24. Graft-transmissible diseases of citrus

Characteristics of the pathogens, economic impact, and management strategies

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24.1 Characteristics of the pathogens

24.1.1 *Citrus tristeza virus* Genus *Closterovirus* (CTV)

Tristeza, meaning sadness in Spanish or Portuguese, was the name first given to a decline of trees on sour orange rootstock in South America. Tristeza was later shown to be caused by *Citrus tristeza virus* (CTV), a member of the closterovirus group within the *Closteroviridae*. Seedling yellows disease is a synonym used for tristeza. CTV is probably the most destructive citrus virus in the world. There have been documented losses of about 30 million trees on sour orange rootstock in Brazil and Argentina in the 1940s and 1950s, of 6.6 million in Venezuela in the 1980, and an estimated 10 million trees in Florida and other Caribbean Basin countries, all these losses were due to the introduction of *Toxoptera citricida*, commonly called the brown citrus aphid (BrCA), which is the most efficient vector of CTV (Rocha-Pena *et al.* 1995). The BrCA has been increasing its geographical area throughout the Caribbean Basin since 1989 and was first found in Florida in November 1995 (Halbert, 1998). Even without the BrCA, CTV has killed about 10 million trees Spain (Cambra *et al.* 1988) and millions more which are not well documented in Israel, Florida, California and in other areas (Bar-Joseph *et al.* 1989).

CTV is present in most citrus areas of the world. It has been reported in the African region, the Eastern Asian region, the Eurasian region, the Mediterranean region, the North American region and the Pacific region (Bar-Joseph and Lee, 1989). If the trees are planted on a CTV tolerant rootstock, such as *Poncirus trifoliata,* citranges, citrumelo, or mandarin, the appearance may not be noticeable unless diagnostic methods are used to detect the virus.

CTV causes several different diseases, depending on the strain of CTV. There are five major disease symptoms: 1) Mild, causing little or no noticeable symptoms on commercial citrus and only slight symptoms of vein flecking, leaf cupping, and stem pitting on very susceptible hosts such as Mexican lime, *Citrus excelsa*, or *Citrus macrophylla*. 2) Decline strains which cause decline and death of trees on sour orange rootstock (Figure 24.1A). Decline strains of CTV can reduce fruit size (Figure 24.1B). Sweet orange, grapefruit, mandarin scions on sour orange rootstock are susceptible to CTV decline. Usually tiny pinholes are produced on the inner surface of a bark patch, which is removed from the sour orange rootstock, and the sour orange will have corresponding tiny bristles projecting from the wood. Often there is a bulge immediately above the budunion, however in areas of Florida the trees are killed by decline strains of CTV so rapidly that the bulge does not develop, instead a brown line forms at the budunion a few minutes of exposure to the air after the patch is removed. 3) Seedling yellows (SY) is a stunting of the plant and chlorosis or yellowing of the leaves, which occurs on sour orange, lemon, and grapefruit following inoculation with some strains of CTV. SY has been used as a presumed marker for identification of severe strains (Dodds *et al.* 1987). Usually SY is a greenhouse disease occurring on indicator plants, however if growers topwork grapefruit or lemon scions onto their trees which are infected with SY strains of CTV, SY symptoms can be expressed in the field. 4) Orange stem pitting (OSP) strains of CTV will stem pit sweet orange varieties and 5) Grapefruit stem pitting (GSP) strains of CTV stem pit grapefruit (Figure 24.1C). Stem pitting is considered the most severe symptom of CTV; this aspect cannot be controlled by growing the scions on a CTV tolerant rootstock. Stem pitting strains reduce fruit size, fruit quality, and overall yield (Rocha-Pena *et al.* 1995). There have been instances where CTV strains will severely stem pit rootstock varieties, which are normally considered to be CTV tolerant. For example, the Capao Bonito isolate in Brazil causes severe stem pitting on Rangpur lime rootstock (Targon *et al.* 2000), strains of CTV causing stem pitting on rough lemon have been reported (Lee *et al.* 1994; S.M. Garnsey, personal communication), and some strains even stem pit rootstocks such as Cleopatra mandarin (Manjunath, *et al.* 1995).

CTV infects all *Citrus* species and *Aeglopsis chevalieri, Afraegle paniculata, Pamburus missiones*, and *Passiflora gracilis* (Bar-Joseph and Lee, 1989).

Molecular Properties

CTV has a positive-sense single-stranded RNA genome encapsidated in flexuous particles about 2000 nm in length (Bar-Joseph and Lee, 1989). The virions contain two capsid proteins (CP) arranged in a "rattlesnake" structure: a 25 kDa CP that encapsidates ~95% of the particle and a 27 kDa minor CP that encapsidates ~5% at one terminus (Febres et al, 1996). The entire sequence of the T36 isolate of CTV was reported by Karasev *et al* 1995.

Genomic Organization and Replication Strategies

CTV is a member of the monopartite genus Closterovirus in the family *Closteroviridae* (Bar-Joseph and Lee, 1989). The genomic RNA (gRNA) of this virus contains from 19,226 to 19,302 nt, depending on the isolate (Karasev *et al.* 1995; Mawassi *et al.* 1996; Suastika *et al.* 2001; Vives *et al.* 1999; Yang *et al.* 1999b), which occur as 12 open reading frames (ORFs), potentially encoding 19 protein products (Karasev, 2000; Karasev *et al.* 1995) (Figure 24.2).

Transmission

The virus is transmitted by grafting and mechanically by a knife-cut and slashes inoculation (Bar-Joseph and Lee, 1989; Garnsey *et al.* 1977; Garnsey and Muller, 1988). It also is vectored by several aphid species in a semipersistent manner with the aphid retaining the ability to transmit the virus for up to 24-48 hrs after acquisition (Bar-Joseph and Lee, 1989; Raccah *et al.* 1989). The most efficient vector for CTV is *T. citricida* (Bar-Joseph and Lee, 1989; Yokomi *et al.* 1994). Other aphid vectors of CTV are *Aphis gossypii* , *Aphis spiraecola* and *Toxoptera aurantii* . *A. gossypii* is the second most efficient vector after *T. citricida.* Epidemiology studies have shown that *A. gossypii* generally transmits to trees, which are 8-10 trees away from the source tree, while *T. citricida* transmits to the nearest 1 or 2 trees. When both vectors are present, the incidence of CTV is greatly accelerated with *A. gossypii* spreading the longer distances and *T. citricida* filling in the spaces (Gottwald *et al.* 1996). *A. spiraecola* may at times be an important vector because of the high populations which can be reached. *T. aurantii* can transmit some isolates of CTV, mostly the more severe isolates, but does not transmit Florida isolates of CTV (R. Yokomi and K. L. Manjunath, personal communication).

Methods of detection

The first method developed for diagnosis of CTV was biological indexing on Mexican lime. While quick methods have been developed for the detection of CTV in infected tissue, the biological activity of an isolate of CTV can only be determined by biological indexing on a battery of different citrus hosts. At the minimum, the hosts should include Mexican lime as a susceptible indicator plant for universal detection, sour orange as an indicator for SY, sweet orange on sour orange rootstock as an indicator for decline strains, grapefruit as an indicator GSP strains, and sweet orange as an indicator for OSP strains (Garnsey et al, 1987). Mexican lime will show vein flecking when infected with mild strains, severe strains will cause leaf cupping, interveinal chlorosis, and pronounced vein flecking and occasionally vein corking on the upper surface of the leaves. It usually takes 3-6 months for the Mexican lime index. Sour orange seedlings will be stunted and display chlorosis or yellowing in the leaves with SY strains of CTV. Non-SY strains do not have a pronounced effect of the growth of sour orange seedlings. The SY index usually required 6-9 months. The sweet orange on sour orange indicator plants will display stunting, chlorosis, and reduced vigor if decline strains of CTV are present, but they rarely die under greenhouse conditions. This index requires 12-15 months under ideal growing conditions. The stem pitting evaluations usually require 12-15 months before rating, severe stem pitting strains of CTV often cause reduced growth on the sweet orange and/or grapefruit indicator plants. Leave chlorosis is often expressed in grapefruit indicator plants infected with GSP strains of CTV. All indicator plants except the Mexican lime are maintained to one growing shoot. When the plants are cut back, the length and weight of the flush removed are recorded.

