Diseases and Pests of Citrus (Citrus spp.)

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ABSTRACT

Citrus spp. are susceptible to a number of destructive diseases that are continuously emerging and which can severely limit production or totally decimate an industry of a country. There is the well known tristeza caused by the Citrus tristeza virus, the crippling bacterial citrus huanglongbing (Candidatus Liberibacter) and canker (Xanthomonas axonopodis pv. citri). Among fungal diseases, soil-borne infections caused by Phytophthora and postharvest pathogens are widespread and lead to heavy yield losses. Several species of phytonematodes have been detected in citrus rhizosphere, but the economic importance is known for only a few species. Tylenthulus semipenetrans occurs worldwide and can reduce yields when populations are dense. Other nematodes of local or regional importance include Radopholus citrphilus, Belonolaimus longicaudatus and species of Pratylenchus and Meloidogyne. Numerous species of insects have attained pest status at various locations at different times because of the damage they cause on citrus trees and fruits and the pathogens they transmit. Lepidopterans of major importance are Papilio spp., particularly the lime swallowtail butterfly, Papilio demoleus, which has recently invaded the western hemisphere. Larvae of coleopterans, Exopthalmus and Pachnus spp. cause extensive root damage, while adults attack young leaves. Of the aphid and psyllid species, Toxoptera citricida and Diaphorina citri, are considered as potentially the most dangerous at this time because they serve as vectors for Citrus tristeza virus and huanglongbing. This paper presents an overview of the important pathogens and pests limiting citrus production, diseases they elicit, genetic structure and diversity, and factors contributing to their emergence.

Keywords: Cachexia, canker, citrus postharvest fungal decays, Diaphorina citri, exocortis, foot rot, gummosis, huanglongbing, leprosis, mal secco, Papilio demoleus, Phyllocnistis citrella, psorosis, Radopholus citrphilus, root weevils, sharpshooters, stubborn disease, tatterleaf, Toxoptera citricida, Triozeryeae, tristeza, Tylenthulus semipenetrans, variegated chlorosis

Abbreviations: ASGV, Apple stem grooving virus; BrCA, brown citrus aphid; CC, citrus canker; CC-A, canker A, asist citrus canker; CC-B, cancerosis B; CC-C, cancerosis C; CC-D, cancerosis D; CC-E, cancerosis E; CEVd, Citrus exocortis viroid; CiLV, Citrus leprosis virus; CL, citrus leprosis; CPv, Citrus psorosis virus; CSD, citrus stubborn; CTLV or CiTLV, Citrus tatterleaf virus; CTV, Citrus tristeza virus; CVC, citrus variegated chlorosis; DAS-ELISA, double antibody sandwich enzyme linked immunosorbent assay; ELISA, enzyme linked immunosorbent assay; EPPO/CABI, European and Mediterranean Plant Protection Organization/CAB International; FAOSTAT, Food and Agriculture Organization of the United Nation Statistical and Data-sets; HLB, citrus huanglongbing or citrus greening; HSVd, Hop stunt viroid; IPM, integrated pest management; ITS, internal transcribed spacers; OEPF/EPPO, Organisation Européenne et Méditerranéenne Pour la Protection des Plantes/ European and Mediterranean Plant Protection Organization; PCR-RADF, polymerase chain reaction-random amplified polymorphism analysis; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism analysis; pthA, pathogenicity locus of Xanthomonas axonopodis; RT-PCR, reverse transcription polymerase chain reaction; SD, spreading decline; SDC, slow decline of citrus; TAS-ELISA, triple-antibody sandwich; TAS-ELISA-HRP, triple-antibody sandwich (horseradish peroxidase)

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Received: 23 July, 2008. Accepted: 15 October, 2008.
INTRODUCTION

Citrus (Citrus spp.) is a woody, evergreen, perennial plant cultivated for its non-climacteric, unique berry-like fruit (Kader 1992; Rieger 2006). Over six million tonnes of fruit were produced worldwide in 2006, mostly in the subtropical regions of Africa (58%), Asia (28%) and the Americas (13%), where there is seasonal variation but little or no freezing weather. Backyard or small scale holdings are more common in tropical regions (FAO/CACT 2008).

All commercial citrus consists of composite trees whereby scions selected for fruit quality are grafted onto hardy, disease-resistant rootstocks (Medina et al. 2005). Commercial citrus species belong to the order Sapindales, family Rutaceae, subfamily Aurantioidea and the genus Citrus, except for trifoliate oranges (Poncirus trifoliata) and kumquats (Fortunella spp.). Trifoliate oranges, as well as C. macrophylla (alemow), C. sinensis x P. trifoliata (citrange), P. trifoliata x C. paradisi (citrumelo), C. reshni (cleopatra mandarin), C. limonia (rangpur lime), C. jambhiri (rough lemon), and C. aurantium (sour orange) are commonly used as rootstocks. Of the species cultivated for their fruit, sweet oranges (C. sinensis) and mandarins (C. reticulata) are grown extensively worldwide, and to a more limited extent, grapefruits (C. paradisi), lemons (C. limon), limes (C. aurantifolia), and the hybrids tangelos (mandarin x grapefruit) and tangors (mandarin x sweet orange) (Rajput and Haribabu 1993).

Like most fruits, citrus is a low-calorie food, a good source of carbohydrates and fibre, is low in sodium and fats and is an excellent source of vitamin C. The fruit is, however, also appreciated for its biologically active, anti-nutrient compounds or phytochemicals (Huang et al. 2007). Citrus phytochemicals have potential antioxidant, anti-cancer, anti-atherosclerotic, and weight loss abilities (Tian et al. 2001).

The fruit crop is widely produced and marketed as fresh fruit. Orange juice is the most important processed product appearing in the market as freshly squeezed juice, frozen concentrate, refrigerated juice from concentrate and not-from concentrate juice, which is perceived as the closest equivalent to freshly squeezed juice. In addition to the pulp, all other parts of the fruit can be consumed; a feature unique to citrus fruits. Flavorings and pectin are extracted from the outer ectocarp and inner mesocarp rind, respectively. Essential oils, for use in cosmetic and pharmaceutical industries, are extracted from the peel. Citrus pulp pellets, the product remaining from peel and pulp once the juice and oils have been extracted, are used as animal feed, while seed oils, seed meals and dried seed-pressed cakes are products derived from the seeds (Kale and Adsule 1995; Ahmad and Rehman 2006; Bampidis and Robinson 2006).

The key to successful production of citrus lies in the effectiveness of long-term pathogen- and pest-management strategies. As with many tropical and subtropical crops, citrus is host to various pathogens and pests, but it is also one of the few crops that is susceptible to a number of destructive diseases that are continuously emerging and which can severely limit or totally decimate production. Production of citrus fruits destined for processing may not require stringent management of pests as those for the fresh fruit market. Nevertheless, a clear understanding of the biology of the pathogen is central to the development of a certification program and maintenance of productive citrus orchards dedicated to processing or fresh-fruit markets. The revision edition of the Compendium of Citrus Diseases (Timmer et al. 2000) describes the etiology and epidemiology of 7 bacterial, 16 fungal and 42 virus or virus-like diseases of citrus. Also described are diseases which are the result of pest-induced injuries, including those caused by 10 genera of nematodes, snails, ants, termites, mites and a number of insects. In 2004, OEP/EPP listed 9 fungal genera, one bacterial (Spiroplasma citri), one viral (Citrus tristeza virus), one nematode (Tylenchulus semipenetrans), and various species of aphids, thrips, whiteflies, leaf roller, leaf miner, moths and scale insects as the principal pests of citrus (OEP/EPP 2004b). This review focuses on recent advances in the knowledge of major pathogens and pests limiting citrus production, the diseases they elicit, their diagnosis, genetic structure and diversity, and factors contributing to their emergence.

Fungal Diseases

Citrus is susceptible to a number of fungal and fungal-like pathogens. Fusarium spp., Phyllosticta spp., Phoma tracheiphila, and postharvest pathogens (Penicillium, Aspergillus, Galactomyces citri-aurantii, Alternaria citri, Glomella citrulina) are regarded as the important pathogens of the fruit crop, responsible for considerable losses in some regions (OEP/EPP 2004).

Foot rot and gummosis

Phytophthora spp. cause serious soilborne diseases of citrus affecting the roots, trunk, branches, shoots and fruits worldwide (Erwin and Ribeiro 1996; Graham and Menge 2000). Although ten oomycete species are reported to be pathogenic on citrus, three of these cause the major damage economically and include Phytophthora citrophthora, P. nicotianae (syn P. parasitica) and P. palmivora (Erwin and Ribeiro 1996; Graham and Menge 2000). Distribution of the species, however, is dependent on their specific temporal and climatic requirements; P. nicotianae occurs frequently in subtropical areas, P. palmivora in subtropical and tropical areas, while P. citrophthora is common in Mediterranean areas (Ricci et al. 1990; Graham 1995; Graham and Menge 2000). P. nicotianae generally does not infect the above-ground parts of citrus trees. It causes foot rot or gummosis, which is a bark disease characterized by degraded cambium and phloem tissues. Both P. palmivora and P. citrophthora induce gummosis and brown rot of the trunk and major limbs. The latter pathogen also attacks citrus fruit. Trunk and root rot affections are regarded as most serious on citrus.

The infection cycle of Phytophthora spp. begins with the attraction of moist zoospores to citrus root exudates, their encystment, germination, and entry into the root tip. Infection of the root cortex results in discoloration, softening of tissues and decay of fibrous roots. Eventually the cortex sloughs off leaving vascular tissue. Lesions on struc-
tural roots or crown rot may also occur (Fawcett 1936; Sandler et al. 1989; Graham and Menge 1999). Foot rot or gummosis occurs when zoospores are splashed onto the trunk above the bud union. The pathogen enters through wounds or bark cracks and eventually lesions develop that extend to the bud union. Active lesions ooze brown to black gum, hence the name ‘gummosis’ (Graham and Menge 1999). A significant number of damaged roots and the resulting rot, induce water stress which can result in the death of young trees. Mature trees exhibit reduced vigor, decreased fruit production and twig dieback. Low-hanging fruits may also become infected from propagules in rainsplash resulting in brown rot of fruits and leaf fall. Scion lesions have been described in South Africa, Egypt and Belize and more recently in Spain (Alvarez et al. 2008). Sporulation on fruits may be important in the establishment of branch infections.

Fusarium dry rot

Another soil-borne disease, Fusarium root rot, is widespread and problematic on citrus. *Fusarium solani*, associated with Fusarium root rot, sudden death or Fusarium dry rot of citrus, has been described in Australia, North America, Europe, South Africa and India. The pathogen is a primary colonizer of fibrous root systems. Afflicted trees develop blackened, rotted roots. Typically, the region below the scion shows brown discoloration, but this does not extend into the scion. The production of naphthazarin phytotoxins, dihydrofusarubin and isomartocin, during infection also contribute to the decline of citrus trees. The toxins, which are readily transported in xylem, contribute to vessel plugging, reduced water uptake, vein chlorosis, leaf wilt and the accumulation of zinc in the trunk wood of citrus trees. Unlike *Phytophthora* root and root rot, there is no gum exudation and the affected roots are affected. *F. solani* is often associated with other pathogens and pests of citrus, including *Phytophthora* and nematodes which exacerbate disease symptoms. The disease occurs in groves with heavy soils that retain moisture easily. Maintaining soil conditions favorable for root development (e.g. avoiding soil compaction, waterlogging and salinity) is most useful in managing the disease (Nemec et al. 1988; Menge and Nemec 1997; Broadbent 2000; Janse van Rensburg et al. 2001; Naqui 2004).

Other soil-borne fungal pathogens, *Pythium* spp., *Thelephora p. basica*, *Armillaria mellea* and *Dipodula matalen*, affect citrus production and contribute to replant and decline problems. They are, however, regarded as minor root pathogens that are mostly problematic under favorable environmental conditions (Menge and Nemec 1997).

