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INCIDENCE OF TRISTEZA AND OTHER CITRUS DISEASES IN PARAGUAY

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Abstract. **Citrus tristeza virus (CTV) was detected in more than 96% of the citrus trees tested in the eastern regions of Paraguay. *Toxoptera citricida* (Kirkaldy), an efficient vector of the disease, was commonly observed in these regions. Symptoms on naturally infected limes and grapefruit were generally mild. In the Chaco (western) region, only two plants of the 190 tested were found to be CTV positive, and *T. citricida* was not observed. There were numerous grapefruit and sweet orange trees grafted on sour orange rootstock which were healthy. Of the other diseases, leprosis was a particularly serious disease in the Boquerón department. Phytophthora foot and root rot was a common and serious disease in all regions of the country. Citrus canker was the most important foliar disease in the eastern region. Pink disease, areolate leaf spot, sweet orange scab, melanose, greasy spot, and postbloom fruit drop were also recorded in at least some areas in Paraguay.**

Tristeza disease of citrus, caused by citrus tristeza virus (CTV), an aphid-borne closterovirus (Bar-Joseph et al., 1979), occurs in most citrus-producing areas of the world and is the most economically important viral disease of citrus (Bar-Joseph and Lee, 1989; Bar-Joseph et al., 1989; Lee and Rocha-Peña, 1992). In South America, the first serious tristeza epidemic which caused the decline of large new plantings of sweet oranges trees on sour orange rootstock was recorded in 1930 in Argentina. More than 10 million trees in six provinces were lost, all of them in full production (Bar-Joseph et al., 1979). In Brazil, nearly 20 million citrus trees were destroyed or rendered unproductive in the 1930s and the 1940s (Costa, 1956; Lee and Rocha-Peña, 1992). In Venezuela, nearly 6.6 million trees were killed during the 1980s (Ministerio de Agricultura y Cria, 1981). In Peru, since the discovery of CTV in the 1950s, citrus plantings on sour orange rootstock have declined to the point of unproductivity (Wallace, 1968). CTV is also widely distributed in Bolivia, Colombia, and Uruguay (Rocha-Peña et al., 1995; Timmer et al., 1981; Wallace, 1968). In South America, only the central valley in Chile and isolated areas of Bolivia are known to be relatively free of tristeza problems (Timmer et al., 1981).

Optimum conditions for the development of citriculture are present in Paraguay, which possesses tropical and subtropical climates. A wide variety of citrus is grown in the country, but most are nonbudded seedlings and planted in backyards. The total area planted to citrus has been estimated at 15,000 ha.

The purpose of this study was to determine the distribution of CTV in Paraguay and ascertain the presence of other diseases in the country which might be detrimental to the expansion of the citrus industry in Paraguay.

Materials and Methods

Survey. We examined experimental and commercial citrus groves, and citrus trees growing in backyards in the main citrus areas in the eastern and Chaco (western) regions. In this study, only mature citrus trees (more than 10 years old) were sampled. Leaves and young shoots were examined for symptoms of CTV and citrus bacterial canker disease (CBCD) caused by *Xanthomonas campestris* pv. *citri* (Hasse) Dye (Dye et al., 1980) and tissues were collected for assay. All samples were kept in a plastic bag in a cooler until processed. We used the biological indexing and enzyme-linked immunosorbent assay (ELISA) for the detection of CTV (Bar-Joseph and Lee, 1989; Rocha-Peña and Lee, 1991) and CBCD (Civerolo and Fan, 1982), respectively. Diagnosis for fungal and other diseases reported was based on the symptomatology.

In the **Eastern region,** the following departments and cities were surveyed (Fig. 1): **Central**—several commercial orchards and nurseries in the vicinity of Asunción; **Cordillera—**from the Instituto Agronómico Nacional and from the vicinity of Eusebio Ayala and Itacuruby de la Cordillera; **Caaguazu—**from San Jose, Coronel Oviedo, Caaguazu, J. E. Estigarribia, J. D. Ocampos and from the Instituto Agronómico Salesiano; **Alto Parana—**from the vicinity of J. L. Mallorquin and Minga guazu; **San Pedro—**from the area of San Estanislao, Tacuara, Chore, Jejui, San Pedro de Ycuamandiyu and Cororo; **Concepcion—**from the locality of Paso Tuya, Yby Yau, Horqueta, Belen, and Concepcion; **Amanbay—** from Chiriguelo, Cerro Corá and Pedro Juan Caballero; **Itapua—** from Mayor Otaño and Carlos Antonio Lopez.

In the **Western regions,** collections were made from the followings departments: **Presidente Hayes—**the area of Pozo Colorado and of Teniente Irala Fernandes; **Boqueron—**the area of the Colonias Mennonitas de Filadelfia, Neuland, Yalve Sanga and in Estacion Experimental del Servicio Agropecuario (SAP).