CTV may be detected by staining thin sections cut from tissue with Azura A, which stains the inclusion bodies present in CTV-infected tissue. They may be observed using a light microscope (Christie and Edwardson, 1977).

Several serological methods have been developed for the detection of CTV (Rocha-Pena and Lee, 1991). SDS-immunodiffusion was one of the early methods developed, presently for large-scale assays ELISA is the method of choice, a modification of this procedure is the dot immunobinding assay on nitrocellulose or equivalent membrane, or a direct tissue immunoblot. A monoclonal antibody has been selected which reacts preferentially with severe (decline) isolates in Florida (Permar *et al.* 1990), more recently an ELISA system was developed for selective detection of CTV strains causing sweet orange stem pitting (Nikolaeva *et al.* 1999). Serologically specific electron microscopy is useful, especially when monitoring the effect of additives on purification (Lee *et al* 1987). Staining

with fluorescent-labeled antibodies to detect inclusion bodies in CTV infected tissue also has been used (Brlanksy *et al.* 1988).

Reverse transcriptase polymerase chain (RT-PCR) assay has become the method of choice for ultra-sensitive detection of CTV. RT-PCR has been used to detect CTV present in single aphids (Mehta *et al.* 1997). Modifications of the RT-PCR have resulted in the development of some procedures, which are useful to predict quickly the biological activity of an unknown isolate of CTV (Niblett *et al.* 2000; Lopez *et al.* 1998; Hilf *et al.* 2000).

24.1.2 *Citrus psorosis virus*

Psorosis was first described in 1898 by Swingle and Webber, and in the early 1930s was the first citrus disease proven to be graft transmissible (Fawcett, 1934). It was present almost universally in old-line budwood sources before the development of certification programs in the early 1950s (Roistacher 1993). It is considered the most important disease of citrus in Argentina and Uruguay where the disease has a natural, unknown means of spreading from tree to tree.

Psorosis was present in many old-line bud sources, and thus, the movement of budwood distributed it almost worldwide especially before the nature of graft transmissible pathogens was understood.

Psorosis symptoms include bark scaling, with the wood beneath the scaling remaining alive. If a cross section is cut through a limb having bark scaling, gum impregnation of the wood is apparent. The leaves will show flecking and vein clearing under cool weather conditions, such as the spring growth flush. The tips of new flush growth often become necrotic and die. In the literature, psorosis A is described as a disease having psorosis-like leaf and foliar symptoms, but little bark scaling occurring on the limbs and trunk whereas psorosis B had both leaf and foliar symptoms plus a lot of bark scaling with symptoms also occurring commonly on the fruit. Wallace (1957) demonstrated that both psorosis A and B were strains of the same virus by preinoculating psorosis A into indicator plants, later when psorosis B was inoculated into the same plants, psorosis A protected against the display of bark scaling commonly expressed for psorosis B when inoculated by itself.

Citrus ringspot virus (CRSV) used to be considered a different virus, although it was accepted as psorosis-like based on production of psorosis-like symptoms on indicator plants. CRSV commonly produces ringspot-like symptoms on the leaves of infected plants and on fruit. CRSV isolates produce necrotic local lesions when upon mechanical inoculation of the older, fully expanded leaves of *Chenopodium quinoa* or *Phaseolus vulgaris* cv. Red Kidney. Some isolates of CRSV produce bark scaling on citrus

hosts whereas other isolates seldom if ever produced bark scaling (Garnsey and Timmer, 1988).

Psorosis virus infects all *Citrus* species and produces typical symptoms on sweet orange, grapefruit and mandarin varieties. Other varieties and *Citrus* species may remain asymptomatic, but they are latent hosts for the virus. Additionally there are several herbaceous hosts: *C. quinoa, P. vulgaris cv.* Red Kidney are common diagnostic hosts, other hosts reported are *Catharanthus roseus, Chenopodium amaranticolor, Crotalaria spectabilis, Cucumis sativus, Gomphrena globosa, Helianthus annuus, Nicotiana benthamiana, N. clevelandii, N. megalosiphon, N. rustica, N. tabacum, Petunia hybrida, Pisium sativum, Poncirus trifiliata, Sesamum indicum,* and *Zinnia elegans* (Timmer *et al.* 1978)

Molecular properties

In 1988 Derrick et al reported the purification of CRSV, isolate 4E, which had two components, both components were required for infectivity. A 48 kdalton protein was isolated from purified CRSV, and a monoclonal antibody was made against this protein. The resultant antibody was very isolate specific and could not be used for broad scale detection of other psorosis isolates. Following N-terminal sequencing of the 48 Kd protein, enough genome sequence was obtained to enable a RT-PCR assay to be developed with amplified the capsid protein of CRSV and other isolates of psorosis (Barthe *et al.* 1998). Based on molecular comparisons (Gracia *et al.* 1997; Barthe *et al.* 1998; Legarreta *et al.* 2000), it appears that psorosis A, psorosis B and CRSV are all strains of psorosis, but that concave gum, impietratura, and cristacortis, all of which produce similar symptoms in indicator plants, are distinct viruses, and they are not members of the psorosis group.

Means of spread

The long distance spread of psorosis and psorosis-like viruses has been by movement of infected nursery material. The virus may be mechanically transmitted to citrus and herbaceous hosts. Clearly a natural means of spread occurs in Argentina and Uruguay, but the means of this spread has never been identified (Roistacher 1993). It most likely is an insect although it could be by pollen or other means.

Detection

Antisera have been developed in Italy (Garcia *et al.* 1997), which permits serological detection of psorosis by ELISA methodology. RT-PCR is probably the method of choice (Garcia *et al.* 1997; Barthe *et al.* 1998; Legarreta *et al.* 2000).

CRSV isolates produce necrotic local lesions on the inoculated leaves on Chenopodium quinoa, but fully expanded leaves must be inoculated. If younger leaves are inoculated, no lesions will develop. On Dweet tangor, sweet orange, or grapefruit indicator plants, psorosis, including CRSV, produces a shock symptom on the first flush which emerges after inoculation; the tip of the flush often necrosis and dies. The leaves usually show pronounced vein clearing and flecking and/or transient oak leaf patterns. Bark scaling on the stem and twigs of the indicator plants may or may not be expressed, depending on the isolate. Concave gum, which produces symptoms on the same indicator plants, usually shows oak leaf patterns and little vein clearing and flecking, and the shock symptoms are now expressed.

24.1.3 Citrus viroids including Citrus exocortis viroid and Cachexia

. The first viroid to be described from citrus was citrus exocortis viroid (CEV). The discover of CEV subsequently lead to the finding of several additional viroids which have been grouped into five viroid groups based on size plus CEV (Duran-Vila *et al.* 1988). CEV produces the strongest symptoms on indicator plants of any of the citrus group of viroids. Citrus viroids seldom are found naturally occurring as single viroids in infected trees, rather they usually occur as a mixture of two or more viroids. Thus, it is hard to say, based on field observations and biological indexing, that CEV or any one of the other viroids produces a given symptom. There has been some work done to establish a correlation between a known viroid and symptoms expressed, but there is need for more of this correlation between specific viroids and combinations of viroids and the expression of symptoms on susceptible hosts from different citrus areas.

Citrus viroids are almost universally distributed and present in all citrus areas of the world. CEV is most destructive to sweet orange, grapefruit, or mandarin on *Poncirus trifoliata* rootstock, or a rootstock containing *P. trifoliata* as one of the parents of a hybrid, such as citranges or citrumelos. Affected trees on these rootstocks show dwarfing and stunting, bark scaling and cracking occurs on the rootstock portion of the tree, and productivity is reduced with small fruit and reduced yield (Figure 24.1 D).

Citron plants infected with CEV display severe leaf epinasty and rugosity with the midrib showing browning and cracking on the underside, especially at the leaf tip and the petiole (Figure 24.1 E). On lemon plants infected with CEV, bark shelling and cracking occur, and often the leaves have a blotchy mottle.

Tomato variety Rutgers displays leaf epinasty, rugosity and stunting when infected with CEV. *Gynura aurantiaca*, velvet plant, is used as the

principal herbaceous indicator, it shows leaf epinasty and rugosity upon inoculation with CEV.

Cachexia, also known as xyloprososis, is caused by a Group II viroid. While cachexia, by itself, will not cause obvious problems to trees on *P. trifoliata* rootstock, or citranges or citrumelos, it does cause stunting, dwarfing, and very poor growth of trees on mandarin or *Citrus macrophylla* rootstocks. Gum pockets and pitting occur in the bark over the rootstock of these rootstocks.