Mal secco

The vascular fungal disease Mal secco, Italian for dry disease, was first recorded in the 1880s in Greece (Solé and Salerno 2000). Currently, the disease has maintained limited distribution and is confined to citrus growing regions of the Mediterranean Basin, around the Black Sea, and in Asia Minor (Solé 1976; Palm 1987; Timmer et al. 1988; EPPO/CABI 1997b). Despite prevailing suitable disease-promoting environmental conditions and cultural practices, the disease has not been reported in the Americas and Oceania (EPPO/CABI 1997b). Species of *Poncirus, Severinia, F. tulipera* and *Citrus* are vulnerable, but lemon (*Citrus limon*) is particularly susceptible to this vascular disease.

Mal secco is caused by the imperfect fungus, *Phoma tracheiphila*. Pycnidiospores are dispersed by rain, then the germ tubes enter leaf tissue through stomata or wounds, invade the xylem and the fungus gradually spreads systemically. Sections through twigs reveal the characteristic salmon-pink or orange-reddish discoloration of the wood, which is associated with gum production. In time, impairment of the water transport system leads to wilting of branches. Typical symptoms on the foliage include vein chlorosis, leaf wilt and die-back of twigs and branches. Eventually the bark ruptures and exposes numerous black pycnidia. Death of the tree then follows with root rot and root galling.

Two other forms of the disease have been documented; mal fulminate, a rapid fatal form of the disease that occurs when the fungus infects the base of the trunk or the roots of the tree, and mal nero, a chronic infection characterized by browning of the heartwood. Latent infections of the disease have also been described. In these instances, a healthy tree appears to develop symptoms suddenly and then collapses (Timmer et al. 1987). Infections by *C. tracheiphila* in California and Salerno (EPPO/CABI 1997b).

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Ezra et al. (2007).

The strategy commonly used to manage mal secco infections involves the reduction of fungal inoculum by pruning withered shoots bearing pycnidia, removing suckers, and spraying with fungicides (OEP/Eppo 2005a). Early diagnosis is of paramount importance. Traditionally, identification of *P. tracheiphila* has relied on isolation of the fungus on potato dextrose agar, carrot agar, or malt extract agar. *P. tracheiphila* produces mal secco-infected twigs, mal nero-infected twigs, and mal dermatophilya, followed by identification of cultural and morphological characters (OEP/Eppo 2005a). However, this approach is effective only at the later stages of infection at which time the application of countermeasures to limit spread are no longer effective. Early molecular methods involving dot blot assay and PCR, developed in the late 1980s, proved unreliable for routine diagnosis because of the generation of non-specific amplimers (Rollo et al., 1987; 1990; Balmas et al. 2005). In 2005, Balmas and coworkers described a more specific PCR-based assay using primers designed to the consensus sequence obtained from the alignment of the ITS region of the nuclear rRNA genes of 17 *P. tracheiphila* isolates and of single representatives of six additional *Phoma* species (*P. glomerata*, *P. exigua*, *P. betae*, *P. cava*, *P. fimeti*, and *P. lingam* isolates). Using this method, the fungus was detected in wood tissues of mal secco-infected twigs, mal nero-infected twigs, and mal dermatophilya twigs. Luciardiello et al. (2006) and Demontis et al. (2008) have since reported on a fast and reliable method for the quantification of *P. tracheiphila in planta* by a real-time PCR assay using a TaqMan® hybridisation probe.

**Postharvest fungal decays**

Other major fungal pathogens affecting citrus fall into the category of postharvest pathogens. Their prevalence is, however, variable and dependent on the citrus variety, weather conditions during the growing and harvest seasons, degree of damage during harvest, and conditions of the postharvest facility. Generally preharvest pathogens, such as *Colletotrichum gloeosporioides*, infect fruits in the field between fruit set and harvest. They transition later to necrotrophs and, along with wound pathogens (e.g. *Penicillium digitatum*, *P. italicum*), attack fruits during postharvest handling, transportation, storage and marketing (Naqvi 2004). *Alternaria citri*, the etiological agent of Alternaria rot, causes premature coloring of fruits in the field, the development of light brown to black lesions of the rind at the button or stylar end and internal black discoloration mainly along the central axis of the fruit during storage (Brown and MacCornack 1972; Brown and Eckert 2000b). Alternaria rot and rot over the button or olive green when caused by *Aspergillus niger* are held at high storage temperatures (> 15°C) and causes aspergillus rot. Infection occurs through damage incurred during handling. Similar to *Penicillium* spp., *Aspergillus* secretes large amounts of gluconic and citric acids during decay development and compromises citrus fruit defenses in the field. Conidia are typically spread by rainsplash over short distances, whereas ascospores are spread in air currents. Although conidia can be deposited in the styrar region of fruits while they are in the orchard, the fungus does not invade their tissues until after harvest when the button abscisses. Decay subsequently progresses unevenly over the fruit and is more severe during ethylene degreening. Early abscission of the button results in a site of entry into fruit during the degreening process. The fungus is conducted under favorable conditions of high temperatures (> 21°C) and relative humidity (> 90%). All citrus fruits are vulnerable to stem-end rot disease (Brown and Eckert 2000a). *Botrytis cinerea* (*Botrytis cinerea*) is a heterothallic ascomycete that attacks over 200 host plants (MacFarlane 1968). It exhibits great genetic variability (van der Vlugt-Bergmans et al., 1993; Diolez et al. 1994) and morphological variability (Grindle 1979; Di Lenna et al. 1981; Lorenz and Eichhorn 1983; Movahedi and Heale 1990; Leone 1990), presumably due to heterokaryosis and aneuploidy (Lorenz and Eichhorn 1983; van der Vlugt-Bergmans et al. 1993). *B. cinerea* causes gray mold or botrytis blossom and twig blight in citrus. Lemons are particularly affected. The disease progresses with the development of patches of a gray mold associated with brown leathery decay of fruits. A distinct aroma of ozone odor is non-infected with the infection. Apart from fruit decay, fruit set is reduced and premature fruit drop is common. Spores produced on organic debris in the orchard are dispersed to flowers by wind or rainsplash. Colonization of floral parts results in quiescent infections of the stem end of flowers and, in time, may cause postharvest decay (Eckert and Brown 2000).

*Galactomyces citri-aureantii* (anamorph Geotrichum citri-aureantii) is associated with sour rot, which is regarded as the most unpleasant of all the citrus decays. *G. citri-aureantii* affects mature or over-mature citrus fruits and may be found in mixed infections with *P. digitatum* and *P. italicum*. The fungus is soil-borne and spreads to fruits by wind or rainsplash. Entry into the rind is through mechanical or insect injuries. Disease symptoms include the initial development of water-soaked, light- to dark-yellow, slightly raised lesions and, over time, the fruit disintegrates into a slimy, watery mass (Brown and Eckert 2000).

*Penicillium* infections, otherwise referred to as blue and green molds, cause very important postharvest decays in citrus-producing regions. The disease cycles of the causative agents, the imperfect fungus *Penicillium digitatum* (green mold) and *P. italicum* (blue mold), are similar but the latter fungus grows better at lower temperatures (10°C) and is more readily dispersed in packing, storage, and transportation facilities (Smith and Maisonneuve 1991). Both phytopathogens secrete large amounts of gluconic and citric acids and actively taking up ammonium ions. Presumably, ambient pH plays a major regulatory role in endopolygalacturonase gene expression in *Penicillium* spp. (Prusky et al. 2004). More recently, Macarismis et al. (2007), using laser-scanning confocal microscopy, demonstrated a 42-hour suppression of defense-related hydrogen peroxide burst in citrus fruit exocarp by *P. digitatum*. *P. digitatum* and *P. italicum* persist in orchards as conidia and are dispersed in air currents. Infections initiated through wounds are followed with the appearance of soft, discolored areas on which powdery mycelia develop. Sporulating areas are blue when infections are caused by *P. italicum* or olive green when caused by *P. digitatum*. Rotted fruits eventually become dry mummies (Brown and Eckert 2000d). Green mold and sour rot are the most economically important postharvest diseases of citrus in arid growing regions (Smilanick and Sorenson 2001). *Aspergillus niger* is generally problematic when fruits are held at high storage temperatures (> 15°C) and causes aspergillus rot. Infection occurs through damage incurred during handling. Similar to *Penicillium* spp., *Aspergillus* secretes large amounts of gluconic and citric acids during decay development and compromises citrus fruit defenses.
(Prusky and Lichter 2008). Aspergillus survives saprophytically in the orchard and its conidia are dispersed by air currents. Symptom development is similar to that of sour rot and starts with the appearance of light-brown to orange-colored lesions that later become sunken as decay progresses. Eventually, soft decayed fruits are covered with black powdery layers of spores (Brown and Eckert 2000e).

Good cultural practices, along with preventive and post-harvest application of fungicides and biological control agents, aid in the control of a number of the postharvest fungal pathogens affecting citrus. Fungicide treatments are generally incorporated in fruit waxes which are, in some cases, used in combination with biological control formulations containing Candida oleophila or Pseudomonas syringae (Droby et al. 1998). Fruits are also immersed in sodium bicarbonate at ambient temperatures or heated in sodium carbonate or borax and boric acid mixtures (Eckert and Eaks 1989; Smilanick et al. 1999). The search for treatments against postharvest fungal pathogens is a continuous challenge. Recently, investigations of other biological control formulations with Bacillus spp., Pantoea agglomerans, new fungicides (fluidoxonil) and the application of ionizing radiation have been reported (Palou et al. 2007; Zhang 2007; Leelasuphakul et al. 2008). However, several issues surround the development and release of new treatments. These include concerns about human health risks associated with residues, particularly in the diets of children, the widespread occurrence of fungicide-resistant isolates and environmental problems associated with the disposal of water used in packing operations (Eckert and Eaks 1989; National Research Council 1993; Eckert et al. 1994).

**BACTERIAL DISEASES**

*Xanthomonas axonopodis*, *Xylella fastidiosa*, and *Candidatus Liberibacter* are listed as etiological agents of economically important citrus diseases. *Candidatus Liberibacter*, by far, is the most destructive to citrus production globally. Various forms of the pathogen are situated in specific regions based on prevailing environmental conditions and insect vectors. Similarly, *Xanthomonas* and *Xylella*, members of the same family, are made up of several species that infect the same host, but induce different pathologies, and are found in different environments.

**Citrus canker**

Citrus canker (CC), asiatie citrus canker or cancrosis A, false citrus canker or cancrosis B, ‘Mexican lime’ cancrosis or cancrosis C, is a very important disease of most commercial cultivars of citrus (Gottwald and Graham 2000). CC is endemic to regions of south-east Asia and occurs in 30 countries throughout Asia, Africa, Oceania and South America. CC has a long history in the southeastern US. The etiological agent, believed to have been imported on nursery stock from Japan, was first reported widespread in Florida and neighboring states in the 1910s. Through nursery and orchard inspections, removal of infected trees and quarantines, CC was declared eradicated from the region in 1933. Later incidences were reported in the same area in Florida in the late 1980s and new outbreaks have since been discovered in urban Miami and Palm Beach County in the mid 1990s and 2000s, respectively (Gottwald et al. 2001). The disease continues to spread worldwide in spite of heightened quarantine regulations imposed by many countries to prevent its introduction (Gottwald et al. 2002).

