrus greening disease in plants is difficult because of the irregular distribution of the pathogen in the host. While the currently available molecular detection methods have provided us with a better understanding of the taxonomy of the organism and confirmation of the disease in various parts of the world, there is a need for robust universal diagnostic methods for quarantine and eradication purposes. The presence of the vector in Florida and the increasing numbers of interceptions of illegal plant materials have made it impossible to neglect the need for development of sensitive detection techniques for citrus greening disease. The new PCR methods or hybridization techniques should be sensitive enough to detect very low levels of the pathogen in both host and the vector, and not give false positives. This requires development of sequence information that is unique to citrus greening pathogens. Citrus greening isolates are available at present in the USDA quarantine facility, Beltsville, MD. Genome sequencing of citrus greening disease has made it impossible to neglect the need for development of sequence information that is unique to citrus greening disease. The new PCR methods or hybridization techniques should be sensitive enough to detect very low levels of the pathogen in both host and the vector, and not give false positives. This requires development of sequence information that is unique to citrus greening pathogens. Citrus greening isolates are available at present in the USDA quarantine facility, Beltsville, MD. Genome sequencing of citrus greening disease has made it impossible to neglect the need for development of sequence information that is unique to citrus greening disease.

Efficacy of Psyllid Control

Removal of infected and exposed host plants could be extensive and expensive. It is not known how far Asian citrus psyllid vectors can fly, but informal estimates suggest that they might fly several kilometers. Removal of all citrus trees within a radius of several kilometers, especially in an urban environment, is likely to provoke extreme public outcry. If psyllid vectors can be controlled successfully, only infected plants would have to be removed. Thus, safe and extremely effective psyllid control probably is needed for successful eradication. Depending upon the efficiency and seasonality of transmission, it may not be necessary to control citrus psyllids 100% all the time, but research is needed on field infectivity of vectors and seasonality of transmission.

Determining the host range of the psyllid vectors is relatively easy compared with determining the host range of the pathogens. Knowledge of both, however, is needed for successful eradication. Hosts of vectors at least would need to be treated to eliminate the insects within the regulated area. Hosts of the pathogens would need to be tested and removed if found to be infected.

Quarantines

Citrus greening pathogens are transmitted only by psyllid vectors, grafting, dodder, and possibly seed. Dodder transmission probably can be discounted in the field. Thus, only grafting, seed, and vectors need to be considered in quarantine regulations. Nurseries that produce hosts of either pathogens or vectors would have to be quarantined very strictly, similar to citrus nurseries in citrus canker quarantine areas (Florida Department of Agriculture and Consumer Services 2000). Our experience has shown that D. citri can move on unprocessed fruit. Numerous D. citri were intercepted in boxes of grapefruit picked in the Bahamas and shipped to Ft. Pierce, Florida for packing (Halbert & Núñez 2004). Similarly, D. citri certainly can move on leaf and twig material. Thus, it may be necessary to quarantine all citrus plant material within the regulated area, including both citrus yard trash and fruit. Quarantine treatments may be feasible for commercially produced fruit.

D. citri was able to colonize much of the state of Florida as a result of shipments of orange jasmine (M. paniculata) plants produced in southern Miami-Dade County and distributed through discount chain stores (Halbert et al. 2002). During the time that the distribution of D. citri in Florida was classified as limited, the DPI required pesticide treatment of M. paniculata if D. citri was found in a nursery. However, the actual producers of the plants in Miami-Dade County frequently were hard to find and proved impossible to regulate. It may be necessary to prohibit movement of all potential hosts of citrus greening pathogens or their vectors within the quarantine area. Murraya paniculata now is considered a Category II...
invasive plant (Florida Exotic Pest Plant Council 2003), which might give additional leverage to a program to regulate its sale and distribution.

CONCLUSIONS

Citrus greening is a devastating disease, and the prospects for maintaining an economically viable citrus industry at current production levels in Florida if citrus greening disease becomes established are very poor, given current knowledge. Keeping citrus greening pathogens out of Florida should be given very high priority. In the meantime, surveys are necessary to find infected plants as soon as possible if the disease becomes established. Given a limited epidemic, eradication may be possible, depending on circumstances. Scientific research on the disease complex is greatly needed, particularly in the areas of rapid field diagnosis, disease epidemiology and efficacy of psyllid vector control.

ACKNOWLEDGMENTS

We thank John V. da Graça, Texas A&M University; Mohammad Afunian, University of Florida, Lake Alfred; Timothy S. Schubert, DPI; Wayne N. Dixon, DPI; Richard Lee, USDA, Riverside, California; Daniel Burkhardt, Naturhistorisches Museum, Basel, Switzerland; Chester Roistacher, University of California, Riverside; J. P. Michaud, Kansas State University; and Xiaoan Sun, DPI, for reviews of the manuscript; Mark Garland and Richard Weaver, both DPI, for botanical help; Douglass Miller and Debra Creel, both USNM, and Daniel Burkhardt (see above) for finding original descriptions of obscure psyllids; Ian Millar, ARC-Plant Protection Research Institute, South African National Collection of Insects, Queenswood, Pretoria, South Africa and Dr. G. J. Begemann, Transvaal Sugar Ltd., Komatipoort, South Africa for photos of Diaphorina citri; and Beverly Pope and Alice Sanders, both DPI for library assistance. We thank Xiaoan Sun, DPI, for checking our Chinese translations. We thank Dr. Thomas Dobbs, USDA/APHIS/PPQ, Miami, for providing interception information. We thank CSREES T-STAR for partial support for this project. This paper is Florida Agricultural Experiment Station Journal Series No. R-10083.

LITERATURE CITED


AUBERT, B. 1990b. High density planting (HDP) of Jiaoagan mandarin in the lowland area of Shantou (Guangdong China) and implications for greening control, pp. 149-157 In B. Aubert, S. Tontyaporn, and D. Buangsuwion [eds.]. Rehabilitation of Citrus Industry in the Asia Pacific Region. Proc. Asia Pacific International Conference on Citrus culture, Chiang Mai, Thailand, 4-10 February 1990. UNDP-FAO, Rome.


