Virus or Virus-like Diseases of Citrus in Tropical and Subtropical Zones

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Abstract

Citrus greening disease caused by fastidious bacteria that infect sieve cells is the most serious problem in tropical and subtropical Asia and Africa. The greening organisms are transmitted by insect vectors, *Diaphorina citri* in Asia and *Trioza erytreae* in Africa as well as by grafting or layering. There is a great diversity in susceptibility among citrus cultivars. 'Ladu' and 'Som-pan' mandarins, Calamondin and rough lemon are resistant. Transmission in fields is enhanced by strong wind due to the ability of the vector to move over long distances.

Tristeza virus is also prevalent in the tropical and subtropical zones. *Toxoptera citricidus*, the most effective vector, is spreading from South America to Central America. Changes in transmissibility of the virus by aphid were observed in many countries. In recent years severe strains have become popular and caused serious problems.

Citrus variegated chlorosis is becoming serious in subtropical Brazil. The causal agent is a xylem-limited bacterium, *Xylella fastidiosa* that is transmitted by grafting. Presence of insect-vector is assumed but has not been confirmed.

Witches' broom disease of lime trees caused by mycoplasma-like organisms (WBDL-MLO) is prevalent in the Sultanate of Oman and the United Arab Emirates. A leafhopper, *Hishimonus phycitis* is considered to be the vector of WBDL-MLO.

Introduction

Annual production of citrus in the world increased year by year and reached 57 million tons in 1992. Many countries including developing countries would like to promote citrus cultivation. However, the occurrence of several severe diseases is a major constraint, i. e., greening disease in tropical and subtropical Asia and Africa; tristeza and stem pitting disease in Central America, Florida, Peru and Australia; citrus variegated chlorosis in Brazil, and witches' broom disease in the Arabian Peninsula. Although their incidence is limited in the early stage, outbreaks occur due to the presence of vectors and increase of national and international movement of citrus budwood.

The paper reviews recent publications about these diseases.

Greening disease

1. History, distribution and economic importance

Citrus greening disease (CGD) was first described in South Africa in 1937. However, a similar disease had been known in China for more than 100 years under the name of Huanglungbin (yellow shoot disease) (Garnier and Bove, 1993). Independently, citrus dieback in India, likubin in Taiwan, citrus leaf mottle in the Philippines and vein phloem degeneration in Indonesia were described as virus or virus-like diseases. Based on etiological studies, symptoms, transmission mode and indexing, these diseases were found to be

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caused by a similar pathogen and they were designated as greening disease (Garnier and Bove, 1993). Up to the present, CGD had been largely distributed from China to Pakistan in Asia. Recently, it was detected in the southern islands of Japan (Miyakawa and Tsuno, 1989). In Africa, CGD was found in southern and eastern countries and in Cameroon. The disease has also been reported in Yemen and Saudi Arabia in the Arabian peninsula and in Sri Lanka, Madagascar, Reunion, and Mauritius in the Indian Ocean (Garnier and Bove, 1993).

The most typical symptom is chlorotic leaf mottle with green blotches on the pale green background. Zinc-deficiency-like symptoms are usually associated. Early symptoms appear in small parts of the canopy, like a yellow shoot. Affected tree is stunted, defoliated and finally dieback is observed. Severely infected trees show a sparse yellow foliage and low fruit sets. The fruit is small, lopsided, dull green on the stylar end when it matures, hence the term "greening". Many abortive seeds in the fruit are observed. CGD leads to the destruction of a citrus orchard within several years.

2. Pathogens and vector

Citrus greening organism (GO) is a kind of Gram-negative bacterium with a 25 nm thick membranous cell wall, different from mycoplasma-like organisms (Garnier *et al*., 1984). Therefore observation by electron microscopy is the most reliable method for GO detection. *In vitro* culture of GO has not been successful.

