Additional Evidence that Tristeza Virus Multiplies in *Passiflora* spp.

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Repeated attempts have been made to extend the host range of tristeza virus outside the family Rutaceae. Over 200 species have been tested. None showed symptoms attributable to tristeza virus, nor could the virus be recovered from inoculated plants.

In 1970, Dr. Arturo Osores, plant pathologist from Peru, informed the writers that he had infected a *Passiflora* spp. with tristeza virus. This observation led us to test several species, including *P. edulis,* the commercial passion fruit. Results are reported here.

MATERIALS AND METHODS

Seedlings of *Passiflora* spp. were raised in the greenhouse in clay pots 15 cm in diameter, filled with a mixture of compost and soil.

Passiflora seedlings (about one month old) were inoculated with the tristeza virus by means of the Oriental citrus aphid, *Toxoptera citricidus* Kirk., collected from colonies formed naturally on Barão sweet orange trees growing in an orchard of the Centro Experimental de Campinas of the Instituto Agronômico. These trees had been tested in the past and found to be infected with a severe isolate of tristeza virus. Leaves and/or young twigs of the Barão sweet orange virus source, bearing 30 to 50 aphids, were placed on the *Passiflora* plants in cages. Each plant was inoculated three different times, and with each inoculation, aphids remained on the plants 48 hours. Five plants of each species were inoculated, and five were kept as uninoculated controls.

We attempted to recover the tristeza virus from inoculated plants and transmit it to Galego lime seedlings by means of the aphid vector obtained from non-viruliferous colonies reared on squash. Aphids were left on *Passiflora* plants for 12 hours, then transferred to Galego lime seedlings. Controls consisted of Galego lime seedlings colonized with aphids previously fed on uninoculated *Passiflora* plants.

Electron microscopy studies were made with negatively-stained leaf-dip preparations (*3*). For histological studies, small pieces of leaf tissue were fixed in 3 per cent glutaraldehyde and post-fixed in 1 per cent OsO_4 (both in phosphate buffer), dehydrated in acetone, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and leaf citrate before examination in a Siemens Elmiskop IEM.

RESULTS

Ten *Passiflora* spp.* were tested: *P. alata* Dryander; *P. bryonioides* H. B. K.; *P. caerulea* L.; *P. edulis* Sims; *P. foetida* L.; *P. gracilis* Jacq.; *P. macrocarpa* Mast.; *P. maliformis* L.; *P. suborosa* L.; and one undetermined. Two months after inoculation, all five inoculated *P. gracilis* plants developed symptoms of stunting and yellowing of the interveinal areas. Veins and veinlets re-

* Species names are those accompanying the original seed samples received from various parts of the world by the Botany Department, Instituto Agronômico de Campinas. *Passiflora gracilis* was introduced from the Jardin de Aclimatación de La Orotava, Puerto de La Cruz, Tenerife, Canary Islands, Spain.