The causal agents of CC are classified as three pathovars of a single species of the gram-negative bacterium, *Xanthomonas axonopodis* (synonym *X. campestris*). These agents elicit five forms of the disease that are differentiated primarily on the basis of host range. Strains of *X. axonopodis* pv. *citri* cause the most severe and widespread disease, CC-A (canker A, asiatie citrus canker), which affects most citrus varieties. Variants of CC-A include A* and A**. A* is limited to ‘Mexican lime’ and has been described in Oman, Saudi Arabia, Iran and India. A** was recently reported in Florida and appears to have originated in south-west Asia. *X. axonopodis* pv. *aurantifolii* causes cancrosis B (CC-B), a disease of lemons, ‘Mexican lime’, sour orange, and pummelo in South America. Cancrosis C (CC-C), caused by *X. axonopodis* pv. *aurantifolii*, is associated with ‘Mexican lime’ in Brazil. Grapefruit and trifoliate orange have been reported to be susceptible to CC-C. A fourth type, CC-D, was described in Mexico. However, identification of the strains eliciting CC-D remains controversial; presumably the agents are of the pathovar *aurantifolii*. CC-E, or nursery canker, is caused by *X. axonopodis* pv. *citri melo* (Medina-Urrutia and Stapleton 1986; Gabriel et al. 1989; Graham et al. 1990a, 1990b; Gottwald et al. 1991; Vernière et al. 1998; Schubert et al. 2001; Mohammadi et al. 2001; Graham et al. 2004; Polek et al. 2007). Of interest is that non-quarantine xanthomonads are also classified in the same groups. Thus, in 2003, Brunings and Gabriel (2003) proposed a change in nomenclature and suggested the use of *X. citri ex Hasse* to refer to species that are responsible for CC only. Later, Schaad et al. (2005, 2006) proposed grouping of the strains...
of the five forms into three taxa based on data from DNA-DNA relatedness assays, sequence analysis of 16S-23S ITS regions, AFLP, serology and pathogenicity. It was suggested that all A strains be placed in one taxon, strains B, C and D in another, and E strains in the third. The names proposed for the three taxa include: X. citri subsp. citri, X. fastidiosa subsp. aurantifolii, and X. alfalfae subsp. citrumelo, for the first, second and third groups, respectively.

The X. fastidiosa strain is characterized by the formation of leaf and fruit blisters. However, under favorable conditions, defoliation, shoot die-back and fruit drop can occur. The first manifestations of CC are seen on lower leaf surfaces. Light-colored, pin-point lesions initially develop, and over time, the lesions become raised, tan- or brown-colored pustules. At this stage, the lesions are visible on the upper leaf surfaces and may be surrounded by a chlorotic halo. Eventually, the lesions take on a corky or spongy crater-like appearance (Fig. 1). This ability to induce coral lesions and programmed cell death is associated with the pathogenicity locus, pthA (Greenberg 1997; Gilchrist 1998). Lesion size depends on the citrus cultivar and age of the plants, but it generally ranges between 2 and 10 mm in diameter. Similar lesion formation occurs on twigs and fruits (Fig. 1, Timmer et al. 1991). Chlorosis is not typically associated with twig lesions, but can be found around the periphery of fruit lesions.

Bacterial cells are released from a dense matrix of extracellular polysaccharides within lesions during wet conditions and are the source of inoculum for further disease development (Timmer et al. 1996; Brunings and Gabriel 2003). Agents of CC are primarily dispersed up to 32 m restricted to the American continent.

A. Citrus variegated chlorosis

Citrus variegated chlorosis (CVC), a leaf scorch and decline disease of citrus, is caused by another xanthomonad that is more restricted geographically than that causing CC. CVC was first described in one of the major citrus-growing regions of northern Brazil in the late 1980s (Rossetti et al. 1990). Since then, the disease has been recorded in all citrus-growing regions of Brazil and has been documented in Argentina (Bransky et al. 1991), Paraguay (Ayres et al. 2013), and more recently, in Costa Rica (Aguilar et al. 2008). The disease is caused by Xanthomonas citri pv. citri, a Gram-negative, fastidious bacterium, which induces chlorotic lesions to develop on the upper surface of leaves of young trees between nursery age and ten years. Leaves of trees 15 years and older never totally become affected and often show limited symptom expression on one branch. Over time, small, slightly raised, gummy, necrotic lesions develop on the underside of leaves corresponding to the chlorotic lesions on the upper surface. New leaves are generally smaller than usual. Although the external fruit symptoms are not visible, fruit size is greatly reduced, sugar content increased, the rind hardened, and fruits cluster in bunches that resemble grape clusters. Trees are generally stunted, exhibit die-back of twigs and branches and a reduced canopy, but they do not die. Symptoms are attributed to water-stress because of limited xylem flow. The agent of CVC is strictly confined to the xylem vessels of the host. Once delivered there by the feeding insect vector, multiplication of bacterial cells results in the formation of cell aggregates, matrices of extracellular polysaccharides and the blockage of water transport (Hopkins 1989; Marques et al. 2002; OEPP/EPPO 2004a; Chatterjee et al. 2008). Pathogenicity is also aggravated by high temperatures, water deficit, root injuries and over-production of fruit (Hopkins 1989).

X. fastidiosa was the first agriculturally important plant bacterium for which the genome sequence was published (Simpson et al. 2000). The genome of 2.67 Mb has a total of 2,249 ORFs, 58.4% of which has been assigned putative functions based on homologies with known genes (Simpson et al. 2000). The bacterium also contains two circular plasmids, pXfF1.3 and pXF51, of 1.3 and 51.1 Kbp, that code for 65 and 65 proteins, respectively. The function of pXfF1.3 is not known, but the plasmid is believed to confer a selective advantage to the strain (Pooler et al. 1997). Apart from the sequences encoding one virulence-associated protein, sequences of pX51 are organized into four regions that code for genes that are related to replication, mobilization and conjugative transfer and they do not play a role in pathogenesis (Marques et al. 2001). Genomes of several strains have since been partially or completely sequenced (Bhattacharya et al. 2002; Nunes et al. 2003; van Sluys et al. 2003), including a non-pathogenic strain (Koside et al. 2004). Comparisons of pathogenic and non-pathogenic strains show that 14 genes are highly divergent or absent from the non-pathogenic strain. Among the absent genes are genes that code for an arginase and a fimbrial adhesin precursor, which may correlate to decreased aggregation of the non-pathogenic strain in xylem vessels and its inability to multiply in host plants, respectively. Sequences coding for an enzyme, UDP-3-0-(R-3-hydroxymyristoyl)-glucosamine N-acetyltransferase, are present in the genome of CVC but absent in the genome of X. fastidiosa. This enzyme catalyzes the final step in the synthesis of a lipopolysaccharide component of the outer membrane; its absence may result in the bacterium being more sensitive to plant antimicrobial compounds. Ten of the genes do not show similarities to known genes.

Genetic comparisons to the closely related X. axonopodis pv. citri reveal 1495 genes in common between X. axonopodis pv. citri and X. fastidiosa, but both species are over-represented in X. fastidiosa and not Xanthomonas axonopodis pv. citri relate to virulence and adaptation to the plant host (Moreira et al. 2004). They include (i) a type IV pili system involved in the attachment to the insect vector as well as adhesion and colonization of host tissue leading to cell aggregates, (ii) colicins, which play a role in bacterial antagonism and which may be more important to xylem-dwelling Xylella pv. citri than to cell-aggregating Xanthomonas, and (iii) restriction and modification systems responsible for the protection of genetic material against invasion of exogenous DNA that may relate to the different plant environments inhabited by the two pathogens. Overall, the genome of X. axonopodis pv. citri is more complex than that of X. fastidiosa. The larger genome of 5.2 Mb encodes for a type II secretion system, a number of cell-wall-degrading enzymes and avirulence effector proteins associated with a type III secretion system, and a complete flagellar
and chemostatic system.

The first line of defense against CC and CVC is exclusion through ongoing inspections of nurseries and orchards, quarantine and eradication campaigns (Gottwald and Timmer 1995; Gottwald and Graham 2000; Graham et al. 2004). Regions in which CC is established have adopted a number of cultural practices involving planting of non-citrus trees or shrubs as windbreaks (e.g. saw pine, red cedar and eucalyptus), leafminer control, especially during periods of flush growth, copper sprays to reduce infection and the use of citrus cultivars with some degree of resistance. Similar management strategies are used against CVC. Pruning is particularly effective during the early stages of CVC infection, before infection becomes systemic. Special attention is also paid to testing of asymptomatic ornamentals, growing in the vicinity of citrus orchards, for the bacterium. Vector control is difficult. Xylella is transmitted by xylem-feeding homopteran sharpshooter leaffoppers (family Cicadellidae, subfamily Cicadellinae) and spittlebugs (family Cercopidae) (Redak et al. 2004). Roberto et al. (1996) have shown that 12 of 16 sharpshooter species in Brazil are vectors of Xylella. Transmission by these insects is unique in that multiplication of the pathogen within the host is not accompanied by a latent period (Purcell and Finlay 1979). The rates of transmission are, however, low and variable, ranging from 0.3 to 30% (Krügner et al. 2000; Yamamoto et al. 2002; Marucci et al. 2003). Moreover, Marucci et al. (2005) have provided evidence that CVC-infected citrus plants growing under greenhouse conditions are not the preferred feeding hosts of sharpshooters and the selection of diseased plants has been influenced by the level of infection. These authors have proposed that vector feeding may be more difficult in diseased plants and that the nutritional quality of xylem of these plants is reduced. Morphological and biochemical changes associated with CVC-infected citrus include increased levels of lignin, chitinase and β-1,3-glucanase (Martins et al. 1999).

Vector activity, dispersion of the insects, and selection of feeding sites within hosts are considered more important to CVC disease dynamics than population densities of the insects (Redak et al. 2004).

Monitoring of CC and CVC is facilitated by reliable detection of both pathogens with various PCR methods using primers designed for unique regions in the genome of X. fastidiosa or for plasmid-borne genes of X. axonopodis pv. citri, rDNA sequences and general or pathogenicity regulatory factors (Hartung et al. 1993; Minsavage et al. 1994; Pooler and Hartung 1995; Chen et al. 2000; Oliveira et al. 2000; Cubero and Graham 2002; Schaad et al. 2002; Rodriguez et al. 2003; Cubero and Graham 2004; Mavrodieva et al. 2004; Cubero and Graham 2005; Coletta-Filho et al. 2006; Francis et al. 2006; Golmohammadi et al. 2007).

**Citrus huanglongbing or citrus greening**

Citrus huanglongbing (HLB), also known as citrus greening disease or yellow-shoot disease, is one of the most extensively studied bacterial diseases of citrus. HLB is known as the century-old malady of citrus in Asia. Apart from the Indian subcontinent, China, and South East Asia, other regions with a long history of the disease include East Africa, Cameroon in West Africa, Madagascar, Reunion and Mauritius islands, and the south-western Arabian Peninsula (Garnier and Bové 2000; Polek et al. 2007). Recently in 2004, HLB was recorded in the citrus-growing regions of São Paulo State, Brazil and in the Florida State, USA, in 2005 (da Graça 1991; da Graça and Korsten 2004; Halbert and Manjunath 2004; Coletta-Filho et al. 2004; Teixeira et al. 2005; Knighten et al. 2005). The Mediterranean basin, the Middle East, Oceania (Bové 2006) and Jamaica (S. Brown, pers. comm.) apparently remain free of the disease.

HLB infection of young citrus trees is generally marked by the appearance of a yellow shoot composed of blotchy, mottled leaves which later progresses across the entire canopy (Fig. 2). Young diseased trees never come into production. Disease symptoms in older trees, on the other hand, are at first confined to the initial area of infection, while the remaining canopy shows normal vegetative growth and fruit production; hence the name ‘huanglongbing’ or ‘yellow dragon disease’. Severely infected trees develop more extensive yellowing, and are sparsely foliated, stunted and non-productive. Generally, severe symptom expression of dieback and severe decline are more prevalent in Asia than in other regions. Associated with the mottle foliar symptom expression is the premature shedding of green fruit, hence the name ‘greening’. Fruits that remain on the tree are small, misshapen, contain aborted seeds and mature only on one side (Fig. 3). They are mainly inedible, high in acid content.
and very bitter. Greening symptoms of fruits occur mainly in Africa (Garnier and Bové 2000; da Graça and Korsten 2004; sweet oranges, mandarins, and mandarin hybrids are most affected by HLB regardless of the rootstock whereas grapefruits, ‘Rangpur lime’, lemons, and pummelos develop less severe disease symptoms. ‘Mexican lime’, trifoliate orange and their hybrids are regarded as most tolerant to HLB which causes only leaf mottling symptoms on these citrus cultivars. HLB affects other plants of Rutaceae, such as the ornamental shrub *Murraya paniculata* (orange jasmine), *Severinia buxifolia* (Chinese box orange), *Vitis vinifera*, *Clausena anisata*, *Zanthoxylum capense*, *Limonium acidissima*, *Calodendrum capense* (Cape chestnut), and the common Chinese landscape plant and a popular medicinal herb, *Atalantia buxifolia* (Moran 1978; Korsten et al. 1996; Garnier et al. 2000; Hung et al. 2001; Li and Ke 2002; Zhou et al. 2007; Deng et al. 2008). Members of other plant genera may be infected experimentally by the parasitic plant *Cuscuta* spp. (dodder), albeit at a lower titer in some instances than in citrus hosts, and include tobacco (*Nicotiana xanthi*), tomato (*Lycopersicon esculentum*) and perennial (*Catharanthus roseus* (Ke et al. 1988; Bové 2006; Zhou et al. 2007; Francischini et al. 2008; Duan et al. 2008).