In African greening disease the symptoms develop at a lower temperature than in the Asian type. The former is transmitted by African citrus psylla, *Trioza erytreae*, while Asian greening is transmitted by *Diaphorina citri*. Recently it has been experimentally shown that each psylla species is able to transmit both African and Asian GO. Serological studies and DNA-probe hybridization indicate that Asian and African GOs are different in major properties (Garnier and Bove, 1993).

In addition to the CGD endemic area *Diaphorina citri* is observed in Brazil and the Ryukyu Islands including Tanegashima in Japan, where CGD could become prevalent if it was introduced.

3. Host resistance

The greening organism may infect all the citrus species and kumquat. Trifoliate orange was infected without distinct symptoms (Miyakawa, 1980). Field evaluations in Thailand showed marked differences in the susceptibility (Koizumi *et al*., 1993). The cultivars Wilking, Ellendale, Pet-yala, Beauty, Onesco, Pongchieng-ga (Tankan), Nian-ju and sweet orange were most susceptible, followed by many mandarins including Fairchild, Murcott, Kinnow, Clementine, Fremont, Ponkan, King, and Som-keo-wan. Avon Ever Bearing (Calamondin) and rough lemon grew well with mild symptoms. Ladu mandarin and Som-pan mandarin were the most resistant, displaying a vigorous growth, few symptoms and yielding healthy fruits. Pommelo (*Citrus grandis*) was mostly free from the disease, but some trees in mixed-planting with infested sour lime or mandarin trees were severely affected. In India, sweet lime and Mosambi sweet orange trees on sweet lime, Eureka lemon or Italian lemon rootstock showed very mild symptoms (Nariani, 1981). These findings suggest that resistant rootstocks could be used to mitigate the damage from greening disease.

4. Epidemiology and control

In the Philippines it is considered that the disease originated from China or India, since citrus nursery plants had been imported from those countries before 1950 and the disease appeared thereafter (Gonzales, 1989). A similar mode of dissemination was proposed in Thailand, Malaysia, the southern islands of Japan and Nepal. Thus long distance dissemination of GO could occur by transportation of infested citrus budwood or plants, or occasionally by vectors.

A single adult is able to transmit GO very efficiently within 1-2 days and a maximum period of 25 days after acquisition. The infected adult retained the infectivity throughout its life (Xu *et al*., 1988). Adults of *D. citri* collected from a field with CGD in Thailand showed a high potential to transmit the disease (unpublished). Detection of GO from *D. citri* with DNA probe showed that 30% of the psylla insects collected in Malaysia in September 1991 were infected with GO, while only 3-5% of psylla insects were infected in May and October 1992 (Garnier and Bove, 1993).

Observations of greening disease in a field where disease-free citrus trees had been planted showed heavy transmission from surrounding infested area and rapid dissemination throughout the field. In Malaysia and Thailand the dissemination of the disease was rapid in flat areas, while it was very slow in a field between mountains or behind hills, presumably due to the dissemination of the vector by the strong wind (unpublished).

Occasional sprays of insecticides which are frequently used to control the vector are not effective in the protection from infection. Farmers observed the vector on the trees a few days after chemical spray, especially after strong wind (unpublished).

Biological control of psylla was achieved successfully in Reunion Island with *Tamarixia radiata* introduced from India against *D. citri*, and with *Tamarixia dryi* introduced from South Africa against *T. erytreae* (Aubert, 1984). Eradication of psylla from the affected trees coupled with biological control resulted in the eradication of the disease from Reunion. However, biological control can not be achieved in many countries due to the coexistence of the parasitic insects with the psylla parasite (Garnier and Bove, 1993).

Elimination of GO from budwood can be achieved by heat therapy, dipping into antibiotics, and shoot-tip grafting.