Gum pocket disease on grapefruit trees propagated on *P. trifoliata* rootstock has been associated with a group III viroid in Swaziland (Marais *et al.* 1996).

All citrus species may be infected with citrus viroids including CEV and cachexia. Herbaceous hosts include tomato, *Petunia hybrida*, *Gynura saramentosa*, *Gynura aurantiaca*, *Chyrsanthemum morifolium*, *Daucus carota* (carrot), *Solanum melongena* (eggplant), *Brassica campestris* (turnip), *Vica faba* (broad bean), *Tagetes patula* (French marigold), and *Vitis* species (Sanger, 1988).

Molecular properties

The viroids are small species of circular RNA, which are highly base paired. The largest citrus viroid at 371 bases is CEV, and the smallest is a Group IV viroid with about 235 bases. While the viroids are circular, they are also infectious if they are nicked and appear in their linear form.

Means of spread

The long distance distribution has been by movement of infected nursery material. Because they are very easily mechanically transmitted, it is not uncommon for a viroid infection to spread to surrounding trees unless great care is taken to disinfest clippers and cutting tools between trees.

Detection

Biological indexing may be used for the detection of citrus viroids (Duran-Vila *et al.* 1993). All viroids should be biologically indexed under warm growing conditions, 30 C night – 38 C day. Etrog citron is a commonly used indicator plant especially for CEV. For cachexia indexing, Parson's special mandarin is grafted onto rough lemon or other vigorous rootstock. Once the Parson's special mandarin bud is growing off, the inoculum is grafted into the rootstock. After a few months, the typical gumming and pitting will occur at the budunion if cachexia is present. Orlando tangelo may also be used as a biological indicator, but this is a longer-term index taking 2-3 years. Scion trees are grafted onto Orlando tangelo as a rootstock, these trees are inoculated with the source to be tested,

and bark patches removed at internals beginning 18 months after inoculation. Severe isolates of cachexia are easy to diagnose, mild isolates producing slighter symptoms are harder to detect using biological indexing. *Gynura aurantiaca* and Rutgers tomatoes are commonly used as herbaceous indicator plants for CEV, the other viroids usually do not express symptoms.

Viroids may be detected by purification and electrophoresis on polyacylamide gels. There have been several extraction methods reported. For best results, the viroid extraction should be made from Etrog citron tissue. Commonly the viroid is affinity purified using CF-11 cellulose powder or by portioning into the 2.0 M lithium chloride soluble fraction. Two dimensional polyacylamide gel electrophoresis is performed, the first gel is a native, non-denaturing gel. The viroid band is removed with a portion of the first dimension gel, and then the viroid is run in a denaturing gel, achieved by use of SDS and urea or by heated return gel electrophoresis (Asia *et al,* 1998). The circular forms of the viroids are retarded on the denatured gel and are well separated from host nucleic acids, the gel is silver-stained and the viroids may be identified by their respective rate of migration on the gel. CEV, being the largest viroid, migrate the least distance whereas the smallest viroids, Group IV, are the smallest and migrate the greatest distance.

RT-PCR assays have been developed (Gillings *et al.* 1988; Yang *et al.* 1992). Hybridization assays have been used to detect CEV and other viroids using various labels, such as biotin, digoxigenin, or radioactive labeled (Palacio *et al.* 2000). Viroid group specific primers are available for RT-PCR of each viroid group, and multiplex RT-PCR assays have been developed which enable simultaneous detection of several viroid groups at once.

24.1.4 Citrus variegated chlorosis (CVC)

Citrus variegated chlorosis (CVC), caused by a strain of *Xylella fastidiosa*, was first found in northern Sao Paulo state and in Minas Gerais in 1987 (Lee *et al.* 1991). The disease spread rapidly in Sao Paulo state and to other growing regions in Brazil through movement of infected nursery material. It soon became one of the economically most important diseases of citrus in Brazil with annual losses now estimated at $100 million per year (Donaldio and Moreira, 1998). Strains of *X. fastidiosa* cause other important diseases such a Pierce's disease of grapevine, phony peach, leaf scorch diseases of almond, coffee, oak, plum and sycamore (Hopkins, 1989; Purcell and Hopkins, 1996). Coffee leaf scorch (CLS) disease was first reported in Brazil, and the CVC strain of *X. fastidiosa* is closely related to the CLS strain (Beretta *et al.* 1996). Inoculation of the CVC strain into coffee results

in CLS-like symptoms, but the CLS strain does not cause CVC symptoms when inoculated into citrus.

CVC is found in throughout the citrus areas of Brazil, in Argentina, Paraguay (Donaldio and Moreira, 1998). Recently it was reported to be present in Costa Rica, Central America (Moriera *et al.* 2003).

The symptoms of CVC usually begin with a zinc deficiency-like chlorosis appearing on one sector of the trees; trees become stunted upon infection (Figure 24.2 A). The leaves develop gummy raised lesion on their underneath side with a corresponding yellow chlorosis appearing on the upper surface of the leaf (Figure 24.2 B). As the symptoms spread, the new leaves are small and tend to point upward, twig dieback occurs, the fruit size is greatly reduced, and the fruit has a hard rind (Figure 24.2 C). The sugar content of the fruit is higher than in non-affected fruit, and the fruit ripen earlier. Once infected with CVC the trees have reduced growth and are rendered nonproductive in three years. Younger trees are more susceptible to CVC that trees which are 10 years of age or older. Symptom expression and incidence of CVC appear to be greater in warmer climates (Lee *et al.* 1991; Donaldio and Moreira, 1998).

CVC infects most species of citrus, but symptoms are displayed most on sweet orange cultivars. Lemons, limes, mandarins, mandarin hybrids such as Murcott and Sunburst, kumquats, trifoliate orange and grapefruit usually do not show symptoms of CVC but allow some multiplication of the bacterium (Donaldio and Moreira, 1998).

Molecular properties

The entire genomes of three strains of *Xylella fastidiosa* have been sequenced: CVC (Simpson *et al*, 2000), Pierce's disease of grapevine, and Oleander leaf scorch.

Means of spread

The disease is easily graft transmitted if xylem tissue is included in the bud chip used for propagation. The long distance spread of CVC is by movement of infected nursery material.

The CVC bacterium is transmitted by several species of sharpshooters which are common in Brazilian citrus areas. The efficiency varies among species. Ultrastructure studies have shown that the bacterium may attach to the inside of the stylet, pump organ (cybarium) and pre-cybarium of the sharpshooter. The sharpshooter loses the ability to transmit *X. fastidiosa* whenever a molt occurs. Once an adult acquires *X. fastidiosa*, they retain the ability to transmit for life. At least 11 species of sharpshooter have been identified to transmit CVC in Brazil (Donaldio and Moreira, 1998). The most important vectors in Brazil are *Acrogonia terminalis*, *Dilobopterus*

costalimai, and *Oncometopia fascialis*, other common vectors present are *Sonesimia grossa*, *Hortensia similis*, *Ferrariana* sp. and *Molomea* sp. The glassy winged sharpshooter, *Homoladisca coagulate,* present in the southeast United State and now in California has been shown to be capable of transmitting CVC (R. H. Brlansky and V. Damsteegt, personal communication).

Detection

Diagnosis of CVC in the field can be confused with other decline diseases of citrus. CVC infected trees will take up water by the syringe injection test while blight infected trees do not. Diagnostic field symptoms are the small fruit having high sugar content, the gummy lesion on the underside of the leaves, and the small, pointy leaves at the top of the tree. Laboratory detection methods include serological assays, culturing the bacterium, microscopy, and PCR (Donaldio and Moreira, 1998). The problem with most of these detection methods is that it is difficult to differentiate between the CVC strain and other strains of *X. fastidiosa*. PCR methods have been reported which allow specific detection of the CVC strain.