The causal agent of HLB is a phloem-limited, Gram-negative, fastidious prokaryote of the phylum *Proteobacteria*. In 1994, Jagoueix et al. defined the ‘Candidateatus’ generic name ‘Liberobacter’. However, the nomenclature of the species was modified to ‘Liberibacter’ by Garnier et al. in 2000. Although the bacterium has not been cultured and Koch’s postulates never satisfied, three species have been proposed based 16S rDNA analysis, namely ‘*Candidateatus Liberibacter asiaticus*’, ‘*Ca. L. africanus*’ (da Graça 1991; Planet et al. 1995), and ‘*Ca. L. americanus*’ (Teixeira et al. 2005), which occur in Asia, Africa, and Brazil, respectively. The disease elicited by these three forms of the pathogen differs by a combination of environmental conditions and insect vectors. Those HLB infections caused by the heat-tolerant forms of *Ca. Liberibacter asiaticus* and *Ca. L. americanus* are vectored by *Diaphorina citri*. Symptom expression occurs under both warm (27 to 35°C) and cool conditions (20 and 24°C). The presence of *Ca. Liberibacter asiaticus* was confirmed in Florida along with the psyllid vector, *D. citri* (Halbert 1998; Bové 2006). The African form of HLB, caused by *Ca. L. africanus*, is a heat-sensitive form that is vectored by the psyllid, *Trioza erytreae*. Symptoms are expressed only under relatively cool conditions (Bové et al. 1974). Heat tolerance of both psyllid vectors corresponds to that of the HLB forms. *D. citri* thrives in hot, low-lying areas of south and south-east Asia, eastern Iran, Saudi Arabia, Argentina, Brazil, Honduras, Mexico, Texas, Florida, the Bahamas, Cuba, Puerto Rico, Costa Rica and Guadeloupe (Knapp et al. 1998; Cermeli et al. 2000; French et al. 2001; da Graça and Korsten 2004). The African *T. erytreae* prefers cooler conditions at elevations ≥ 500 m above sea level (Bové 2006; Le Roux et al. 2006).

Nucleotide sequence analysis of 16S rDNA of HLB strains from Asia (Poona strain, India) and Africa (Nelspruit strain, South Africa) in the early 1990s showed similarity of 97.7% between the strains (Jagoueix et al. 1994). Similarly, later reports in 2000 suggested high sequence identities (99.4-100%) between Asian strains (Okinawa, Irimote, Indo-China, Thailand, Nepal and the Philippines), but lower sequence identities with African strains (97.5%) (Subandiyah et al. 2000). More recently, Coletta-Filho et al. (2005) reported on two distinct strains of HLB bacteria, designated as LSG1 and LSG2, in Sao Paulo, Brazil. LSG1 was 100% identical to Asian strains, whereas the LSG2 strain shared high similarities of 95 to 96% with African strains. The American strain shares 93.7% and 93.9% similarities with the African and Asian forms, respectively (Coletta-Filho et al. 2005). Sequences of cloned fragments of the genomes of an Indian strain (Poona) have been reported (Villechanoux et al. 1995). In general, the organization of the fragment containing the *nisG-rpK* operon has proven to be similar to that of *Eubacterium* and is 74.2% similar to the same region in an African strain (Nelspruit) (Planet et al. 1995). Sequences of another fragment of the Indian HLB, not found in an African strain of HLB, resembles that of a DNA polymerase of bacteriophage SPO2. Given that field diagnosis of HLB is difficult because of the non-specific nature of foliar symptoms and the similarities to nutrient deficiencies, probes and primers designed to the *nisG-rpK* operon or to the ribosomal protein genes *rplA* and *rplB* are used for confirmation of HLB Liberibacter, or to differentiate between the African and Asian strains, respectively (Jagoueix et al. 1996; Hochquell et al. 1997).

As with CC and CVC, sound integrated-pest-management strategies are recommended for the management of HLB. Establishment of orchards with disease-free materials, diligent scouting of citrus and non-citrus hosts for the identification and removal of infected branches or trees, and a psyllid control programme employing insecticides or biocontrol agents have proven effective in some regions (da Graça and Korsten 2004).

### Citrus stubborn

HLB-like leaf symptoms, along with misshapen fruits that do not colour at the stem end, are induced by the pathogen *Spiroplasma citri*, on citrus. Similar to HLB bacteria, *S. citri* attacks the phloem of citrus trees. Young trees are often severely stunted, with dense, abnormally upright leaves. Symptoms are less conspicuous on mature trees, but fruits of poor quality are produced and yields are reduced (Timmer 1999). Off-season flowering is common, resulting in various ages and sizes of fruit on trees. The disease, referred to as citrus stubborn (CSD) is, however, rarely lethal to citrus and symptom expression is more predominant under warm weather conditions (Bové and Garnier 2000). All major commercial citrus species are susceptible to CSD. Other rutaceous hosts include *Fortunella* spp. and interspecific rootstock hybrids, such as citranges (*C. sinensis × Poncirus trifoliata*). *P. trifoliata* is often asymptomatic when infected (Bové and Garnier 2000). Many other cultivated or wild plants are also hosts of *S. citri* and include members of the families Amaranthaceae, Apocynaceae (*Catharanthus roseus*), Chenopodiaceae, Brassicaceae (*Armoracia rusticana*) and Plantaginaceae. CSD is an important disease in several hot and arid regions of southwestern US, most of North Africa, the eastern Mediterranean Basin, and the Middle East (Bové and Garnier 2000). Neither mechanical nor seed transmission of *S. citri* has been demonstrated. Natural spread is via leafhoppers (*Scaphytopius nitrades, Circulifer tenellus*) and is apparently controlled by the interac-
tion of *S. citri* with two leafhopper glycoproteins (Killiny *et al.* 2005). Management of CSD is achieved by the use of disease-free budwood.

*S. citri*, the type species of the genus *Spiroplasma* (Spiroplasmataceae, Mollicutes), is a wall-less eubacterium, with helical morphology and motility (Woese 1987; Weisburg *et al.* 1989). Ye and coworkers determined the genomic map by restriction fragment analysis in the 1990s (Ye *et al.* 1992). The size varies among strains, ranging from 1.6 to 1.9 Mbp, and is characterized by numerous extrachromosomal elements (Ye *et al.* 1995). These plasmids encode for proteins involved in DNA-element partitioning, cell-to-cell DNA transfer and insect transmissibility. Presumably, they contribute to efficient transmission by different insect vectors to various plant hosts and thereby assist in the rapid adaptation of the mollicute (Calavan and Bové 1989).

**Virus and Virus-like Diseases**

Citrus is host to over 40 virus, virus-like and presumed virus pathogens (Timmer *et al.* 2000). However, three pathogens, *Citrus tristeza virus*, *Citrus psorosis virus*, and *Citrus leprosis virus*, responsible for the diseases tristeza, psorosis, and leprosis, respectively, are considered economically important and cause major losses in important citrus-growing regions. Tatterleaf, exocortis and cachexia are also quarantine risks.

**Tristeza**

*Citrus tristeza virus* (CTV), a phloem-limited virus, is known to infect, naturally, all citrus species, hybrids or cultivars, and other closely related *Citrus* and *Fortunella* genera of the Rutaceae family. Experimental hosts of CTV include *Aegle, Aeglopsis, Afraegle, Citropsis, Clausena, Eremocitrus, Heesperthusa, Merrillia, Microcitrus, Pamburus, Pleiospermum* and *Swinglea*. Experimental infection of non-citrus hosts, *Passiflora* spp., has been achieved (Yoshida 1996; Lee and Bar-Joseph 2000). Symptoms of CTV are dependent on environmental conditions, citrus species and scion-rootstock combination and virus strains (Garnsey *et al.* 1987). The four major strains of CTV include mild, decline-inducing, stem-pitting and seedling-yellows strains (Roistacher 1991). Mild strains induce no discernable symptoms in citrus. Decline strains cause tristeza disease on sweet oranges, mandarins, grapefruits, kumquats and limes propagated on sour orange or lemon rootstock. Infection results in quick decline (Fig. 4), wilting and death of the tree. Girdling at the bud union (Fig. 4) due to non-functional phloem is accompanied by a reduction in the root system, dull-green or yellow foliage, leaf drop, twig dieback and the production of unmarketable, small, pale-colored fruits. All citrus cultivars are affected by stem-pitting strains of CTV, regardless of the scion-rootstock combination. Unlike decline strains, infection with stem-pitting strains does not generally culminate in tree death, but results in poor vigor, the production of small fruits containing little juice and chronic yield reductions. Presumably, interruptions in meristematic activity in areas of the cambium cause irregular radial growth and local depressions or stem pitting (Fig. 4). In extreme cases, there is the distribution of deep pits and longitudinal depressions in the bark, distortion and twisting of the main branches and the development of many fine pits. Moreno *et al.* (1989) have documented changes in protein profiles, in particular, a 20-kDa protein in the bark of infected sour orange rootstock. Additionally, Gandía *et al.* (2007) have illustrated differential changes in gene expression between 'Mexican lime' plants infected with severe (T305) or mild (T385) CTV strains. While no significant change in gene expression has been observed in response to T385 in transcriptome analysis using a citrus microarray, a 28% increase in expression of genes involved in stress and defense has been noted in plants infected with T305. On the virus side, the molecular determinants for symptom expression have not been defined, but expression of the virus coat protein in a number of citrus species induces CTV-like symptoms (vein-clearing, leaf epinasty, apical necrosis and stunting). The fourth CTV strain, seedling yellows, induces a disorder that causes stunting and chlorosis (Moreno *et al.* 2008). Of the four strains, stem-pitting and decline strains are the most economically important.

CTV is a member of the *Closteroviridae* family. The virus consists of a monopartite positive-sense, single-stranded, RNA genome comprised of 19,296 nucleotides encapsidated in flexuous, rod-shaped particles of 2,000 × 11 nm (Bar-Joseph *et al.* 1989; Karasev 2000). Two capsid proteins, of 25 and 27 kDa, coat approximately 95 and 5% of the particle, respectively (Pappu *et al.* 1989; Febres *et al.* 1996). The virus uses three replication strategies (proteolytic processing, translational frameshifting, and formation of subgenomic RNAs) and, at any one time, there are usually more than 30 different species of RNA in an infected cell (Hilf *et al.* 1995; Mawassi *et al.* 1995; Karasev 2000; Che *et al.* 2001; Gowda *et al.* 2001, 2003). In addition to genomic and subgenomic RNAs, multiple defective RNAs (D-
The complete nucleotide sequence of CTV genomic RNA has been reported for mild and severe isolates from Florida, i.e. T36 and T30 (Pappu et al. 1994; Karasev et al. 1995; Albiach-Marti et al. 2000), a severe isolate from California i.e. SY568 (Yang et al. 1999) and Israel (VT, Mawassi et al. 1996), mild and severe isolates from Spain, i.e. T385 and T318A (Vives et al. 1999; Ruiz-Ruiz 2006), a seedling-yellow isolate from Japan, i.e. NUagA (Suastik et al. 2001), and an isolate from Egypt (Qaha, GenBank access number AY 340974). Low genetic variation exists between T36 and Qaha as well as T30 and T385 isolates. The genome of SY568, however, appears to have been generated from recombination between T385 or T30 and an unrelated genotype (Vives et al. 1999, 2005). T318A sequence shows high nucleotide identity with the severe isolates SY568R (98.4%) and NUagA (97.9%) and lower identity values with VT, T385 and T30 and T385 respectively. Apparently, the isolates from different geographical locations share a common ancestor and high selective pressure on the virus genome maintains a repertoire of genotypes with appropriate fitness for survival (Ruiz-Ruiz et al. 2006). Previous genetic analysis of the 5’ half of the genome of over 300 CTV isolates from 30 citrus-growing regions indicated grouping of isolates into (i) T30 and VT genotypes and (ii) T385 and T36 genotypes (Hill et al. 1995).