Serological test for CTV. All trees were tested by double-antibody-sandwich ELISA (Rocha-Peña and Lee, 1991) using a kit called "Ingezim-CTV" (Ingenasa, Madrid, Spain) and following the specific instructions provided by the suppliers. Plant extracts were prepared with 0.5 g fresh weight of leaves in a small plastic bag with 1.5 ml of extraction buffer (phosphate-buffered saline, pH 7.4, with 2% polyvinyl-pyrrolidone). Duplicate assays were performed, using a negative (healthy leaves and buffer control) and positive (infected leaves) control each time. These tests were scored visually as positive or negative in routine work.

Biological test for CTV. Citrus mother plants, previously selected in the eastern and Chaco regions for use in the Citrus Improvement Programme conducted in Facultad de Ciencias Agrarias(UNA), were indexed on Mexican lime (*Citrus aurantifolia* (Christm.) Swingle) seedlings (Timmer et al., 1981). These seedlings were grown and maintained in a screenhouse, graft-inoculated with bark from test trees, and maintained for 12 months.

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Plants were examined periodically for typical vein-clearing symptoms of CTV infection.

Serological test for CBCD. Leaves with symptoms that resembled CBCD were collected along the main road in the eastern region and tested by ELISA using specific antibodies and conjugate (provided by Servicio de Sanidad Vegetal, Montevideo, Uruguay). Leaf extracts were prepared by macerating 10 lesions in 1.5 ml of phosphate-buffered saline, pH 7.4, with 0.05% Tween 20 and 2% polyvinyl-pyrrolidone in a small plastic bag. All samples were tested twice with a positive (samples with CBCD) and a negative (healthy leaf and buffer) control.

Biological test for CBCD. All samples collected with symptoms of CBCD were also bioassayed. Young leaves of grapefruit (*Citrus paradisi* Macf.), which is highly susceptible to CBCD, were cut into small pieces with a paper punch and the pieces placed in wells of ELISA plates previously filled with water agar (0.7%). Five to seven lesions were macerated in 1.5 ml of sterile water and then 50 µl of the extract were placed on the leaf disc in each well. Positive (with CBCD) and negative (healthy) controls were included in all tests. After covering the plates with clear plastic wrap, they were incubated at 28°C with continuous light and periodically examined for symptom development for 15 days.

Figure 1. Map of Paraguay showing the departments sampled for citrus tristeza virus and surveyed for other diseases.

Results

Tristeza and other viral diseases. Results from ELISA were obtained for CTV in 685 samples in the eastern and Chaco regions of Paraguay. Diagnosis based on ELISA was confirmed by biological indexing in many cases. We examined 8 departments in the eastern area and two in the western area (Table 1). A high percentage of CTV trees in the 8 departments of the eastern region were infected with CTV. Of 357 mature seedling trees, 92% were infected and of 123 mature grafted trees, all were infected. Mild tristeza vein clearing was observed in Mexican lime and Tahiti lime (*Cit-*

Table 1. Citrus tristeza virus in citrus species and hybrids in the eastern and western regions of Paraguay as determined by ELISA.

	No. positive/total	
Species[z]	Eastern	Western
Seedlings		
Mexican lime	76/76	0/60
Rangpur lime	16/16	0/6
Sweet lime	2/2	—
Grapefruit	117/128	0/17
Sour orange	43/48	—
Sweet orange	63/63	1/12
Tangerine	36/38	—
Rough lemon	8/8	—
Citron	0/1	—
Kumquat	1/1	—
Budded trees (Scion/rootstock)		
Tahiti lime/Rangpur lime	6/6	—
Tahiti lime/rough lemon	14/14	—
Mexican lime/rough lemon	—	0/3
Grapefruit/rough lemon	2/2	1/60
Grapefruit/sour orange	—	0/12
Grapefruit/Troyer citrange	—	0/6
Sweet orange/rough lemon	58/58	1/1
Tangerine/rough lemon	16/16	0/4
Lemon/rough lemon	2/2	—
Pummelo/rough lemon	2/2	—
Tangor/rough lemon	3/3	—

[z]Kumquat (*Fortunella margarita*), tangerine (*Citrus reticulata* Blanco), Rangpur lime (*Citrus limonia* Osb.), tangor (*C. reticulata* × *C. sinensis*), lemon (*Citrus limon* (L.) Burm. F.), Troyer citrange (*C.sinensis* × *Poncirus trifoliata*).

Table 2. Severity of fungal and bacterial diseases of citrus in six departments of Paraguay.

