

# Transmission of Citrus Leprosis Virus by *Brevipalpus phoenicis* (Acari: Tenuipalpidae)

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**ABSTRACT.** This work demonstrated the occurrence of populations of the *Brevipalpus phoenicis* mite that carry citrus leprosis virus (CiLV) in mandarin and the transmission of leprosis to mandarins from symptomatic mandarin trees. Mites were transferred to healthy Cleopatra mandarin and *Ligustrum lucidum* (Oleaceae) seedlings. Leaves of Cleopatra, infested with these mites, showed yellowish punctuations after 60 d. that developed into lesions with concentric rings, typical of citrus leprosis. Electron microscopy of thin sections from the lesions of mandarin leaves, both from field plants and experimentally inoculated by mites, revealed the presence of short bacilliform particles within membrane bounded cavities in the cytoplasm, as well as the dense cytoplasmic inclusions, characteristic of citrus leprosis virus infection. Symptoms were not observed in *L. lucidum* and in the healthy Cleopatra control plants for up to 4 mo. In another experiment, greenhouse-grown 2 yr-old Willow leaf mandarin plants grafted on Rangpur lime, infested with mites collected from sweet orange trees with leprosis, also developed symptoms in leaves and fruits. This is the first report of the experimental transmission of leprosis from mandarin to mandarin and from sweet orange to mandarin.

*Index words:* mites, *Ligustrum*, IPM.

The mite, *Brevipalpus phoenicis* (Geijskes), has an extensive host range and may cause economic damage depending on the host. Pritchard and Baker (10) list over 65 hosts and Ochoa et al. (8) list 114 hosts. In Brazil, there are reports of diseases associated with *B. phoenicis* infestation in important crops such as citrus, coffee (*Coffea arabica* L.) and passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg) (3, 4, 7) and ornamentals such as *Ligustrum* (11) and *Hibiscus* (6). In these cases, *Brevipalpus* mites act as vectors for diseases such as citrus leprosis, coffee ringspot, *Ligustrum* ringspot and passion fruit green spot. This mite in other parts of the world is a common and important pest on tea and citrus (9, 12).

Citrus leprosis (CiLV), a disease characterized by ring-like lesions in leaves, twigs and fruits, commonly occur in sweet orange, being caused by short bacilliform particles resembling rhabdovirus (2), but has not been frequently reported in other citrus species, especially in mandarins which were considered resistant and a possible source for genetic resistance for this virus (1, 13).

In this work we report experimental data showing that mandarins are susceptible to citrus leprosis virus by observation of infected field plants and the experimental transmission of the virus.

## MATERIALS AND METHODS

**Transmission from mandarin to mandarin.** Several field surveys in different mandarin orchards in the State of São Paulo (Cordeiropolis and Piracicaba) detected plants with leaf lesions similar to those caused by citrus leprosis virus in sweet oranges associated with a *B. phoenicis* mite infestation. Leaf samples with lesions and harboring mite populations were collected and brought to the laboratory. These mites were transferred to ten healthy Cleopatra mandarin and *Ligustrum lucidum* seedlings. As control, the same number of mandarins and *L. lucidum* plants were also infested by mite populations originated from eggs and raised on *L. lucidum*. Non-mite infested plants consisted another control plot. All the plants were maintained in the laboratory at room temperature under fluorescent light.

**Transmission from sweet orange to mandarin.** In another experiment, six 2 yr-old Willow leaf mandarin plants grafted on Rangpur lime kept in pots in the greenhouse were infested with mites collected from greenhouse sweet orange plants with leprosis. Four plants without mite infestation and four plants infested by mites raised on healthy plants served as controls.

**Electron microscopy.** Samples from leaf lesions from original field plants (mandarins and sweet oranges) as well as from the experimentally inoculated mandarins were processed for electron microscope histological studies (5). Small pieces of leaf tissues from the lesions (and from healthy leaves as controls) were fixed in a modified Karanovsky solution (2.5% glutaraldehyde, 2.5% paraformaldehyde in 0.05M cacodylate buffer pH 7.2), post-fixed in 1% OsO<sub>4</sub> in the same buffer, dehydrated in acetone, embedded in Spurr's medium, sectioned in an ultramicrotome with diamond knife, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 900 transmission electron microscope.

## RESULTS

**Mandarin to Mandarin transmission.** Leaves of Cleopatra mandarin seedlings infested with mites collected from Cleopatra plants in the field with CiLV-like symptoms showed yellowish punctuations after 60 days. These spots developed into lesions with concentric rings typical of CiLV one week later; essentially similar to the CiLV lesions in sweet orange plants. However, no lesion developed on infested *L. lucidum* plants (Fig. 1A and B). Also, no lesions appeared on mandarins infested by mites raised on *L. lucidum*, or kept mite free.

**Sweet orange to mandarin transmission.** Willow leaf mandarin infested by *Brevipalpus* mites collected from greenhouse sweet

orange infected with CiLV, developed typical leaf symptoms of CiLV in the leaves and fruits about 4 mo after initial infestation (Fig. 1 C and D).