Spread of CTV by propagation of virus-infected planting materials accounts for most CTV introductions in a country, whereas local spread is primarily by vector transmission (Moreno et al. 2008). CTV is transmitted by seven aphid species, Aphis gossypii, Aphis spiraecola, Aphis craccivora, Toxoptera aurantii, Myzus persicae, Macrosiphum euphorbiae and Toxoptera citricida, in a semi-persistent manner (Bar-Joseph et al. 1983). The latter is the most efficient vector, especially of severe strains (Rocha-Peña et al. 1995; Karasev 2000). A. gossypii is the second most efficient vector of CTV and is the major vector in the Mediterranean basin and areas of North America (Cambra et al. 2000). However, the two species cause different patterns of virus spread, presumably related to their feeding habits. A. gossypii is a migrating vector, whose movements to trees several meters away is affected by wind, while T. citricida is the semi-persistent vector to adjacent trees (Gottwald et al. 1996). Increases in CTV of 5 to 95% in 8 to 15 years between random trees several meters apart have been documented in regions where A. gossypii was predominant. In contrast, similar increases in disease have been observed in a shorter period of 2 to 4 years with spread between trees in close vicinity (Gottwald et al. 1996, 1998; Hughes and Gottwald 1998; Gottwald et al. 1999). Other than the presence of aphid species, factors affecting the rate of CTV spread include aphid population density, environmental conditions, such as moderate temperatures, irrigation, and fertilization that results in new flushes, and citrus cultivar (Moreno et al. 2008). However, in some countries, such as Israel, a lag period of more than 30 years has been observed between the introduction of CTV and noticeable field spread of the virus, which would indicate the requirement of an adaptation period of virus strains to the local vector (Bar-Joseph 1978).

CTV is distributed worldwide in tropical citrus-growing regions such as North, Central and South America, the Caribbean, Asia, Africa, Oceania and the EU (Bové and Vogel 1981; Roistacher 2004; Roye et al. 2007). Traditionally, CTV diagnosis has been performed by biological indexing on sensitive citrus indicators, such as ‘Mexican lime’ (C. macrophylla). ‘Mexican lime’ seedlings exhibit vein clearing, leaf cupping, reduced internodes, and stem pitting upon CTV infection. Grapefruit, sour orange and sweet orange are used to differentiate between mild, decline, and stem-pitting inducing strains (Roistacher 1991). Diagnostic symptoms include stunting and small yellow leaves in grapefruit and sour orange. Pitting is observed in the stems of grapefruit and sweet orange. Iodine testing on plants for starch depletion and microscopic detection for inclusion bodies have also been used (Garnsey et al. 1980; Beleksky 1988; Roistacher 1991). Serological detection using ELISA techniques with polyclonal and monoclonal antibodies specific for CTV strains are routinely used today (Perrin et al. 1990; Garnsey and Cambra 1991). Molecular techniques such as RT-PCR, hybridisation and dsRNA patterns are also employed in the detection of CTV (Dodds et al. 1987; Sekiya et al. 1991) including the differentiation between mild and severe strains (Mehta et al. 1997; Huang et al. 2004). CTV is the most destructive and economically important virus affecting citrus varieties worldwide. It has been responsible for the death of over 50 million trees worldwide and has affected citrus production significantly especially in regions where widespread use of the most susceptible rootstock, that is sour orange, occurs (Rocha-Peña et al. 1995). Hence, quarantine measures have been put in place to limit the spread of the virus into regions where it is absent or only the mild strains are present. Other precautionary measures include the introduction of official virus-free certification programs for monitoring local distribution of citrus materials, prohibition of importation of citrus varieties from countries with CTV and treatment of exported fruits for pests in order to prevent the introduction of the vector into other countries. Surveys for CTV using ELISA detection are encouraged at regular intervals to detect new outbreaks of the disease (Navarro et al. 1984, 1988; Frison and Taher 1991). There is no single effective strategy for managing the dreaded disease once CTV has been introduced into a region. Current methods employed include mild-strain cross-protection; the use of biological-control agents of the vector, tolerant cultivars, chemical control of vectors and clean stock or certification programs (Rocha-Peña et al. 1995; Lee and Bar-Joseph 2000; Walker and Hoy 2003). Although genetic resistance is the most effective way to manage the disease, the complex reproductive biology of citrus has impeded the genetic improvement of the crop through conventional breeding methods (Peña et al. 2007). As a result, several research groups have examined pathogen-derived resistance and direct genetic manipulation of citrus for the development of transgenic resistance against CTV. Varying levels of resistance have been reported with transgenic plants (Citrus aurantifolia) carrying the coat protein p25 following exposure to the virus via aphid and graft inoculations. The phenotypes included no-symptom expression, or delay in symptom expression and virus accumulation (Dominguez et al. 2002). Resistance has also been obtained against non-homologous strains (Dominguez et al. 2002). However, other transformation experiments with citrus (Citrus aurantifolia) transgenic for the silencing suppressor and pathogenicity determinant, p23 (Lu et al. 2004) have resulted in developmental abnormalities in the host, closely resembling virus-like symptoms (Fagoaga et al. 2005). Subsequent studies with similarly transformed citrus showing normal phenotypes have yielded strong levels of resistance against CTV (Fagoaga et al. 2006). Transgenic plants that produce dsRNA transcripts of p23 transcripts have not provided resistance against CTV (Batuman et al. 2004). Whether or not the use of transgenic plants could be an alternative strategy for managing CTV in the field remains to be tested.

Psorosis

Citrus psorosis virus (CPsV) is responsible for the first and longest-known virus disease of citrus. It is speculated that the virus originated in Asia and was spread worldwide through the distribution of citrus planting materials (Roistacher 1991).
Two species differing in virulence have been described; psorosis A, the more common form which induces a mild form of the disease and the more severe form, psorosis B. The latter strain has been referred to over the years as *Citrus ringspot virus*.

Bark scaling is the major and diagnostic symptom in mature (> 10 years) orange, grapefruit and occasionally mandarin field trees infected with psorosis A (Fig. 5). Gumming later appears and, in advanced stages, accumulates below the scales, impregnating the xylem which results in the staining of the wood of the main trunk and limbs and vessel occlusion. Leaf symptoms may also occur as chlorotic flecking on young leaves on new flushes (Fig. 5), but they gradually fade as the leaves mature (Roistacher 1993; Derrick and Barthe 2000). Symptoms of psorosis B, the more aggressive form of the disease, include persistent chlorotic flecking on leaves with gummy pustules on the under side, extensive bark scaling of the trunk and secondary branches, and, occasionally, ringspot blemishes on fruits. Neither form kills citrus trees, but afflicted trees exhibit reduced canopies and twig dieback and produce fruits with reduced weights, especially when infected with psorosis B (Fawcett and Klotz 1938; Klotz and Fawcett 1941; Fawcett and Bitancourt 1943). Disease development is slow in citrus, with symptoms being manifested several years after exposure to the CsPV. Bark-scaling symptoms are often confused with those of Rio Grande gummosis and leprosis. However, gum exudation is more copious and eruptive with Rio Grande gummosis than those of Psorosis, and more localized lesions are formed in leprosis-infected trees.

CsPV affects a wide experimental host range, including the plant families Amaranthaceae, Apocynaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae-Papilionoideae, Pedaliaceae, Rutaceae, Solanaceae (Timmer et al. 1978). CsPV is mainly spread by propagation of infected planting materials, but observations in Argentina, Texas and California implicate an unknown vector (Timmer et al. 2000). The single-stranded negative-sense RNA genome is divided into three segments; (i) RNA1 of 8,184 nucleotides which has two ORFs, one of which potentially encodes an RNA-dependent RNA polymerase, (ii) RNA2 of 1,650 nucleotides, which codes for a putative 54 kDa protein of unknown function, and (iii) the smallest component, RNA3 of 1,454 nucleotides, which encodes for the coat protein (Barthe et al. 1998; Sánchez de la Torre et al. 1998, 2002; Naum-Ongania et al. 2003). Genetic analysis of the RNA species of 22 isolates from Argentina, California, Florida, Italy and Spain has revealed three clades; (i) group I, comprising isolates from California, Florida, Italy and Spain; (ii) group II, the Argentian isolate, which consists of a homogenous population, and (iii) group III, which is distant from the other two groups and which contains a single isolate from Texas (Martin et al. 2006). Martin and coworkers (2006) have suggested that the populations examined have been shaped by gene flow between countries, genetic exchange, and the effects of selection for amino-acid conservation.

Psorosis is controllable by budwood certification. Indexing is generally conducted by graft-inoculating citrus indicator plants (sweet orange, mandarin, sweet tangor) and observing the resulting flecking of young leaf leaves (Roistacher 1993; Derrick and Barthe 2000). Quicker methods available are serological, such as double-antibody sandwich (DAS-ELISA), triple-antibody sandwich (TAS-ELISA) and TAS-ELISA-HRP (triple-antibody sandwich linked to horse-radish peroxidase) (Garcia et al. 1997; Alioto et al. 1999; Zanek et al. 2006). Primers designed to the coat protein gene are also used for RT-PCR diagnosis (Garcia et al. 1997; Barthe et al. 1998). TAS-ELISA and RT-PCR have proven to be the more reliable tests for detecting the virus in field trees (Barthe et al. 1998; Legarreta et al. 2000; Martin et al. 2002). Shoot-tip grafting, thermotherapy (40°C for 16 hours with lights and 30°C for 8 hours in the dark), and somatic embryogenesis can be used for the elimination of CPsV from propagative materials (D’Onghia et al. 1997; Derrick and Barthe 2000; Roistacher 2004). Transgenic materials have not provided resistance against the virus thus far. Transgenic sweet orange plants carrying the coat protein gene of CPsV from Texas failed to confer resistance against the homologous isolate when graft-inoculated under greenhouse conditions. A combination of factors, including a high dose of challenge virus, variable transgene expression and resistance-breaking proteins of the challenge virus have been cited to be responsible for the reaction of the transgenic plants (Zanek et al. 2008).

**Leprosis**

Citrus leprosis (CL) or scaly bark is an economic disease of mainly sweet orange and mandarins (*C. reticulata*, *C. reshmi*, *C. deliciosa*). The disease is characterized by the formation of local lesions on citrus leaves, stems and fruits. Leaf lesions of 2 to 3 mm in diameter are initially chlorotic and later become necrotic in the center, but maintain a chlorotic halo. Larger lesions of 10 to 20 mm may develop due to coalescence of smaller leaf lesions. Lesions on immature fruits develop similarly to the leaf lesions, but they become

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**Fig. 5 Leaf (A, B, C) and bark symptoms (D) of psorosis. (Photos: Jose Guerri)**
depressed and are associated with gum exudation. Protruberant, necrotic lesions develop on stems. Coalescence of numerous stem lesions leads to death of the twig, premature fruit drop, and leaf abscission. In 2006, de Andrade Maia and de Oliveira (2006) investigated whether plants commonly used as hedge rows or windbreaks could serve as alternative hosts for CL. A number of weeds found in citrus orchards were also included in the study. Mites reared on infested with mites from different hosts (Magome et al. 2003) developed the first, specific, molecular, diagnostic tool for the detection of cytoplasmic-type CiLV. Two primers were designed to amplify the movement protein and replicase of CiLV in RT-PCR. The use of both primers in assays was recommended to reduce false negatives and to guarantee reliability of detection. Management of the disease employs the use of resistant citrus cultivars, windbreaks, control of weeds that may be reservoirs for the vector (and possibly for the virus), pruning to reduce disease inoculum and seasonal acaricide sprays to manage mite populations (Childers et al. 2003; Rodrigues and Martinez 2000). Alternative strategies for managing mite populations involve the preservation of entomopathogenic fungi and predaceous mites (Phytoseiidae) in citrus orchards (Bastianel et al. 2006; Chen et al. 2006).