24.1.5 Huanglongbing (Citrus greening disease)

Citrus greening is referred to also as Huanglongbin, yellow shoot, likubin, leaf mottling, and vein-phloem degeneration (da Graca, 1991; Garnier and Bove, 1993). The disease is caused by a systemic phloem-inhabiting bacterium, *Candidatus* Liberobacter. In thin sections under the transmission electron microscope (TEM), this fastidious bacterium is pleomorphic having a three-layer envelope. There are two forms of greening, each form has a similar host range but they differ in the temperature under which they express strongest symptoms. The African form, *Candidatus* L. africanus causes symptoms under cool conditions while the Asian form, *Candidatus* L. asiaticus causes symptoms under warm conditions. The Asian forms, in general, express stronger symptoms than African forms.

HLB is one of the most destructive diseases of citrus. Once established, the management of the disease to achieve continued production of citrus is difficult and expensive. The HLB bacterium is spread by citrus psyllids (McClean and Oberholzer, 1965). When the psyllid vector is present and under ideal climatic conditions, HLB can rapidly decimate productive citrus plantings. If trees are infected while young, they often have no fruit production.

Present in most areas of Africa, Asia, Southeast Asia, Arabian Peninsula, Indonesia and the Philippines.

Symptoms of Asian greening include leaf chlorosis and canopy dieback, the early symptoms usually appear only on one sector or branch of the tree (Figure 24.2D). The chlorosis spreads, often resembling a zinc deficiency symptom. Twig dieback occurs, and the affected trees decline to a non-productive state. Fruit is small, lopsided, with the basal end often remaining green, and the seeds are usually aborted. The fruit has a bitter taste. The symptoms of the African form of greening are similar except they are expressed during cooler weather, and a blotchy mottle symptom is common on the leaves, especially young flush leaves grown under cool temperature (Oberholzer *et al.* 1965; da Graca, 1991).

The HLB bacterium infects nearly all citrus species, cultivars and hybrids and some citrus relatives. Sweet orange, mandarin and mandarin hybrids are most susceptible; lemons, grapefruit and pummelos are moderately affected; and Mexican lime, trifoliate orange, citranges and citrumelos are more tolerant, often expressing foliar symptoms but little twig dieback (da Graca, 1991). Periwinkle and tobacco are experimental hosts, infected by use of dodder (Gao *et al.* 1993).

The bacterium causing greening has not been cultured, and Koch's postulates have not convincingly been fulfilled although there are reports of bacterium having the traits of greening being cultured (Garnet *et al.* 1985).

Means of spread

Greening is graft transmissible. The distribution of the greening bacterium within an infected tree can be irregular so not all buds will contain the bacterium or transmit the disease. The more phloem tissue that is included in the inoculum, the greater the probability of graft transmission when transmitting by grafting. Transmission from plant to plant and to experimental species has been done by the use of dodder (Gao *et al.* 1993).

Greening bacterium is transmitted by psyllids. *Trioza erytreae* occurs in Africa, Yemen, islands in the Indian Ocean, and is associated with the African form of greening. *Diaphorina citri*, commonly called the Asian citrus psyllids, is better adapted to warm humid climates and occurs in Asia, the Indian Subcontinent, Saudi Arabia, Reunion, Mauritius, South America, and more recently in several Caribbean islands, areas in Central America and Florida. Both psyllids have been shown to transmit either form of greening, Asian or African. The bacterium is transmitted in a persistent manner with a latent period occurring after the psyllid acquires the bacterium before the insect is able to transmit. The bacterium multiplies in the psyllid vector. Because of the latent period, most psyllids capable of transmitting greening are either late-stage nymphs or adults. The psyllids remain capable of transmitting greening for the duration of their life once the bacterium has been acquired. Citrus species is the primary host for feeding of both psyllid

species; other hosts include *Clausena anisata*, *Vepris lanceolata* and *Murraya paniculata* and a few other species of Rutaceae. The psyllids prefer to feed on young flush tissue. Hosts, which are vigorous and always flushing, such as lemon, lime, and *M. paniculata* are ideal hosts to look at for presence of psyllids.

Detection

The quickest and most reliable method for detection and diagnosis of greening is by PCR using symptomatic tissue as the source (Jagoueix *et al.* 1994: Harakava *et al.* 2000). Biological indexing is often difficult because severe strains of citrus tristeza virus (CTV) occur in most areas where greening occurs, and the symptoms of CTV can mask the presence of greening. Cool greenhouse conditions, 24-27 C, are required for symptom development of African forms of greening, while warm conditions, 30-37 C, are required for symptom development of Asian forms of greening. Asymmetric growth of young leaves, especially on greenhouse indicator plants, is indicative of presence of greening, in addition to a blotchy mottle leaf symptom for African forms and zinc deficiency-like symptoms for Asian forms. Grapefruit and lemon plants are good indicator plants, showing good symptoms under greenhouse conditions. Mandarin indicator plants may be useful if severe strains of CTV are present as the mandarins are tolerant of CTV and most of the symptoms being expressed are due to greening. From studies using *in planta* cultures in the quarantine facility, Beltsville, MD, dweet tangor has proven to be a sensitive indicator plant (M. Hooker and R. Lee, unpublished). Other methods of assay which have been reported are by the use of transmission electron microscopy (Moll and Martin, 1973), a method not useful for a large number of samples, serological detection (Sdoodee and Garnett, 1994) and assays for presence of gentisic acid, a method which is not as accurate and sensitive as PCR, but it does not require elaborate equipment (Hooker *et al.* 1993).

24.1.6 Witches' broom disease of lime (WBDL)

In the early 1980s a devastating disease of lime, now known as witches' broom disease of lime (WBDL), occurred in the Liwa area in Batina, Oman (Figure 24.3E) (Garnier *et al.* 1991). This disease was never reported elsewhere before that time. WBDL is caused by a mycoplasma-like organism know by the *Candidatus* name Phytoplasma aurantifolia (Zreik *et al* 1995)

Up until the early 1990s, citrus ranked as the second most important crop in Oman, after dates. The area used for citrus production was around 2500 hectares. Ninety percent of the citrus grown is a small-fruited acid lime (*Citrus aurantifolia*) known locally as Omani lime. The Omani limes, either

fresh or dried and pulverized, are an important food seasoning throughout the Near East. Farms affected with WBDL showed an infection rate of 1.2-3.3% in 1993. This rate increased rapidly and by 1996 the infection rate in surveyed farms was almost 100%. In 1998 the export of limes was ceased and the price of lime increased markedly (Bove, 1986).

There are other reports of phytoplasmas producing witches' brooms in citrus:

In India: a witches' broom in acid lime shows initial symptoms of highly proliferated shoots and shortened internodes with small chlorotic leaves. The leaves drop prematurely and infected twigs become distorted, resembling a broom-like appearance. In advanced stages the infected branches develop dieback. Infected branches do not produce any flowers or fruit. Symptom development is restricted to a single branch while the rest of the branches have normal vegetation and fruiting. Also in Nagpur mandarin (*C. reticulata*) the symptoms are of two different types. The first symptom has the whole plant showing excessive sprouting with small, thick leaves. The branches are very flexible with no flowering or fruiting. The second type of symptoms are similar to those described above in acid lime with the development of highly proliferating shoots with small chlorotic leaves which become distorted, followed by a dieback that give a dry broom-like appearance (D. Ghosh, personal communication). A similar witches' broom has been observed in mandarin in Oman.

In Jamaica a phytoplasma-like disorder has been seen. The disorder was first observed on a 20-year-old Cleopatra mandarin tree. Galls were formed, followed by bud proliferation forming witches-broom like structures but without the usual pronounced internode shortening. As high as five percent incidence of this disorder has been observed on citrumelo and mandarin rootstocks and on Valencia sweet orange budlings in nursery situations. The disorder also has been observed on Clementine trees and older Swingle seed source trees (Figure 24.2 F). The broad host range and rapid spread makes this disorder a threat to citrus production (R. Lee, unpublished).

WBDL occurs in Oman, United Arab Emirates. A disease with all the symptoms associated with WBDL has been reported in southeast Iran and in the Nagpur region of India, but there is no molecular evidence that these diseases having similar symptoms are caused by the same phytoplasma.

The production of witches-brooms symptoms is the main characteristic of WBDL infections. The brooms are compact with very small, pale green leaves. In early symptom trees, there are only a few witches'-brooms with the rest of the canopy looking normal. The first witches' brooms to form are often in the interior of the tree. Additional witches-brooms develop over time (Figure 24.2 E). In the advanced stages of the disease, the leaves of the older witches-brooms die, but some dry leaves remain attached for a period

of time before they finally fall off. The disease is lethal in 5 - 10 years after the first witches-broom is found. All ages of trees appear to be susceptible to WBDL.