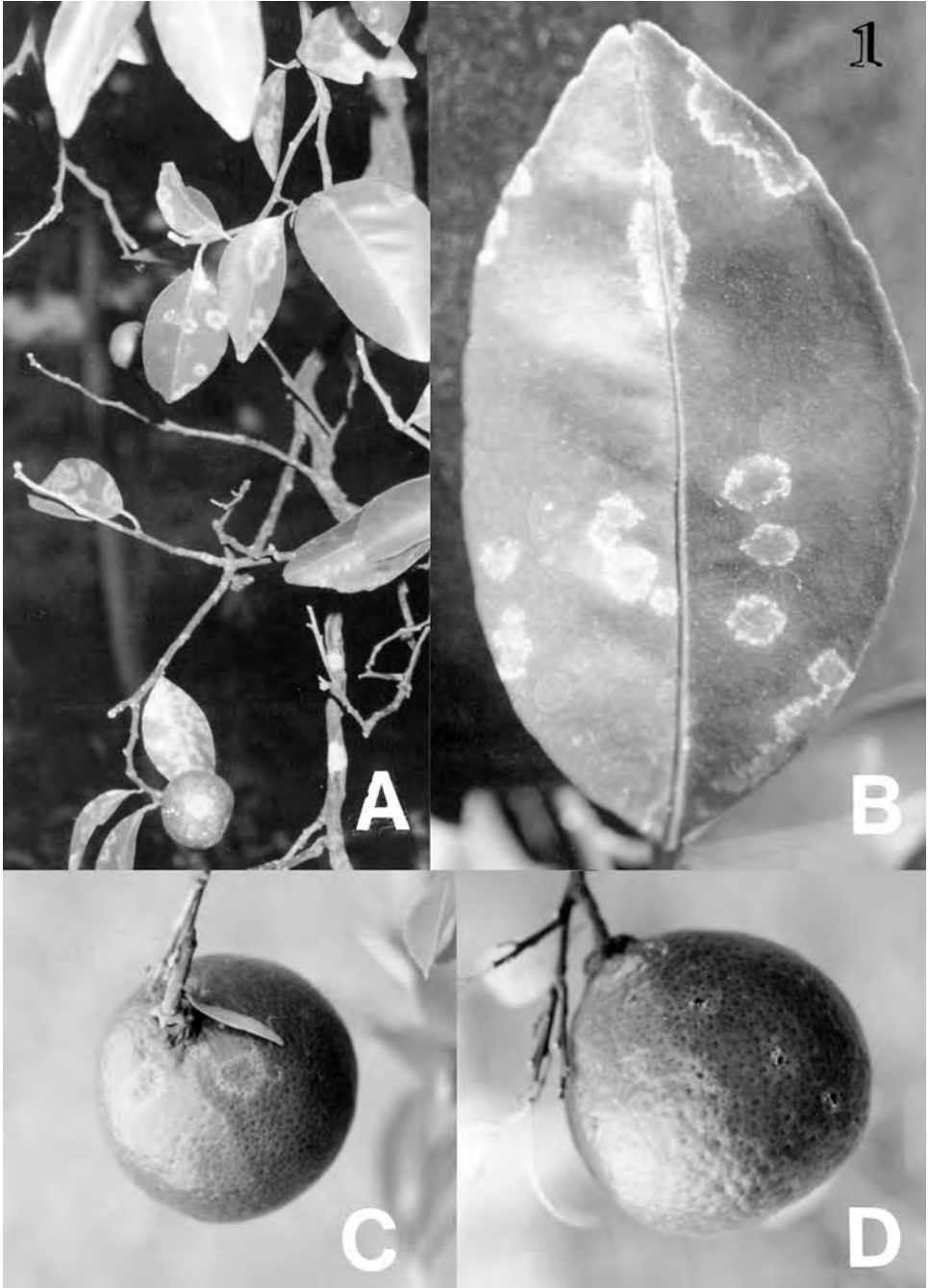
**Electron microscopy.** Typical cytopathic effects of CiLV infection, characterized by the presence of short, bacilliform particles (ca. 40 to 50 nm × 100 to 120 nm) within membrane bounded cavities of the endoplasmic reticulum and the presence of dense, viroplasm-like masses in the cytoplasm were found in cells from leaf lesion from field affected and experimentally infected mandarins (mandarin to mandarin and sweet orange to mandarin) (Fig. 2).

## DISCUSSION

The experimental data here reported clearly indicates that mandarins (both *C. reshni* and *C. deliciosa*) are susceptible to CiLV infection when inoculated with viruliferous *B. phoenicis* mite populations derived either from infested mandarin or sweet orange plants. The infrequent observations of leprosis symptoms in mandarins, in the field, may be due to other factors like more efficient elimination of affected tissues in mandarin trees, different levels of sensitivity or different management practices used for fruit production mandarins.

*Ligustrum* was revealed to be immune to CiLV inoculation by *B. phoenicis* suggesting that this virus is different from *Ligustrum* ringspot virus, which is morphologically and cytopathologically very similar to CiLV, being also transmitted by *B. phoenicis* (11).