**Tatterleaf**

The virus formerly known as Citrus tatterleaf virus (CTLV or CTLV) is presently classified with the Apple stem grooving virus (ASGV) of the family Closteroviridae and genus Capilloivirus (van Regenmortel et al. 2000). Since literature on the virus is mainly under the former acronym, the virus will be referred to as CTLV.

CTLV has long filamentous particles with a modal length of 600–700 nm and a width of 12 nm. Its genome is made up of a single species of single-stranded RNA (~6,500 Kb) with two overlapping ORFs (Yoshikawa et al. 1992; Ohira et al. 1995). The virus is believed to have originated in China, and to have been imported into the USA in 1962 in latent infected ‘Meyer lemon’ materials and subsequently distributed thereafter to other countries. CTLV has been reported in Japan, Africa and Australia, and is restricted to Morocco in the Mediterranean (Marais and Lee 1986; Miyakawa and Ito 2000), in citrus and non-citrus hosts. Complete genome sequences of isolates from kumquat in Taiwan (GenBank accession number AY646511) and lily in Japan (Ohira et al. 1995) have been reported. Phylogenetic analysis show large divergence between CTLV isolates from different hosts (Magome et al. 1997; Hilf 2008).

CTLV causes the disease tatterleaf or cricnge stunt on many citrus species, cultivars, and hybrids (Adams et al. 2004). Although the virus is symptomless in most citrus species (C. sinensis, C. aurantium, C. paradisi, C. reticulata, and C. limon), it causes bud-union abnormality or flushing when these varieties are grafted onto a trifoliate or trifoliate hybrid rootstock. The scion can easily be severed at the bud union under high wind conditions (Roistacher 1991). Stems of afflicted citrus plants may also be deformed and exhibit a zig-zag pattern of growth in chloride regions. Plants also generally show stuntling. Common foliar symptoms include chlorotic spotting or mottling and leaf deformities or tattered leaves, the symptom after which the disease is named (Garsey 1964). CTLV can be transmitted to 19 herbaceous hosts, including Chenopodium quinoa, Vigna unguiculata, Phaseolus vulgaris and Nicotiana spp. Typical symptoms displayed on these plants, respectively, are chlorotic lesions and a systemic mottle, local lesions and variable systemic necrosis, and mild necrotic etching (Roistacher 1991). Spread of the disease agent is purportedly by mechanical transmission via pruning tools or razor cuts. No invertebrate vector has been implicated in the transmission of the disease.
spread of the virus (Miyakawa and Ito 2000).

CTLV can be detected by DAS-ELISA using antisera developed in Japan, Germany and the United Kingdom. Molecular techniques, such as RT-PCR and immunocapture RT-PCR, have also been successfully used to detect CTLV (Magome et al. 1997; Hilf 2008). Biologically, CTLV can be detected by grafting infected tissue onto rusk citrange (C. exzela), citrange or citron seedlings (Roistacher 1991).

CTLV is symptomless in economically important crop plants (de Noronha Fonseca et al. 2005; Rizza et al. 2007; Benett et al. 2008; Wang et al. 2008). The etiological agents, Citrus exocorticis viroid (CEVd) and Hop stunt viroid (HSVd) synonym: citrus viroid [CVd]-II, classified in the family Pospiroviridae, of the genera Pospivirion and Hostuviroid, respectively, are pathogenic to all citrus species and varieties (Frison and Taher 1991; Flores et al. 2001). CEVd-infected sweet orange, grapefruit and mandarin (C. mandarina cv. 'Tangelo' indicator species and 'Parson’s Special' mandarin, respectively. Symptoms are generally visible on Tangelo within 2 to 6 months and 1 year on 'Parson’s Special' mandarin (Lee et al. 2002). Biophysical techniques such as gel electrophoresis, hybridization and RT-PCR are also commonly used in the detection of these infectious agents, but their sensitivity depends on viroid titers in plant tissues (Duran-Vila et al. 2000).

**Exocortis and cachexia**

Viroid diseases of exocortis and cachexia are also prevalent wherever citrus is grown (Wallace 1978; Roistacher 1991; Kyriakou 1992; Najar and Duran-Vila 2004; Ragozzino et al. 2006). Exocortis or the citrus root nematode, and Radopholus citrophilus (Loof 1991) (synonym R. similis), the burrowing nematode of citrus, are considered to be preponderantly responsible for damage to Citrus spp. While T. semipenetrans is a widely occurring nematode that is found at every location where Citrus spp. are grown, R. citrophilus affects citrus crops only in Florida. T. semipenetrans causes the condition referred to as ‘slow decline of citrus’ (SDC), whereas R. citrophilus causes the condition called ‘spreading decline’ (SD). From as far back as 1913, there is evidence that T. semipenetrans is pathogenic to citrus (Siddiqui 1973). SD has been known since 1928, but was not linked to the nematode until 25 years later (Orton Williams and Siddiqui 1973).

**NEMATODE DISEASES**

Eight genera of plant parasitic nematodes of destructive potential are associated with various citrus crops (Hutton et al. 1982; Duncan 1999). However, two nematode species, Tylenchulus semipenetrans, commonly called the citrus nematode or the citrus root nematode, and Radopholus citrophilus (Loof 1991) (synonym R. similis), the burrowing nematode of citrus, are considered to be preponderantly responsible for damage to Citrus spp.

**Slow decline of citrus**

SDC is characterized by nonspecific indicators of inadequate roots, which include a general reduction in tree growth and vigor, yellowing, shedding of leaves, and undersized fruits. The decline symptoms are often more pronounced in the tops of the trees. Below-ground, there is poor growth of feeder roots and the adherence of soil to roots in regions of accumulated gelatinous nematode egg masses. Overall, the symptoms are very similar to those elicited by biotic and abiotic factors that impair root function. SDC is not lethal to citrus and, as the name of the disease suggests, there is a gradual increase in unthriftiness of trees. Additionally, decayed roots succumb to invasion by secondary organisms (e.g., Fusarium spp. and Sclerotium spp.), resulting in the development of lesions or cortical sloughing and root death under light or heavy infestations, respectively (Kaplan and Duncan 2000b; Duncan 2005).

A direct relationship between nematode population density and tree health has been documented. It has been found that as much as 5% loss in weight of citrus seedlings is attributable to every 1000 T. semipenetrans in the soil. Disease severity is influenced by various soil and soil nutrient conditions. Citrus in soils with 10-15% clay show more severe decline from infestations by T. semipenetrans than citrus in soils with lower or higher proportions of clay. Dry, fine-textured soils favor the nematode, but damage to trees is more pronounced in wet soils. In addition, when calcium, sodium, or potassium concentrations in soils are borderline or unfavorable for citrus, the nematode is more damaging than when occurring in soils of good nutrient status (van Gundy et al. 1964; Bello et al. 1986; Masheka et al. 1992; Navas et al. 1992). As in other cases, the physiology of the entire tree is influenced by nematode-induced root damage, and crop loss is accentuated under conditions of stress (Siddiqui 1973; Droopin 1980). Estimated yield losses of up to 30% are due to SDC (Cohn 1972; Tarjan and O’Bannon

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1974; Timmer and Davis 1982; Childers et al. 1987; Duncan 1989; Greco et al. 1993; Duncan et al. 1995). *T. semipenetrans*, the causative agent of SDC, is a sedentary endoparasite. The females are obligate parasites and their development depends on successful establishment and maintenance of feeding sites within the root cortex. Reproduction generally occurs by parthenogenesis despite the presence of male nematodes. Both males and second-stage juveniles infect and feed by puncturing the root cortex. The latter, as well as third- and fourth-stage juveniles, feed on the hypodermal cells of citrus roots while young female adults penetrate deeper into the root cortex, leaving a half of their bodies extending outside the root. Between 75 and 100 eggs are laid in gelatinous masses in nurse cells by young adult females. Although development to the second-stage juvenile occurs within the egg, this stage hatches in free soil water. Upon hatching, the second-stage juveniles then attack citrus roots. Adult males do not penetrate roots. They pass through three molts without feeding and attain maturity within 12 days of hatching. Spread of the nematode pathogen is mainly via infested seedlings and rootstocks (Siddiqi 1974; Kaplan and Duncan 2000b; Skaria 2004).

Based on differential host tests, three biotypes of the *T. semipenetrans* have been described on citrus (Baines et al. 1983; Duncan et al. 1988). The ‘Citrus’ and ‘Mediterranean’ biotypes reproduce on *Citrus* spp. and grapes; they do not reproduce on trifoliate orange, and only the ‘Mediterranean’ biotypes do not infect olive. ‘Citrus’ races are distributed throughout the USA and Italy while the ‘Mediterranean’ races are located in the Mediterranean, South Africa, and India (Duncan 2005). The ‘Poncirus’ biotype, on the other hand, is found in California, Florida, Israel, Japan and South Africa and infects *Citrus* spp., including trifoliate citrus, and does not reproduce on olive. Presumably the distribution of the biotypes correlates with the predominant rootstock. Limes, rough lemon and sour orange are more commonly used than *P. trifoliata* worldwide, except in Japan (Duncan 2005; Kwaye et al. 2008).

Sanitation is important to protect against the introduction of the pathogens of SDC on seedlings at planting. The citrus nematode is easily transported through infested seedlings and rootstocks. Nurseries should, therefore, never be established on or near to old citrus orchards and nursery soil should be sterilized before planting. Care should be taken not to spread the nematode through tools, machinery and irrigation water from infested groves. Hot water or nematicide dips can be used to treat bare-rooted seedlings. In soils, the citrus nematode may occur as deep as 4 meters, so fumigation is not always effective. Initially, the most frequently used control measure in fields is the application of nematicides, either as preplant soil treatments or as drenches for established trees (Siddiqi 1973; Dropkin 1980), but mechanical root pruning is being used because of environmental concerns (Lee 2004). Damage thresholds vary between regions and are dependent on prevailing factors, such as nematode reproduction rate, susceptibility of the rootstock, climatic conditions, soil properties, presence of other pathogens and optimal management practices (Duncan and Cohn 1990; Duncan 1999). In California, treatments are recommended when populations exceed 400 females per gram of root in February or April or 700 in May to June. In South Africa, the figure is 100 females per gram of root whereas in Cyprus it is 20 juveniles/cm² of soil (Philis 1989; Le Roux et al. 2000; Sorribas et al. 2008). Rotation with annual crops for one to two years prior to planting with citrus has been found useful in reducing nematode populations. Knowledge of pathogen biotypes is also useful in the selection of resistant rootstocks for production as well as in breeding programs (Inserra et al. 1980).

**Spreading decline**

SD is restricted to citrus crops established in deep, sandy, well-drained areas of central Florida. The etiological agent, *R. citrophilus*, is a migratory endoparasite that enters the citrus root tip from the root cap to the region where epidermal cells have started to suberize. If the apical meristem is destroyed, terminal growth ceases. However, should terminal growth continue, lesions develop several centimeters away from the root tip and invasion via the root tip is not immediately apparent. Lesions of various sizes occur, which may coalesce to form large cankers with calloused margins. An infected tree may have approximately half as many functional feeder roots as a healthy tree. At depths between 25 and 75 cm, 25 to 30% of the feeder roots are destroyed; below 75 cm, 90% of feeder roots are destroyed. Above-ground symptoms of SD are similar to, but more pronounced than those of SDC. Trees infested with SD show sparse foliage, dead twigs with branching ends and dead limbs. Trees appear undernourished and leaves are small. Although blooming is profuse, few fruits set and these are usually small in size. SD causes yield reductions of 40 to 70% in oranges, and 50 to 80% in grapefruit. Above-ground symptoms usually appear one year after initial infestation of roots. Decline symptoms are generally apparent on a group of trees. However, with continued burrowing of the nematodes down and across the soil, the infested area continuously increases in size every year. This spread, in all directions (trees per year), is the most characteristic feature of the disease, hence its name. Parasitized, but healthy-looking trees occur one to three rows in advance of those showing visible decline. In Florida, minimal rainfall from February to May puts additional stress on trees already weakened by nematode infestations and affected trees will readily under drought conditions. During summer rainfall, trees recover partially, but never recover normal growth (O’Bannon 1982; Duncan 1999; Kaplan and Duncan 2000c).