Small-fruited acid lime (*Citrus aurantifolia*), Palestine sweet lime (*C. limetta*) and sweet limetta (*C. limettoides*) are naturally infected and show symptoms. Experimentally, WBDL has been transmitted by graft inoculation to: *C. aurantifolia, C. excelsa, C. hystrix, C. ichangensis, C. karna, C. macrophylla,* Etron citron (*C. medica*), Meyer lemon (*C. limon*), Rangpur lime (*C. limonia*), rough lemon (*C. jambhiri*), and Troyer citrange (*Poncirus trifoliata* x *C. sinensis*) (Bove *et al.* 1996).

WBDL is caused by a mycoplasma-like organism know by the *candidatus* name Phytoplasma aurantifolia. The phytoplasma has not been cultured. The phytoplasma is closely related to the phytoplasmas of alfalfa, sesame, and sunhemp phyllodies based on sequencing of the 16S rDNA genes (Zreik *et al.* 1995).

Means of spread

WBDL has been shown to be graft transmissible. Recently there have been reports that WBDL is transmitted through seed collected from WBDL affected trees. The WBDL is thought to be transmitted by the leafhopper *Hishimonus phycitis*, but this has not been verified by transmission tests. WBDL may be transmitted by dodder.

Detection

The most common method of detection of WBDL is by PCR amplification of the 16S rDNA gene. Primer pairs are available which enable selective amplification of a region of the 16S rDNA gene (Khan *et al.* 2003). TEM examination of fixed tissue, immunofluorescence using monoclonal antibodies, and the use of labeled DNA hybridization probes have also been used for detection (Bove *et al.* 1993)

24.1.7 Citrus blight (CB)

Citrus blight, also called young tree decline and sandhill decline in Florida and declinio in Brazil, is a chronic decline disease of unknown etiology. It appears in trees on susceptible rootstock between 6-8 years of age when the tree is bearing, but the tree does not usually die. The disease causes a plugging of the xylem vessels.

Citrus blight occurs in most citrus areas having a warm, humid climate, but it has not been reported from countries having a Mediterranean-like climate. There are reports of blight from Florida, Texas, Louisiana, Hawaii, throughout the countries in the Caribbean Basin and Central America,

Colombia, Argentina, Brazil, South Africa, and Australia (Timmer *et al.* 2000).

The first visual symptom of blight is the development of a slight grayish color change of the canopy (Figure 24.1 F). Symptoms are preceded by an increase in zinc in the phloem, then in the wood of the tree. Often one sector of the tree will develop zinc deficiency-like symptoms in the leaves. In the northern hemisphere this sector is usually in the southwest quadrant of the tree while in the southern hemisphere, the first sector to show zinc deficiency symptoms is in the northwest quadrant. The tree then begins to decline with twig dieback, reduced fruit size, high sugar content in the fruit, delayed blossom set, and the leaves at the top of branches become small and point upward. Within 1-2 seasons, the tree is rendered non-productive but the tree does not die.

Citrus blight affects most commercial varieties on rootstocks as well as seedling trees which are bearing fruit. In the field some rootstocks are very susceptible to blight, such as rough lemon, Carrizo citrange, Rangpur lime, *Poncirus* trifoliate and Volkamer lemon while other rootstocks have a good field tolerance, such as sour orange, Cleopatra mandarin, and Swingle citrumelo.

Citrus blight is a disease of unknown etiology, but it has been shown to be transmitted by grafting roots from a blighted tree onto the roots of a healthy tree, but the disease has not been transmitted by any above ground parts of the tree (Timmer *et al.*. 1987; Derrick and Timmer, 2000).

Means of spread

Citrus blight has been shown to be graft transmitted using roots from blighted trees to graft to roots of healthy trees. With some rootstocks such as Cleopatra mandarin and sour orange, which normally show field tolerance for blight, blight will spread down the row tree to tree. This is presumed to be due to natural root grafting between trees, as herbicide applied to the stump of a tree, which has been sawed off, will result in phytotoxicity showing up 1-2 trees down the row. The first occurrence of blight in a grove is randomly distributed, but subsequent spread tends to be 1-2 trees away from the blighted tree (Timmer *et al.*. 2000).

Detection

Because blighted trees have amorphous plugs in their xylem, a water injection test is often used in the field to diagnose blight (Lee *et al.* 1984). Healthy trees will take up 10 ml of water within 30 sec when using a 30 ml syringe to push the water into a $1/8^{th}$ inch diameter hole in the tree trunk, blight trees will not take up water. Blight may also be diagnosed by the high zinc content in the wood, presence of the amorphous plugs in the xylem

vessels, and the presence of blight-associated proteins in leaves and roots using serological methods (Wutscher *et al.* 1977; Derrick and Timmer, 2000).

24.2 Economic impact case studies

It is difficult to get good yield loss data due to the presence of graft-transmissible pathogens. Losses become apparent to the growers who realize that the inputs into their grove are exceeding the income, and the reduced vigor and declining trees are apparent. However, comparable control blocks are usually lacking because the trees are a different scion variety, different rootstock, or under a different management regime. The value of proactive programs, such as mandatory citrus certification programs, comes from maintaining productivity or even increasing productivity over a period of time due to the introduction of superior clones which are free of graft-transmissible pathogens. The value of these proactive programs is difficult to calculate because the potential losses don't occur and cannot be accurately estimated.

24.2.1 Citrus viroids

Low vigor and stunting were apparent in three to five year old plantings of Valencia sweet orange trees grafted onto Rangpur lime or Carrizo citrange rootstock, depending on the grove, in Belize. The budwood for these trees had been imported into Belize from outside the country on the assumption that the budline was superior to clones of this variety already available within Belize. Several groves of Valencia of the same age and same rootstock had been propagated on the same rootstocks, but using a locally available clone of Valencia which was free of citrus viroids. All the groves were under the same management regime. The yield loss was readily apparent and production cost records and yields from similar groves with three groves infected with citrus viroids and three groves which were not affected were analyzed. The groves were all planted at a tree density of 225 trees per hectare. At the end of the eight year period when the viroid infected block was removed, the total loss was US$5,147 per hectare. For the grove planted with the viroid free budwood, the total profit at the end of eight years was US$10,898 per hectare (Roistacher *et al.*. 1996).

In an earlier study (Roistacher *et al.*. 1991), an experimental planting of navel sweet orange was designed to test the effect of citrus viroids on tree growth and productivity on susceptible (Troyer citrange) and tolerant (sour orange) rootstock. Citrus viroid isolates tested included citrus viroid (CV) CV-IIa alone; CVs-IIa and IIIb; CVs- Ia, IIa, IIIb; and CEV with CVs-IIa, and IIIb. The trees were planted in the Central Valley of California, 10 replicates of each treatment and planted in a randomized block. Yield and

tree measurements were made for 10 years, and the trees size measurements represented the average size of all trees in the treatment at the end of 10 years, the yield was expressed as average yield in Kg over the 10 year period for each treatment.

The effect of the various viroid combinations on the tree volume after 10 years of growth on the two different rootstocks is shown in Figure 24.4 A. The effect of the viroid combinations on the 10 year accumulative average yield is shown in Figure 24.4 B. CEV, Cvds- IIa and IIIb together reduced the canopy volume on Troyer rootstock by 59.2% while Cvds- Ia, IIa, and IIIb together and IIa and IIIb together reduced the canopy volume by 49.7% and 43.6%, respectively. It is interesting to note that the canopy volume of the trees on sour orange rootstock was reduced by 36.1%, a significant reduction, especially considering that sour orange is a viroid-tolerant rootstock. Considering the average yield over the 10 year period, CEV with Cvds-IIa and IIIb together reduced yield by 34.2%, Cvds-Ia, Ib, and IIIb by 21%, and Cvds-IIa and IIIb by 19% for the trees on Troyer citrange rootstock. CEV with Cvds-IIa and IIIb reduced yield by 21.6% on sour orange rootstock, a significant reduction in yield. Fruit quality was not reduced by the presence of CEV and the other citrus viroids.