Disease	Central	Cordillera	Caaguazu	Alto parana	San Pedro	Itapua
Foot and root rot	+++[z]	+++	+++	+++	++	+++
Pink disease	+	—	—	+	—	++
Areolate leaf spot	+	+	—	+	+	+
Sweet orange scab	++	++	++	++	+	++
Citrus scab	++	++	++	++	++	++
Melanose[y]	+	+	++	+	+	++
Greasy spot	+	+	+	+	+	+
Postbloom f/drop[x]	—	—	—	+	—	++
Citrus canker	++	+	++	++	++	++

[z]+++ = severe damage; ++ = damage of economic importance on susceptible varieties; + = symptoms observed, but of little economic importance; — = no symptoms observed.
[y]Caused by *Diaporthe citri (*Fawc.) Wolf.
[x]Caused by *Colletotrichum acutatum* J. H. Simmonds.

rus latifolia Tan.) leaves in several places in the eastern region. Stem-pitting was mild or absent on all Mexican limes examined but was observed on some grapefruit and on pera sweet oranges (*Citrus sinensis* (L.) Osb.) in the Central department in the eastern region. Rough lemon (*Citrus jambhiri* Lush.) seedlings in citrus nurseries usually tested negative for CTV. The brown citrus aphid (*Toxoptera citricida* (Kirkaldy), the highly efficient vector of CTV, was observed commonly in the eastern region. Lime indexing tests were conducted for 45 trees in the eastern region. All developed typical CTV symptoms and these results were corroborated by ELISA.

The situation was different in the Chaco area. Only 2 trees tested positive from a total population of 190 trees (94 mature seedling trees and 96 grafted mature trees) (Table 1). We also found numerous locations where grapefruit on sour orange (*Citrus aurantium* L.) rootstock, and Mexican lime seedlings were growing well and did not show any CTV symptoms. The brown citrus aphid was not observed in the Chaco area.

Because most of the trees in backyards and in some commercial plantings are of seedling origin, the common graft-transmissible viral diseases were rare in Paraguay. Symptoms of exocortis and cachexia were seen on trees budded on susceptible rootstocks such as Troyer citrange (*C. sinensis* × *Poncirus trifoliata*) and sweet lime (*C. limettiodes* Tan.) in the Itapua and Boqueron Departments, respectively. Psorosis-like symptoms and concave gum symptoms were observed on budded sweet orange in the Central, Itapua and Boqueron Departments. The presence of exocortis, psorosis and concave gum was confirmed by indexing on Etrog citron (*Citrus medica* L.) and sweet orange, respectively. Leprosis, a virus disease transmitted by mites in the genus *Brevipalpus*, produced extensive crop losses and tree decline in sweet oranges in the Boquerón department of the Chaco region, but also was commonly observed in the Central, Alto Parana, and Itapua departments of the eastern region.

Citrus Canker. Citrus canker (CBCD) was widespread in the citrus area in the eastern regions and was the most important foliar disease particularly on grapefruit and on some varieties of sweet orange. All of samples collected suspected of being citrus canker were confirmed by ELISA and by bioassay.

Fungal diseases. The relative severity of fungal and bacterial diseases in six departments is indicated in Table 2. Foot and root rot caused by *Phytophthora* spp. was a common and serious disease because seedling sweet orange and grapefruit trees and rough lemon rootstock are highly susceptible to the disease. Many young trees were killed and older trees were weak and unproductive. The high rainfall and fine-textured soil created ideal conditions for *Phytophthora* spp. A bark rot, known as pink disease, caused by *Corticium salmonicolor* Berk. & Br., was particularly serious on sweet orange trees in the Itapua department. Sweet orange scab, caused by *Elsinoe australis* Bitancourt & Jenkins, occurred on sweet oranges in most of the citrus production area and affects the marketability of the fruit. Citrus scab, caused by *E. fawcettii*, is also a serious disease of rough lemon seedlings in seedbeds and nurseries. Other common fungal diseases like areolate leaf spot, caused by *Pellicularia filamentosa* (Pat.) Rogers, and greasy spot, caused by *Mycosphaerella citri* Whiteside, were present but not serious in most regions. Other diseases were absent or unimportant in citrus culture.

Discussion

Citrus tristeza virus was found to be widespread in the major citrus area in eastern Paraguay. All grafted trees tested were infected with CTV which probably occurred because nurseries imported young grafted trees and budwood from Brazil and Argentina, countries where CTV has been endemic and widespread since the 1930s. In spite of the fact that CTV is not a seed-transmitted disease (McClean, 1957), we also detected CTV in a high percentage in mature seedlings in this region (92%) which demonstrates the vector efficiency of brown citrus aphid that is very common in this area.