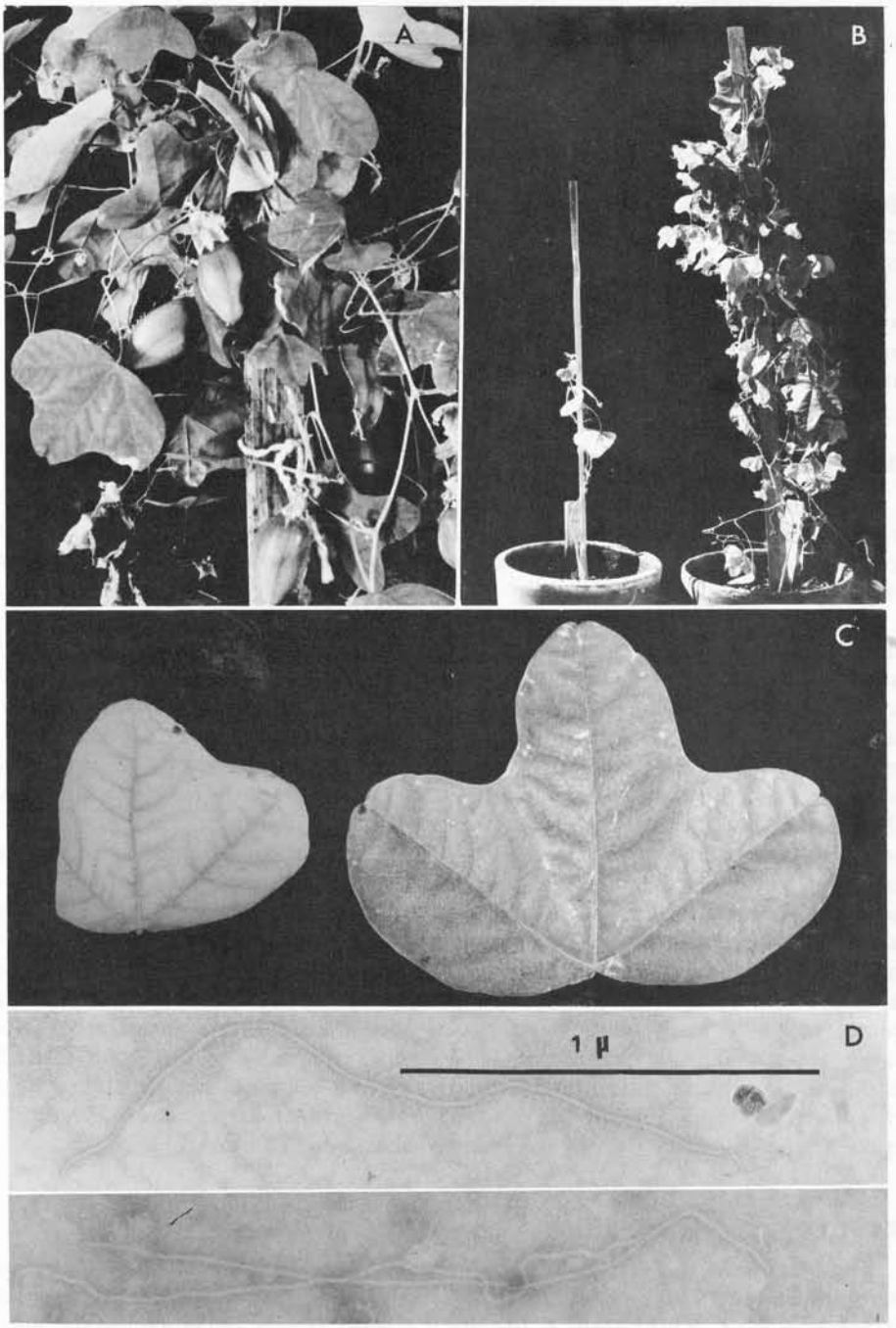

Fig. 1. ***A.*** **Uninoculated** ***Passiflora gracilis*** **plant.** ***B.*** **Tristeza-infected** ***P. gracilis:*** **left, with severe chlorosis and stunting; right, uninoculated control plant.** ***C.*** **Left, leaf of** ***P. gracilis*** **with symptoms of tristeza. Note reduced size and severe interveinal chlorosis when compared with leaf from uninoculated control plant (right).** ***D.*** **Electron micrographs of tristeza virus particles in leaf-dip preparations. Top, from infected** ***P. gracilis;*** **bottom, from Galego lime seedling.**

mained green. Faint vein clearing could be seen in some very young leaves. Leaves eventually became completely chlorotic (fig. 1A) and plants died about one month after appearance of the first symptoms (fig. 1B). Uninoculated control plants developed normally (fig. 1A, B), and were still alive several months after the inoculated plants had died.

We collected seeds from the healthy plants, and attempted to transmit tristeza virus to them by means of the Oriental citrus aphid. In this second attempt, all inoculated plants developed symptoms described above.

Transfers of tristeza virus from affected *Passiflora gracilis* to Galego lime seedlings by means of the aphid vector induced vein clearing symptoms, typical of tristeza virus, in at least one of six Galego lime seedlings inoculated. None of the aphids fed on uninoculated *P. gracilis* induced tristeza symptoms.

Leaf-dip preparations from *Passiflora gracilis* plants showing chlorosis and stunting consistently contained thread-like particles about 10 nm wide and varying in length from 1,000 to 2,000 nm (fig. 1D). No particles were present in control plants. These particles were identical with those reported in citrus plants infected with tristeza virus (*1, 2, 5, 6, 7*). In ultrathin sections of infected leaf tissues of *Passiflora* plants, cytoplasm in some cells adjacent to sieve tubes contained a mass of flexuous, elongated particles, 9 nm wide, similar to those previously described for tristeza-infected citrus plants (*2, 4, 6, 7*).

DISCUSSION AND CONCLUSIONS

Results of inoculation tests, recovery attempts, and electron microscopy indicate that *Passiflora gracilis* plants were infected with tristeza virus. The presence in infected *P. gracilis* of thread-like particles similar to those found in infected citrus plants supports the previously reported association of such particles with tristeza disease (*5*).

Tristeza virus was recovered from an infected *Passiflora gracilis* plant. Poor virus recovery from this plant is attributed to the feeding habits of *Toxoptera citricidus,* which did not feed well on the diseased plants, and usually did not remain on them longer than 12 hours.

That a species outside the family Rutaceae is a host plant of the tristeza virus is now a fact. Studies aimed at extending the host range should be carried out. This might lead to finding a better assay and source plant for the virus.

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LITERATURE CITED

1. Bar-Joseph, M., G. Loebenstein, and J. Cohen
1970. Partial purification of viruslike particles associated with the citrus tristeza disease. Phytopathology **60:** 75–78.
2. Chen M., T. Miyakawa, and C. Matsui
1971. Tristeza virus in *Citrus reticulata* and *C. tankan*. Phytopathology **61:** 279–82.
3. Kitajima, E. W.
1965. A rapid method to detect particles of some spherical plant viruses in fresh preparations. Jour. Electronmicroscopy (Tokyo) **14:** 119–21.

4. KITAJIMA, E. W., AND A. S. COSTA
1968. Electron microscopy of tristeza infected leaf tissues. *In:* Proc. 4th Conf. Intern. Organ. Citrus Virol. (J. F. L. Childs, ed.) Gainesville: Univ. Florida Press, pp. 59–64.
5. KITAJIMA, E. W., D. M. SILVA, A. R. OLIVEIRA, G. W. MÜLLER, AND A. S. COSTA
1964. Thread-like particles associated with tristeza disease of citrus. Nature **201:** 1011–12.
6. PRICE, W. C.
1966. Flexuous rods in phloem cells of lime plants infected with citrus tristeza virus. Virology **29:** 285–94
7. SHIKATA, E., AND A. SASAKI
1969. Long flexuous threads associated with Hassaku dwarf disease of citrus trees. Jour. Fac. Agric. Hokkaido Univ. **56:** 219–24.