24.2.2 Citrus tristeza virus

It is very difficult to determine yield losses due to stem pitting strains of CTV because in the areas where severe stem pitting, there are no healthy trees for comparison. Some estimates may be made however, by examining the yield and performance data from mild strain cross protection experiments. A good example is the summary of 20 years production from mild strain cross-protected Marsh grapefruit trees in Australia by Broadbent *et al.* (1991). *Toxoptera citricida* was present in Australia at the time the trials were planted in the field. After 20 years, at the location having the milder climate there was no breakdown in trees, which had been inoculated with mild isolates while no marketable fruits were produced by trees which had been inoculated with a severe stem pitting CTV isolate. At the warmer location all trees were smaller at the end of 20 years. The trees inoculated with the severe CTV isolate began declining and showing symptoms in only the last few years of the trial; the cumulative yield loss for the trees inoculated with the severe strain ranged from 19.4% to 20.3. However, the fruit size was greatly reduced with 79.1% of the fruit being size class 125 or greater compared to 22.5% and 14.4% for mild isolates. At the mild climate location, the cumulative yield for the trees inoculated with the severe isolate ranged from 52.9% to 44.2% when compared against the yield of trees inoculated with the mild isolates. The fruit size was also greatly reduced

with 76.3% of the fruit being size 125 or greater compared to 23% and 17.8% from trees inoculated with mild strains.

In South Africa, Marsh grapefruit trees were propagated on rough lemon rootstock and then inoculated with the Nartia mild isolate of CTV that was used widely in South Africa for several years as an universal mild isolate for cross protection (Marais, 1994; Marais *et al.*1996). When the propagated trees were planted into the field in Nkwaleni Valley, Northern Natal Province, about 25% of them were severely affected by CTV stem pitting. It is thought these plants were probably infected with severe strains of CTV by aphids feeding on the rough lemon liners before the trees were propagated. From the field planting, 10 trees were selected which had mild stem pitting, and 10 trees were selected with had severe stem pitting but yet the tree canopy was vigorous and healthy appearing. Yield data was collected from these selected trees for seven years. The average yield per year over the seven-year period was 221.6 kg per tree for the mild stem pitting trees and 178 kg per tree for the severe stem pitting trees. This is an average yield reduction of 20%. A total of 33.3% of the fruit from the mild stem pitting affected trees was size 40 or smaller compared to 72.6% of the fruit from the severe stem pitting affected trees.

Toxoptera citricida, the efficient vector of CTV, has expanded it's geographical area to the Caribbean Basin beginning in the mid-1980s. *T. citricida* was first found in Jamaica in July 1995, and although it was known that decline strains of CTV were present in the country, there had been no outbreaks of tree losses on sour orange rootstock due to CTV. In 1997, the first outbreak of tree losses was found on sour orange rootstock in the Bog Walk Valley, Jamaica (Lee *et al.* 2002). Based on past performance, the yield losses due to CTV were documented from 1997 through 2001. At one location of 541 acres, a total loss of 489,751 boxes of fruit (standard 90 pound boxes) were lost while at a second location of 618 acres, a total loss of 525,384 boxes of fruit were lost. By November 2001, the total losses for the two locations totalled US$4,413,630, using US$4.35 per box of fruit. These losses did not include the cost of removing the dead trees and replanting with new plants.

24.2.3 Huanglongbing (Citrus greening)

HLB is perhaps one of the most difficult citrus diseases to control. In areas where HLB is endemic, it is rapidly spread by the psyllid vectors. At two locations in India, 67% of the certified HLB-free trees became infected five years after planting (Azzaro *et al.* 1993). A method for living with greening has been developed in South Africa where HLB was first reported in South Africa in 1928/1929 (Buitendag and von Broembsen, 1993). This disease was very severe at elevations greater than 700 m between the years

1932-1936, 1939-1946, and again in 1958. Between the late 1960s and early 1970s, three major citrus production areas were abandoned due to HLB. By 1975, it was estimated that 4 million of 11 million trees in South Africa were infected with HLB (Buitendag and von Broembsen, 1993). Subsequently, a method was developed to live with greening and to grow citrus productively. This method includes planting healthy trees in the field, reduction of inoculum by pruning symptomatic foliage from trees, reducing psyllid populations by removing hosts which are attractive to psyllid breeding and use of systemic insecticides in the citrus trees to reduce psyllid populations.

In Thailand, HLB was first reported by Schwarz *et al.* (1974), who noted that two years appeared to pass between infection of trees and the debilitation of the trees. The trees were not killed, but rendered nonproductive. Marcotting was a common practice in Thailand, and since most trees were infected with HLB, the marcotted cutting was also infected. Su and Tolley (1992) observed that the average life span of a citrus grove was 8 years. They recommended implementation of a clean stock program and citrus certification programs. Roistacher (1994) observed trees declining due to HLB at 5 years of age and even younger. He estimated that between 10-15% of the tangerine trees were removed from productivity annually due to HLB. Grenzebach (1994) studies the economics of producing citrus in Thailand. He calculated the financial returns from an average citrus farm. He noted that if the citrus planting was healthy appearing when the grove was planted, it was about 8 years old before noticeable decline due to HLB occurred and by 12 years, the grove was ready to remove. Individual trees were removed as they declined, so the groves contained trees of several ages ranging from the date of planting to recent replants. Grenzebach used 12.5 ton/ha as the average national production in the country based on data made available by the Department of Agricultural Extension. From his analysis, yields reached 22.75 ton/ha in years 5 through 8, then declined to 6.5 ton/ha by the 12 year due to HLB infections. Roistacher reanalyzed the data from Grenzebach, and based on his personal observations, realized that some plantings went to the field already infected with HLB, and in many instances the decline due to HLB began much earlier than the 8 years used by Grenzebach. A graphic representation of the profit and losses for 6, 8, 10 and 12 years in Thailand are shown in Figure 24.5. Based on the interpolation of Grenzenbach's data, it is estimated that a grove must live for 10 years to make a profit, and if the grove could live and be productive for 20 years, a cumulative profit of about $125,000 would be realized (Roistacher, 1996).

24.3 Management Strategies

For control of graft transmissible pathogens of citrus, the starting point is to be able to plant a healthy tree. This is best accomplished through a mandatory citrus certification program. In addition to providing a cost effective method to enable all growers in a region to plant healthy trees free of graft transmissible pathogens, it also ensures that the grower has planted citrus germplasm of the highest possible quality, which has a proven record of performance.

A citrus certification program is composed of three distinct and different programs: 1) quarantine, 2) clean stock, and 3) certification (Lee *et al.* 1999; Navarro, 1993). These three programs must be integrated to provide for a functional, effective certification program.

1) Quarantine Programs for safe introduction of selected horticultural germplasm. It is often desirable to move citrus species and varieties between different citrus areas for commercial and/or scientific purposes. Uncontrolled introduction of such budwood carries a risk of introduction of new pests and pathogens. Provision has to be made to import such budwood safely to reduce the risk to the importing industry or country. This is usually accomplished by careful introduction through quarantine stations, which perform procedures for the safe importation of the germplasm. There are two main approaches to importing citrus budwood under quarantine conditions: the classic method of propagation of the imported material in an isolated condition until the material can be freed of graft-transmissible pathogens by thermotherapy and/or shoot tip grafting, indexed to verify their pathogen-free status, and then released to the clean stock program for subsequent use in the citrus industry. The second method maintains the imported budwood in tubes. Imported budwood is immediately surface sterilized, placed in culture media in test tubes, buds are forced, shoot tip grafting is performed, and the resulting plants are then indexed to verify freedom from pathogens. This approach is now used in Spain and California, and offers the advantage of more rapid introduction. As an example, one budstick from Texas was introduced into Spain in January 1988, and in May 1989, 10,300 healthy buds were released to citrus nurseries (Roistacher, 1994).

2) Clean Stock Programs for identification and production of desirable sources, and their maintenance as pathogen-free propagating stock.

In a citrus certification scheme, it is desirable to recover healthy plants from the local varieties or cultivars. These varieties may be best adapted for the local climate, soils, and market. Clean stock programs are usually carried out by research institutions with the joint participation of scientists in horticulture, virology, and tissue culture, and in close communication with the Ministry of Agriculture or Commissioner of Agriculture. A clean stock

program consists of six steps: 1) selection of mother trees of local cultivars, 2) indexing of the mother trees, 3) recovery of pathogen-free plants by shoot tip grafting and/or thermotherapy, 5) indexing of the recovered plants, 5) horticultural evaluation of the healthy plants, and 6) maintenance of healthy plants under protected conditions with recurring indexing to ensure they have not become contaminated again. When selecting mother trees, selection of individual trees of the different cultivars should be made according to documented horticultural criteria, such as superior production, higher fruit color, early or late ripening, etc. The presence or absence of pathogens should not be a deciding factor; the therapy treatment will rid the selection from the pathogens.