In the Chaco region, the situation was different, and CTV was found only rarely. This citrus-growing area is isolated, about 300 kilometers from the eastern regions, and there is little interchange between the two areas. There is only one nursery (Servicio Agropecúario del Chaco Central) that propagated budwood free from CTV imported from Texas from 1960 to 1970. The absence of CTV and the brown citrus aphid in western Paraguay is remarkable given that this area is surrounded by affected areas and that the movement of the brown citrus aphid has been so rapid in Central America and the Caribbean (Rocha-Peña et al., 1995). The Facultad de Ciencias Agrarias (UNA) and the Ministerio de Agricultura are trying to promote citriculture in the Chaco region by developing certification schemes coupled with clean stock programs to ensure that all budwood that is introduced into this area is certified free of CTV as well as other graft-transmissible pathogens. For other serious diseases such as foot and root rot, CBCD and leprosis, management strategies such as rootstock selection and integrated management must be implemented in order to reduce the impact of those diseases.

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POTENTIAL USE OF COMPOSTED MUNICIPAL WASTE FOR MANAGEMENT OF PHYTOPHTHORA ROOT ROT OF BEARING CITRUS

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Abstract. **Most citrus grove soils in Florida are infested with *Phytophthora nicotianae*, the causal agent of Phytophthora root rot which results in slow decline of tree condition and fruit production. The potential for composted municipal solid waste (CMW) as a soil amendment to provide long-lasting suppression of *P. nicotianae* and enhance root health and tree yield was evaluated. Composted municipal solid waste was applied to two groves of 25-yr-old Marsh grapefruit trees on sour orange rootstock and one grove of 6-yr-old Valencia orange trees on Carrizo citrange rootstock growing on marginal soils in a stage of decline in tree vigor. Two sources of CMW were applied as 5- to 10-cm-thick mulch layer under the tree canopy. Rhizosphere population densities of *P. nicotianae* were not reduced, but in some cases were increased by CMW. Root density response was site-dependent. Trees treated with CMW were more densely foliated probably due to improved soil moisture availability. Yields were not significantly greater at the second harvest after the application of CMW, but fruit size of Marsh grapefruit and Valencia orange was increased. The third year after application, CMW increased yield and size of grapefruit and size of orange. While CMW did not suppress *P. nicotianae*, the potential for CMW to improve citrus tree condition and productivity on certain marginal soils was demonstrated.**

Citrus, the most economically important and widely planted crop in Florida, covers about 273,000 ha in central and south Florida. The center of the citrus industry is within a 100-mile radius of over 7 million people producing 5 kg of solid waste per person per day. Between 1992 and 1994, over 10.3 million new trees were planted on sandy soils, mostly low in organic matter, and poor in nutrient and water holding capacity. Our previous research has demonstrated that mulch treatments of composted municipal wastes (CMW) consistently stimulate growth of young trees in newly established groves (Widmer et al., 1996, Widmer et al., 1998b). Responses of young trees to CMW were seen on sandy soils typical of both the ridge and flatwoods production areas. Increases in stem diameter and root mass of young trees ranged from 20 to 30% and occurred in response to surface mulch treatments after planting, as well as incorporation treatments at planting.

In bearing citrus, *Phytophthora nicotianae* Breda de Haan (synonym = *P. parasitica* Dastur) is an endemic root pathogen that causes a slow decline in canopy vigor and fruit production (Lutz and Menge, 1986). Applications of the fungicides, fosetyl-Al and metalaxyl, control fungal infection of roots and increase fibrous root density (Timmer et al., 1989). Although fungicides increase yields and fruit size of orange and grapefruit trees on disease susceptible rootstocks, yield response is often variable. Therefore, fungicides are not recommended unless populations of *P. nicotianae* exceed a threshold of 10 to 15 propagules per cm of soil. Fungicides are usually applied to the soil through the irrigation system two to three times over multiple seasons to be effective and economical for application. However, soil-applied pesticides are prone to loss of efficacy after prolonged usage and, since they are water soluble, have the potential to leach into the groundwater after excessive irrigation or rainfall (Kookna et al., 1995). Another recent finding in Florida nurseries is fungal resistance to metalaxyl which raises concerns about the long-term usage of this fungicide in citrus groves (Timmer et al., 1998).

An alternative strategy to fungicides for sustained control of soilborne diseases is to periodically apply composted organic materials as amendments to suppress fungal root pathogens. Composted bark, when incorporated into potting media, suppresses soil borne diseases of ornamental plants (Hoitink and Fahey, 1986; Hoitink and Grebus, 1994). In Australian avocado groves, root rot caused by *P. cinnamomi* has been effectively controlled by intensive mulching with organic amendments and applications of gypsum (Broadbent and Baker, 1974).

In our studies of citrus (Widmer et al., 1996; Widmer et al., 1998a, b), CMW reduced the incidence of infection by *P. nicotianae* on citrus seedlings in the greenhouse and improved growth of newly planted trees in several field trials in spite of inoculation with *P. nicotianae* at transplant. The purpose of this study was to examine the potential for CMW, applied as a mulch layer on the soil surface, to suppress *P. nicotianae* and improve root health and fruit yields of mature citrus trees growing on marginal soil types with damaging soil populations of the pathogen.

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