Up to the present integrated control had been recommended (Aubert, 1988), based on reliable epidemiological information about the disease incidence, vector population and climatic conditions. As a result, the production of disease-free stock and its multiplication became possible under disease-free conditions, through the spraying of insecticides coupled with biological control of the psylla, and cultural environmental management including eradication of infected trees. However, eradication and maintenance of disease-free conditions are very costly and cannot be easily achieved in most of the developing countries.

Tristeza virus

T. citricidus moved from Brazil and Colombia throughout Venezuela, where over 6 million trees on sour orange rootstock have died since around 1976 because of tristeza disease (Roistacher and Moreno, 1991). The aphid is moving to Central America and now it is found in Panama, Costa Rica, El Salvador and Honduras (Lastra *et al*., 1991). Citrus trees in Mexico and USA are under the threat of invasion by the aphid.

Bar-Joseph and Loebenstein (1973) first revealed a change in the transmissibility of CTV by *Aphis gossypii* from 2-5% to 40.7%. Similar changes were observed in California and Florida (Roistacher and Moreno, 1991). The transmissibility appears to depend on the isolate of CTV, from now due to the mutation of CTV. Recently it has been reported that an outbreak of quick decline occurred and severe strains of CTV were becoming more abundant in Florida (Yokomi *et al*., 1991). It has never been confirmed that *Aphis citricola* transmitted CTV in Japan and California, but it is known to be a vector in Florida, Spain and Israel. These facts suggest that the presence of severe CTV in fields threatens citrus trees even if an effective vector has not invaded.

In the countries where CTV-SY and its effective vector have become endemic, resistant rootstocks are used. However, outbreak of severe stem pitting disease occurred in Brazil, Peru, Australia, and in South Africa, due to the prevalence of extremely severe strains of stem pitting tristeza virus (CTV-SP). Affected trees became dwarfed with low yields and small fruits. In Peru and Brazil CTV-SP is considered to spread from satsuma mandarin trees imported from Japan. Severe stem pitting disease was commonly seen on sweet orange in Indonesia (Muharam and Whittle, 1991). In other countries of tropical Asia severe stem pitting disease has not been observed on sweet orange, sour lime and Pommelo.

Citrus variegated chlorosis

Citrus variegated chlorosis (CVC) has been observed since 1987 in Sao Paulo and Minas Gerais states, Brazil (Beretta *et al*., 1993). It has spread rapidly in nurseries and commercial groves. Affected trees show leaf chlorosis similar to zinc deficiency symptoms. Chlorosis appears on young leaves as they mature. Newly affected trees develop the symptoms in small parts of the canopy.

Fruit remains small but ripens early, averaging only 77g whereas normal fruit averages 203g. The sugar content is high, but fruit rind is too hard to squeeze the juice in processing. Pera, Natal, Valencia, Hamlin, and Bhaia-Navel on Rangpur lime, Cleopatra mandarin, and Volkamer lemon rootstocks were susceptible. The disease has not been observed on Tahiti lime or mandarin trees even when they were planted in sweet orange groves severely affected with CVC.

Symptoms of CVC are severe on trees 1 to 8 years old, while milder symptoms are observed in older trees. On trees more than 10 years old, symptoms developed on a few leaves and small fruits where seldom observed.

A gram-negative xylem-limited bacterium, with morphological and structural characteristics of *Xylella fastidiosa* (Lee *et al*., 1991), has been associated with the disease based on electron microscopic observations, culture characteristics and serological reaction. The size of the bacterium ranges from 1-3.5 micrometer x 0.3-0.5 micrometer. Other strains of *X. fastidiosa* cause Pierce's disease of grapevine, plum leaf scald and phony peach. They are known to be vectored by a group of insects commonly called sharpshooters.

The disease is transmitted by grafting. Although spatial analysis of the CVC incidence in Sao Paulo suggested that the disease was transmitted by a vector (Gottwald *et al*., 1993), vector transmission has not been confirmed.

Control of CVC in Brazil remains incomplete. Observations suggest that the rapid spread of the new disease in Brazil probably originates from infected budwood sources (Lee *et al*., 1991). The occurrence and rapid spread of CVC are seriously threatening the citrus production in Brazil.