3) Certification Programs, for maintenance and distribution of virus-free propagating materials for commercial use, guarantee the sanitary status and trueness-of-type of the propagating material during the process of commercial propagation through the nurseries. Additionally, they also control the horticultural quality of nursery plants. These programs have legal regulations governing the different steps of nursery operations and require periodic indexing and inspection of trees of the different blocks used for nursery propagations. .

Certification programs have to be adapted to the specific situation of each citrus area. Organization of the citrus industry, pests and diseases present, and sources of funding so the program will be sustainable are important considerations when developing a citrus certification scheme. Certification programs only give a guarantee for those pathogens, which are actually included and tested for in the program.

The propagation scheme allows for concentration of resources of the expensive and time consuming indexing for freedom of graft-transmissible pathogens, verification of horticultural trueness-of-type, and selection for highest horticultural quality to be focused on the foundation trees, yet the benefits of this effort is realized by all the resulting propagations. Certification programs must have the support of the growers and members of the industry, and funds must be available to sustain operation of the program.

These programs are the foundation on which to build any efforts to reducing damage caused by diseases and pests, and for the improvement and continued productivity of a citrus industry.

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Figure captions:

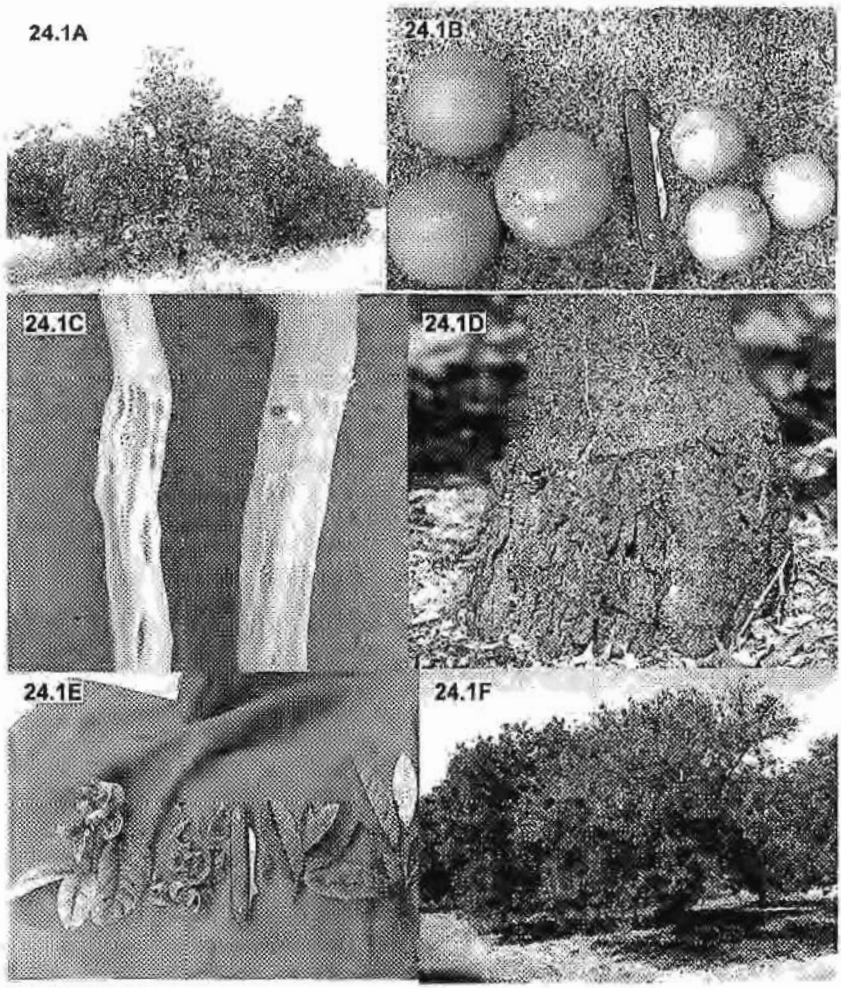
Figure 24. 1. Symptoms expressed by *Citrus tristeza virus* (CTV), citrus viroids, and citrus blight. A. Hamlin sweet orange tree on sour orange rootstock undergoing decline due to decline strains of CTV. B. Fruit size of Hamlin fruit from the tree illustrated in A, compared to fruit from a non-declining tree, immediately behind the declining tree in A. Depending on the time of year, amount of fruit already set, and length of time of the tree to completely die due to decline strains of CTV, the fruit size may be significantly reduced. C. Stem pitting due to CTV on twigs from a Marsh grapefruit tree showing pits with gumming beneath due to stem pitting strains of CTV. Stem pitting due to CTV can occur on sweet orange also. Fruit size and quality is reduced on trees affected with stem pitting strains of CTV. D. Citrus exocortis induced bark scaling on *Poncirus trifoliata* rootstock, note that the bark scaling occurs on the rootstock only and not on the scion. E. Variation of symptoms due to citrus viroids expressed on the viroid indicator plant Etrog citron clone 861. The plant on the right, labeled "H" is healthy, the rest of the plants were inoculated from field sources containing various mixtures of citrus viroids. F. A Valencia sweet orange tree on rough lemon rootstock showing the symptoms of citrus blight (CB), a disease of unknown etiology which may be graft transmitted using root grafting. The trees affected with CB first develop an off color green appearance, often followed by zinc deficiency-like chlorosis on the southwest quadrant of the tree (Northern hemisphere). Canopy dieback then occurs and the tree declines over a period of several years. Fruit size and yield are reduced, blossom set is delayed 2-3 weeks compared to healthy trees.

Figure 24.2. Symptoms expressed by fastidious prokaryotes causing diseases in citrus. A. Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa,* causes a general stunting in affected trees, and the tree takes on a yellow or chlorotic appearance. The tree in the foreground is CVC infected while the tree immediately behind is growing normally. B. With CVC, leaf symptoms begin with an interveinal chlorosis which is apparent on both sides of the leaf. Gummy slightly raised lesions are then formed on the underside of the leaf in the areas which are chlorotic. C. CVC reduces fruit size, the fruit have hard rinds, and the fruit ripen early and have a higher than normal sugar content. The Natal sweet orange fruit on the left came from a tree apparently free of CVC, the fruit on the right came from a CVC infected tree. D. Yellow shoot symptom on a Valencia tree infected with an African strain of Huanglongbing (HLB), commonly called citrus greening. The sectored branches showing general chlorosis are common on early symptomatic HLB affected trees. E. Witches' broom of lime (WBDL) from Oman. The witches' brooms start to develop in the inner parts of the tree canopy, then gradually spread and appear throughout the tree's canopy. The WBDL affected trees eventually die. The WBDL in Oman and surrounding countries is caused by *Candidatus* Phytoplasma aurantifolia. F. A 20 year old Swingle citrumelo tree in Jamaica showing witches' broom symptoms. A phytoplasma was identified from samples taken from this tree (R. Lee, unpublished).

Figure 24.3. Representation of the gene expression and genome organization of citrus tristeza virus. Open reading frames are shown as boxes and the putative domains on ORF 1a and 1b are separated by lines. PRO: papain-like proteases 1 and 2; RdRp: RNA-dependent polymerase. The genomic and subgenomic RNAs are shown by solid lines, the size, in kilobases, is indicated by the scale at the top. Defective-RNA strategy is shown by dashed lines (Ochoa-Corona *et al.* 2000).

Figure 24.4. The effect of different citrus viroid combinations on the canopy area at the end of 10 years (Fig. 6 A) and the cumulative 10 year yield (Fig. 6 B) of Naval sweet orange on Troyer citrange, a viroid susceptible rootstock, and sour orange, a viroid tolerant rootstock. The canopy area is expressed as cubic meters, the yield is expressed as kilograms of fruit. Data from Roistacher *et al.* 1991.