After penetration of citrus roots, adult females of *R. citrophilus* lay eggs, which hatch in 2 to 3 days. Sexual reproduction is usual, but females can reproduce as hermaphrodites in the absence of males. Adult males do not feed. The nematodes remain within the root until overcrowding or extensive damage of cortical tissue necessitates migration. *R. citrophilus* in Florida is morphologically indistinguishable from *R. similis* that is pathogenic to banana worldwide, and it is regarded as the citrus race (DuCharme and Birchfield 1956). Based on biochemical, physiological and karyotypic differences, the taxonomic status of the citrus race was elevated to sibling species as *R. citrophilus* and in 1986, Siddiqi classified the sibling species as *R. similis* subsp. *similis* and *R. similis* subsp. *citrophilus* (Huettel and Dickson 1981a, 1981b; Huettel et al. 1982; Huettel 1983, 1984a, 1984b; Siddiqi 1986). Later in 1996, Kaplan and his colleagues demonstrated that the two sibling species of citrus nematodes from Florida, Puerto Rico, Belize and Hawaii using RAPD. However, the taxonomic status of the nematodes as races of *R. similis* is still recognized.

Initially, local quarantine measures were enforced for the management of SD to limit the spread of the nematode and to limit its establishment in unaffected groves within Florida. To date, all other citrus-growing areas in the USA, and elsewhere, have stringent quarantine and other regulations to prevent the spread of *R. citrophilus* via affected plant materials or growing media. The measures have been extremely successful, since SD has not been reported anywhere outside of the state of Florida. The measures adopted include the ‘push and treat’ method, the use of barriers to prevent the spread of the nematode, the use of nematode-free nursery stock and soil fumigants, certification programs, and the use of resistant or tolerant rootstocks (‘Milam lemon’, ‘Ridge Pineapple sweet orange’, ‘Kuharski Carrizo citrange’). More recent trials have shown some benefit of restricting the growth of the citrus roots across root free buffer zones (Duncan et al. 1990; Duncan 1999, 2005). Other strategies involve ways of encouraging optimum plant vigor through fertilization and irrigation.
Other nematodes

In addition to *T. semipenetrans* and *R. similis*, the lesion nematode (*Pratylenchus coffeae*) and sting nematode (*Belonolaimus longicaudatus*), are pathogenic to citrus. While the sting nematode is limited to Florida, the lesion nematode is prevalent in Florida, Brazil, Japan, India and Taiwan. *Pratylenchus coffeae* affects both young and mature trees throughout one or more growing seasons and causes above-ground symptoms similar to those of SD. However, the pathogen does not move as rapidly through the orchard as that of SD. Belonolaimus longicaudatus is an ectoparasite that feeds at the root tips of lateral fibrous roots of citrus trees resulting in the cessation of growth, formation of short stubby roots and severe stunting (Duncan 1999; Kaplan and Duncan 2000a; Duncan 2005; Han et al. 2006). More recently, a complex of root-knot nematodes, *Meloidogyne spp.*, was reported to be pathogenic on roots of *Citrus* spp. in China and India (Davis and Vinette 2004).

**ARTHROPOD PESTS**

Insect species belonging to various orders are associated with the flowers, shoots and roots of citrus. They often colonize more than one part of the tree, resulting in a multidimensional impact on the productivity and life of the citrus tree (Browning 1999). Infestations of citrus flowers and young fruits can result in scarred and fruit drop. Repeated puncturing of fruits result in discoloration, water loss, and reduced aesthetic quality. Eggs and feeding larvae within the fruit lead to spoilage, decreased soluble solids, altered juice content, color and flavor. Colonization of the leaves reduce vegetative growth, photosynthetic capacity and vigor. The trunk, the main structural component of the tree, is attacked internally and externally by insects that feed on wood or outer bark cells, respectively. Several of the arthropod species colonizing citrus have attained pest status in various citrus-cultivating countries of the world. While many of the insects cause direct damage to the plant, others serve as vectors of various diseases which are usually more debilitating to the plants than the direct effects of insect attack. Some of the insect pests that have attained international pest status and which are most injurious are discussed.

**Citrus leafminer**

The citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) is distributed worldwide, occurring in most regions where citrus is cultivated. *P. citrella* is endemic to India and other Asian countries. In the last 15 years, it has spread rapidly into other citrus-growing areas worldwide. The leaf-mining insect was first recorded in Florida in 1993 (Heppner 1993) and spread thereafter throughout the Caribbean and Central and South America.

*P. citrella* is a small, brownish-grey moth about 2 mm long. The hind wings are fringed with long hairs. The eggs, which look like tiny water droplets, are laid singly on the underside of leaves. They hatch within 3 to 5 days after which the young larvae immediately start burrowing under the surface of the leaf. The entire life cycle lasts about 2 to 3 weeks. The larvae are the economically important stage because of their tunneling activity just below the surface of citrus leaves. Three instars are completed at this stage. The mines have a characteristic silvery appearance and each larva is generally responsible for one mine. The mining activity usually causes leaves to curl (Browning 1999). Although leafminers primarily feed on tender new growth, they can occasionally be found on fruits and stems when their population densities are high. Concerns about citrus leaf miner population densities have surfaced because mines produced by larvae provide CC bacteria with access to ideal growing conditions in the citrus host (Graham et al. 1996; Chagas et al. 2001; Gottwald et al. 2001; Christiano et al. 2007). The feeding galleries result in lifting and tearing of the cuticle thereby exposing leaf mesophyll tissues to splash-dispersed or wind-blown, rain-dispersed CC bacteria.

Systemic insecticides have been used against the citrus leafminer with limited sustained success. As is the case with most pests, the shift is now towards a more integrated approach focusing on biological control. Several general predators, such as spiders, ants and lacewings, play an important role in suppressing the population of the citrus leafminer. Ageniaspis citricola, *Closterocerus cinctipennis*, *Horismenus sardis*, *Horismenus fraternus*, Elasmus tischiae, *Prinagalio minio*, and *Zagrammosoma multilineatum* have been reported as successful parasitoids of this pest and have the potential to play an integral role in its biological control (Michaud 2002; Lacey and Shaprio-Ilan 2008).

**Brown citrus aphid**

Of the several species of aphids associated with citrus, the brown citrus aphid (BrCA), *Toxoptera citricidus* Hemiptera: Aphididae, has the greatest economic importance at present. This is due to its efficiency as a vector of CTV (Herron et al. 2006). The pest, which is thought to have originated in South East Asia (Michaud 1998), is now widely distributed in several countries in Europe, Asia, Africa, North America, South America, Central America and the Caribbean (Rocha-Peña et al. 1995; CAB International 1998). This shiny reddish-brown to black aphid is usually 1.5-2.8 mm in length with a life cycle ranging from 8 to 29 days (Fig. 6). Females are parthenogenetic and a single generation develops in 6 to 8 days. They are capable of producing up to 30 generations per year, depending on temperature and the availability of food. BrCA feeds primarily on *Citrus* species and occasionally on other Rutaceae. Infestations result in distorted leaves, impaired shoot growth, arrested blossom development and blossom drop. However, the major impact of BrCA is due to the transmission of CTV (EPPO/CABI 1997a; OEPP/EPPO 2006).

Biological and chemical control strategies are most frequently used against BrCA and thus an IPM strategy based on these two control strategies is the preferred approach, with greater emphasis on the biological control component. There are several natural enemies of aphids, many of them also attacking BrCA; however, the level of successful parasitism is varied. Parasitoids that have been associated with the BrCA include *Lipolexis oreogena* (Hoy et al. 2007) and *Lysiphleba japonica* (Hymenoptera: Aphididae). Predators include the coccinellids *Cycloneda sanguinea* and *Harmonia axyridis*, the syrphid fly *Pseudodorus clavatus* and the lacewing *Chrysoperla plorabunda* (Michaud and Browning 1999; Michaud 2001, 2002). Several organophosphorus and organocarbamate insecticides have been recommended for use against BrCA; however, the adverse impact of these...
chemicals on the natural enemies of aphids requires that they be used judiciously and as a last resort.

Psyllids

*Diaphorina citri* (Hemiptera: Psylloidea: Psyllidae) has a very wide geographical distribution, with its presence being reported in Europe (France), several countries in Asia, Africa, North America (Florida), Central America, the Caribbean and South America (CAB International 2001; Halbert and Nunez 2004). Adult Asian citrus psyllids are relatively small, yellowish-brown insects (Fig. 7). The wings are transparent with white spots or light-brown with a broad, beige, longitudinal band in the center. The length of the body is about 2.5 mm. The eggs are orange-coloured and almond-shaped (0.31 mm long and 0.15 mm wide) and are laid singly inside half-folded leaves of the buds, in leaf axils and other suitable places on the young tender parts of the tree (Fig. 7). They hatch in 2 to 4 days. There are five nymphal instars, which are completed in 11 to 15 days (OEPP/EPPO 2005b). The total life cycle ranges from 15 to 47 days (Halbert and Manjunath 2004), but may be longer at low temperatures. While large populations of the psyllid feeding on young leaves may cause leaf curling and premature leaf fall, the economic importance of psyllids is based largely on their role in the transmission of the citrus disease caused by the HLB bacterium (EPPO/CABI 1996). Fourth- and fifth-instar nymphs and adults are capable of transmitting the etiological agent of Asian HLB.

*D. citri* is often confused with *Trioza erytreae* (Hemiptera: Psylloidea: Trioziidae), another plant-sucking hemipteran insect which is the vector of African HLB (CAB International 1998; Tamesse et al. 1999; OEPP/EPPO 2005c). *T. erytreae* primarily infests lime and lemon in eastern and southern Africa and mandarin in the Cameroon. It is widespread in sub-Saharan Africa, Madagascar, Mauritius, Réunion, Saint Helena, Saudi Arabia, Yemen, and Madeira (Portugal) (EPPO/CABI 1997c, 1998; Fernandes and Franco-quinho Aguiar 2001) and was recently detected in Tenerife and La Gomera (Islas Canarias, Spain) in 2002 (Padrón and Hernández 2002). Adult *T. erytreae* are light-brown insects about 4 mm in length, with relatively large wings and clearly outlined veins. Males have a blunt tip to the abdomen and are smaller than the females, which have a sharp point at the tip to the abdomen. They fly very well and the adults stand with the abdomen raised at an angle of about 35° to the feeding surface when feeding (Halbert and Nunez 2004). The eggs are yellow or orange, cylindrical, with an upturned, sharp, anterior point, and a short stalk that is inserted into the plant tissue. They are laid on leaf margins and along the midribs of young, tender, actively growing foliage (Annecke and Morán 1982). There are five nymphal instars. The nymphs are dorsoventrally flattened with a distinct marginal fringe of white, waxy filaments and they vary in colour being yellow, olive-green or dark grey. They are largely sedentary and form conspicuous colonies, settling on the underside of young leaves where, after a few days of feeding, they produce distinctive cup-shaped or pit-like, open galls (OEPP/EPPO 2005c). Late-instar nymphs and adults can transmit the causal agent of HLB to citrus (EPPO/CABI 1997c). Feeding damage caused by *T. erytreae* citrus includes leaf distortion, curling, stunting, galling and chlorosis (OEPP/EPPO 2005c). Several organophosphorus, organocarbamate and pyrethroid insecticides used against the Asian psyllid have had varying levels of success (McDaniel and Moran 1972; van den Berg 1990). The parasitoid, *Tamarixia radiata*, has also had different degrees of success in suppressing *D. citri* populations in different parts of the world (Chien et al. 1989) and is considered a suitable candidate for use in IPM programs. Recently, Boykin and coworkers (2007) investigated the genetic diversity of *D. citri* populations from Florida, Texas and Brazil using PCR microsatellite analysis. Twelve polymorphic microsatellite markers were developed. Allelic diversity ranging from three to eight alleles per locus and observed heterozygosities 0.014 to 0.569 and expected heterozygosities of 0.052 to 0.653 were obtained. It was concluded that the microsatellite loci can be used for assessing overall genetic variation and migration patterns for *D. citri*. Data on diversity of the insect species may contribute to further understanding of HLB transmission and parasitoid interactions (Boykin et al. 2007).