Witches' broom disease

Witches' broom disease of acid lime was first described by Bove *et al*. in 1988, but the disease presumably occurred in the 1970s in the northern coastal plain of the Sultanate of Oman. The disease had been restricted to the northern coast of the country but it is spreading towards the southern part. Since 1989 it has also appeared in the oases of the coastal mountain range and in the United Arab Emirates (UAE) (Bove *et al*., 1993). It is now impairing the production of sour lime in those countries.

Affected trees are characterized by the presence of compact witches' brooms, multiple sprouts and small to very small leaves, which are often pale green or yellow in color (Bove *et al*., 1988). In the early stages of the disease, the symptoms were observed on only one or a few branches. In the advanced stages, the leaves with older witches' broom lesions died but remained attached to the branch. In the final stages, the trees showed many dead twigs and died. The disease is observed on sour lime, citron, Indian Palestine sweet lime and sweet limetta. Sweet orange and sour orange are not susceptible (Bove *et al*., 1993).

Mycoplasma-like-organisms (WBDL-MLO) are associated with the disease and have been found to be the causal agents based on electron microscopic observations, graft transmission and dodder transmission (Bove *et al*., 1988). Monoclonal antibodies against the WBDL-MLO were applied to detect the pathogen from various leafhoppers captured in affected orchards. It was found that since only *Hishimonus phycitis* showed a positive reaction at a high incidence, this insect is likely to be a vector but the transmission test has not been developed (Bove *et al*., 1993).

Conclusion

Long distance dissemination is generally due to the transportation of budwood or nurseries infected with the disease or vector. In the countries where the disease is not endemic, strict quarantine measures are necessary. Importation of budwood or nurseries from other countries should be prohibited except for certified materials through plant quarantine procedures. We recommend that budwood should be transported by air after washing, disinfecting and full coating with paraffin wax, followed by shoot-tip grafting procedures in a laboratory after arrival. Navaro *et al*. (1991) reported that citrus germplasm exchange between Spain and USA could be achieved using these methods.

Before the disease and its vector become widespread, an eradication program should be implemented rapidly with the cooperation of growers.

In countries where the disease and effective vector are endemic, integrated control should be developed, including the use of disease-free budstock, propagation under disease-free conditions, vector control, elimination of inoculum source and utilization of resistant rootstock if possible. Preinoculation with protective and mild CTV is very effective against stem pitting disease. Integrated control can not fully control the disease, but it may prolong the commercial production period of citrus for some years. For example in Thailand, infected local mandarins usually died within 5-6 years after planting because of greening disease. That is why the prolongation of the productive cycle is very profitable to the grower.

In conclusion the development of a reliable detection method is most important to implement a control program. Development of a rapid, easy, mass-handling and low-cost method is urgent.

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Discussion

Wiles, G. (Papua New Guinea): Since we share a border with Indonesia, I would like to know what is the distribution of greening disease in Indonesia, in particular, whether greening disease is present in Irian Jaya.

Answer: Five years ago the disease was present in Java, Bali, Sulawesi and most of the islands.

Comment: Lizada, M. C. C. (the Philippines): Most of the technical requirements for the control of viral disease of fruit trees are well documented. However, approaches considering socio-economic aspects need to be given some attention, particularly in developing countries. Perhaps we need to share our experience in this area.

Uritani, I. (Japan): You indicated that in cirtrus many bacterial and virus diseases are transmitted by vectors. Are all of the diseases transmitted by vectors?

Answer: Transmission by vectors has been confirmed only for citrus greening disease and tristeza virus disease. For witches' broom and CVC, vector transmission is assumed but has not been verified. It is very difficult to control diseases transmitted by vectors. Even if we plant virus-free materials by using shoot-tip grafting, the plants may become infected with the pathogen in the field by the vector.