Figure 24.5. Huanglongbing (HLB) is a limiting factor of citrus production in Thailand. Grenzenback (1984) and Roistacher (1996) studied the cost of producing citrus in Thailand, calculated the financial returns from several average citrus farms, and extrapolated the profit or loss in US$ per hectare depending on the life span of the block. The rate of spread of HLB was about 10% per year, the average life span was eight years with a range of five to twelve years. The extrapolated profit/loss for 6, 8, 10, and 12 years of age are illustrated in A, B, C, and D, respectively. The short life span groves coincided with planting trees which were already infected with HLB. This example highlights the need to plant healthy plants.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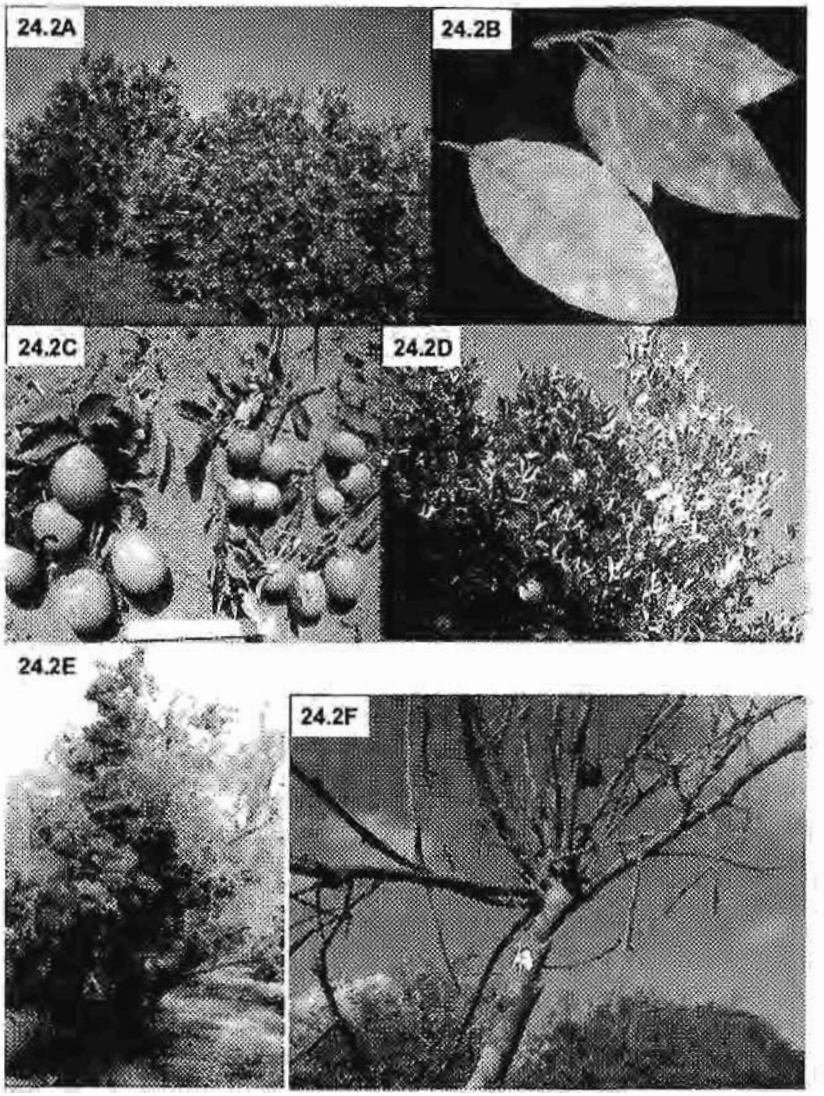
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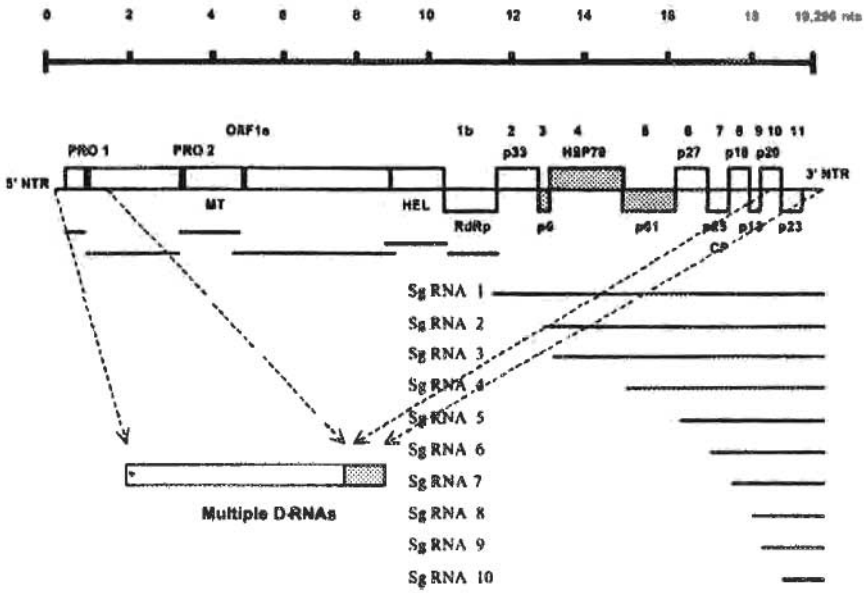
Figure 24.3.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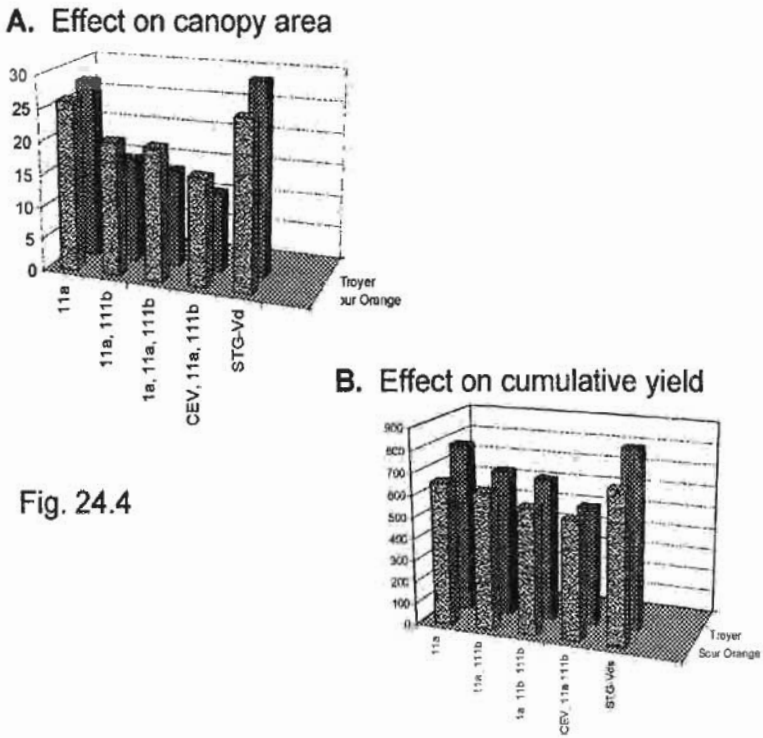

Fig. 24.4

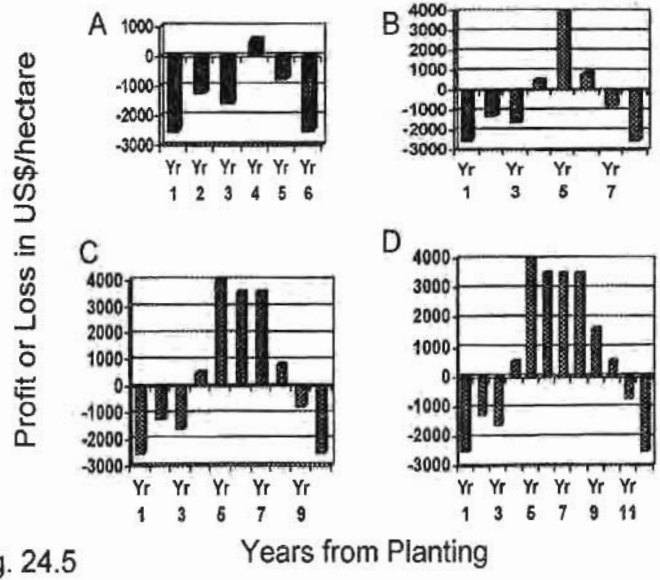

Fig. 24.5