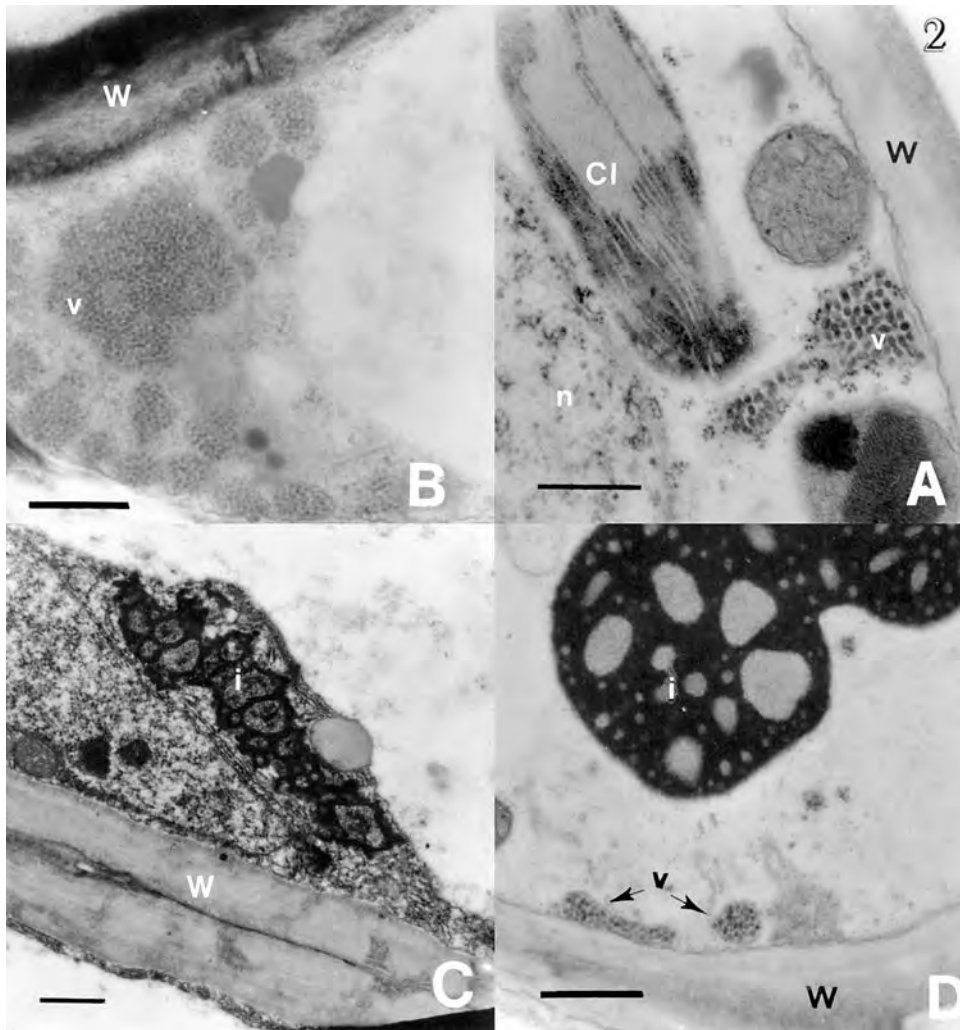
The results presented strengthens the need that the integrated pest management (IPM) and the field inspections routinely carried out in sweet orange orchards in São Paulo for the leprosis mite vector should also include neighboring mandarin orchards that may harbor both the vector and the virus. Transmission tests with CiLV and other similar viruses such as Coffee ringspot (3),



**Fig. 1.** Symptoms of CiLV in Cleopatra mandarin: A) Symptoms in field infected tree, B) Symptoms in Cleopatra inoculated with *Brevipalpus* mites from mandarin tree with leprosis, and C + D) Leprosis symptom fruits of Willow leaf mandarin from plant inoculated by infesting it with mites from sweet orange tree infested with CiLV.

Ligustrum ringspot (11), and Green spot of Passion fruit (4) transmitted by *B. phoenicis* in different hosts

should be intensified to clarify biological relationships of these viruses. Equally, vector efficiency of popula-



**Fig. 2.** Transmission electron micrographs of thin sections leaf tissues showing CiLV symptoms obtained from Cleopatra mandarin experimentally infested with viruliferous *Brevipalpus phoenicis*. A) Particles with round or bacilliform profile contained in cytoplasmic membrane-bounded cavities, B) group of virus-like particles in the cytoplasm, C and D) Viroplasm (i) inclusions and virus-like particles (v) near a large cytoplasmic inclusion (i). Bars = 0.8  $\mu$ m. Cl = chloroplast; n = nucleus; W = cell wall.

tions of mite vectors derived from distinct host species and different geographical localities needs to be determined. Selection of parents of

genetic lines to be utilized in breeding programs to develop resistance to CiLV should be based on intensive testing using different virus sources.

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