Organophosphorus insecticides such as dimethoate and monocrotophos are often used to suppress populations of *T. erytreae*. Cultural practices, involving the removal of alternate hosts from the vicinity of citrus fields, are also employed (van den Berg 1990). There is also the potential for biological control of this pest as at least two parasitoids (*Tamarixia dryi* and *Psyllaephagus pulvinatus*) are reported as having significantly suppressed populations of the pest (McDaniel and Moran 1972; van den Berg 1990).

Sharpshooters

Unlike the aphids and psyllid which are phloem feeders, Cicadellinae leathoppers (commonly named sharpshooters) are xylem-fluid-feeding insects, belonging to Auchenorrhyncha (Hemiptera). They have an inflated clypeus that encloses the strong musculature connected to the pumping diaphragm (cibarium), which enables these insects to feed on xylem at high negative tensions. These insects may serve as vectors of the bacterial plant pathogen, *Xylella fastidiosa*, which causes CVC of citrus and several other plants. The subfamily Cicadellinae includes the most important known vectors of *X. fastidiosa* and is taxonomically diverse, containing approximately 1,950 species. Members of this group vary greatly in size, with some species ranging from 3.4 to 4.5 mm in length, while others may be longer than 20 mm (Redak et al. 2004).

Young (1968, 1977, 1986) organized the subfamily in two tribes: Proconini and Cicadellini. Proconini species are restricted to the New World and are moderate to large in size, with some considered the largest leathoppers in Cica-
dellidae. Cicadellini is taxonomically larger and a cosmopolitan tribe, found in all zoogeographical regions of the world, but species richness is highest in the neotropical region. Among the Proconini and Cicadellini species of the New World, more than 90% are neotropical and around 64% are distributed in South America. In the Old World including Australia, another 83 genera and about 500 species of Cicadellini have been cataloged (Young 1986); a single genus and species (Cicadella viridis L.) is found in Europe (Redak et al. 2004).

Lime swallowtail butterfly

Of the several Papilio spp. that have been reported as pests of citrus, the lime swallowtail butterfly, Papilio demoleus Linnaeus (Lepidoptera: Papilionidae), is of greatest significance to the citrus industry at this time. The pest is widely distributed throughout southern Asia, from the Middle East to India, and from the Indo-Pacific region to New Guinea and Australia (Commonwealth Institute of Entomology 1979). Its presence in the New World was first reported in the Dominican Republic in 2004 (Guerrero et al. 2004), and later in Puerto Rico (Homziak and Homziak 2006) and Jamaica (E. Garraway, pers. comm.). Guerrero et al. (2004) recently documented similarities in morphological characteristics between P. demoleus from the Dominican Republic and Southeast Asia. Eastwood et al. (2006) later confirmed strong similarities between the two populations through sequencing of genetic markers (3′ end of mitochondrial cytochrome oxidase subunit I gene). A single haplotype has been identified, which suggests a single introduction of the species into the Dominican Republic. Various modes of introduction have been proposed: (i) that the insect arrived on its own accord, (ii) accidental introduction of early stages on citrus materials, and (iii) deliberate introduction for hobby interests or for release at celebrations (such as weddings). Irrespective of the mode of entry, P. demoleus is recognized as a major pest of citrus, causing significant losses in production in the Old World (Pakistan Agricultural Research Council 2003; Agribusiness Information Centre of India 2005; Homziak and Homziak 2006). Given its dispersal characteristics, life cycle, and favorable conditions in New World, it will very likely be established as a citrus pest throughout the Caribbean and adjacent mainland locations (Radke and Kandalkar 1988; Bhan and Singh 1997; Chatterjee et al. 2000; Pathak and Rizvi 2003).

The lime swallowtail butterfly is relatively large with a wingspan of 8 to 9 cm. Adults are usually distinguishable from other swallowtails by the basal yellow marks on the forewing and the large, blue eye-spot and the red spot on the hindwings (Fig. 8, Guerrero et al. 2004). The larvae are cream and dark brown in the early stages, becoming green in the later instars (Fig. 8). Mature larvae are about 3 to 4 cm long. It is the larvae that are responsible for the economic impact of this pest; they feed mainly on the young leaves of citrus plants and are therefore most dangerous under nursery conditions. Damage usually results in a loss of photosynthetic leaf area and slowed growth of the plant.

The use of chemicals is the control strategy most frequently employed against the larvae of the lime swallowtail butterfly. While several organophosphorus insecticides have been recommended against the larvae, there has been a move towards a more integrated approach, which allows natural control measures to maintain the pest population at acceptable levels, with interventions being made using selective insecticides. Some braconids have been reported as parasitizing the larvae of P. demoleus (Narayananamma et al. 2003) and biopesticides such as Bacillus thuringiensis, Beauveria bassiana and extracts of the neem plant have provided varying levels of control (Narayananamma and Savithri 2003). With its recent entry into the Caribbean, efforts are still being made to determine a suitable management strategy for the region.

Root weevils

Several species of root weevils (Coleoptera: Curculionidae) have been reported as having attained pest status in different parts of the world. These include Exopithacus spp. (fiddler beetles), Pachnaeus spp. (blue-green root weevils), Diaprepes spp. and Lachnopus spp. The adults of different species vary in size, ranging from 1.1 to 2.25 cm in length with varied colors. They may be black, light-green or cream with different colors of stripes. These beetles are characterized by their specialized, snout-like (prognathous) mouthparts. Citrus root weevils are predominantly tropical. About 150 species have been recorded in the Caribbean, Florida, Central and South America. Diaprepes abbreviatus (sugarcane rootstalk borer weevil) is native to the Caribbean and was introduced into Florida in the mid 1960s. Temperate species are found in the USA, Chile, Argentina, Australia, and New Zealand and include Pachnaeus spp. and Asymochus spp. (Hall 1995; McCoy 1999; Skaria and French 2001; Lapointe 2004).

Mating and the initial stages of the citrus root weevil life cycle occur in the tree canopy, but the remaining stages are completed in the soil. Eggs are usually laid between two leaves that are cemented together. On hatching, larvae fall to the soil, burrow into the soil and begin feeding on small roots. Later larval instars feed on larger roots. The fully-grown larvae move away from the root and pupate in the soil. After emergence, adults feed on buds and young leaves. Damage to the root system of citrus plants by the larvae of the citrus root weevil usually results in reduced water and nutrient flow, which often causes wilting of the plant and subsequent reduction in fruit size (Quintela et al. 1998; McCoy 1999). Secondary infection by root-rot fungi, such as Rhizoctonia solani and Sclerotium cepivorum may result in the death of young trees (Rogers et al. 1996; McCoy 1999; Graham et al. 2003). Infestations with D. abbreviatus often result in rapid tree decline and the decimation of entire orchards within a few years.

Initially, control of citrus root weevils was based on the application of insecticides to the soil for larval control. However, the lack of efficacy of this control method has led to the development of an integrated approach to the management of the pest with greater emphasis on cultural control, such as the replanting of old fields, crop rotation, manual collection of adult weevils and egg masses, and biological control. Several natural enemies have been used in classical or augmentative biological control programs against citrus root weevils. These include the egg parasitic wasp, Tetrastichus kaltiensis and Ufens spp. (Chalcidoidea), and the predaceous Crematogaster ashei (ant) and Battusoctus kelgani (mite). Larval parasites include Tetrastichus marylandensis and Pheidole spp. and entomophagous nematodes, Heterorhabditis heliothidis, Steinernema carpocapsae and Steinernema diaprepsi (Bullock et al. 1999; Nguyen and Duncan 2002; Stuart et al. 2004). Microbial infection of the citrus root weevil is considered a potentially viable control strategy and has been tried with varying levels of success (Lacey and Shapiro-Han 2003, 2008).
CONCLUDING REMARKS

The citrus industry worldwide continues to be an important one, even though it is plagued with many diseases and pests. Economic damage results when pests and disease outbreaks result in decreased fruit yield, quality, and generally a negative impact on the bottom line. Vegetative propagation, cultural practices, international travel, and worldwide exchange of germplasm have contributed to the spread of a number of diseases and pests from their centers of origin to other parts of the world. CTVs, CTVL, viroids, psorosis, tatterleaf and CSD are notable examples. Tristeza, tatterleaf and HLB are age-old maladies in Asia, the center of origin of citrus, whereas CSD originated in the Mediterranean region, which is a secondary center of origin for citrus. New, potentially destructive diseases of citrus have also emerged in regions other than the centers of origin as a result of the crop being exposed to new pathogens or pests, e.g. viroid diseases. Viroid infections of citrus most likely developed because of the planting of citrus groves in close vicinity to grapevines in the Middle East and Asia. Grapevines contain a variety of different viroids and have been cultivated in the Middle East and Asia for millennia (Bar-Joseph 2003). Weather-related factors have also contributed to the development of or have intensified the development of some diseases, such as CC, HLB and foot rot.

Although control of some diseases has been achieved, or the introduced pathogen has been successfully eradicated, albeit at great cost, for other pathogens, the feasibility of eradication is questionable, the possibility of reintroduction is a looming reality, and the issue is whether the country should be resigned to live with the disease. For instance, the industry in several Asian countries has lived with CC for many decades. Although some cultivars are no longer grown, production costs increased, and national and international markets lost (because of quarantines), production continues. Similarly, regions in which CTV is endemic have learnt to live with tristeza, employing replanting, certification and quarantine programs. Brazil has successfully been able to manage HLB, which is regarded as the most serious disease of citrus, through tree removal and insecticide treatments against the psyllid vectors (Bové 2006). The country’s success has also been attributed to favorable factors, such as the absence of tropical storms and hurricanes, which plague other countries year after year and which possibly play a role in the spread of the pathogen as well as the insect vector. In general, knowledge of the centers of origin, the diversity of citrus, their pathogens and pests, together with effective quarantine, certification programs and the use of healthy planting materials are necessary for the maintenance of productive citrus groves.

ACKNOWLEDGEMENTS

Support for work in our laboratory was received from The Principal’s New Initiative Fund, University of the West Indies, Mona (UWI); The School of Graduate Studies and Research (UWI); and principal’s New Initiative Fund, University of the West Indies, Mona

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In 2007, Citrus bacterial canker disease and citrus tristeza virus were discussed in detail. The book "Citrus Diseases and Their Management" provides comprehensive information on the biology, epidemiology, and management of various citrus diseases. The book emphasizes the importance of integrated pest management strategies to control these diseases. It also highlights the role of genetics in disease resistance and provides insights into the latest research in the field.

Relevant articles and publications include:

- "Pathogenicity in hop virus virus" in "Plant Pathology, Volume 60, Transgenic Crops IV" (2007).
- "Regulation of pathogenicity in hop virus" in "Annual Review of Plant Biology, 2005, 56: 281-310".
- "Evidence that Xylella fastidiosa is the causal agent of citrus tristeza virus" in "Phytopathology, 1999, 89: 1051-1060".
- "Diagnosis and management of virus and virus-like diseases of citrus" in "Molecular Plant Pathology, 2004, 5: 339-362".

The book also includes chapters on molecular biology and genetics, biological control, and host resistance. The information is presented in a clear and accessible manner, making it valuable for researchers, educators, and students in the field of citrus biology.

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