Transmission of Citrus Tristeza Virus (CTV) from "Declinamiento"-Diseased Sweet Orange Trees on Trifoliate Orange Rootstocks and its Epidemiology in Relation to the Flight Activity of the Vector, Toxoptera citricida Kirk. (Homoptera: Aphididae) in Misioness, Argentina[1]

O. Nickel, F. Klingauf, and H. U. Fischer

ABSTRACT. Declining and healthy sweet orange trees on trifoliate orange rootstock in Misiones, Argentina, were shown to be infected with citrus tristeza virus (CTV). The virus was acquired readily by the tropical citrus aphid (*T. citricida*) and transmitted to three test plants: a) Key lime seedlings, b) 'Calderón' sweet orange on trifoliate orange, and c) 'Koethen' sweet orange on trifoliate orange. Trifoliate orange seedlings were also inoculated, but could not be infected. Transmission trials were carried out with single aphids and with colonies of the vector. Visual diagnosis of CTV infection in Key lime seedlings was confirmed by serological analysis of the inoculated material. Under field conditions the pattern of natural spread of CTV is seasonal, with winter, spring, late summer, and early fall being the periods of highest infection pressure. The infection of Key lime seedlings used as trap plants in the field was significantly correlated with the period of greatest flight activity of the vector and the number of colonies on the trap plants. The possibility of using mild strains of CTV in virus control strategies is discussed.

Citrus tristeza virus has long been known to be highly unstable (2, 8). Since the tristeza calamity in the 1940's in South America and the replacement of sour orange as a rootstock for sweet orange, grapefruit and tangerine, a number of new strains have formed, which attacked formerly resistant scion-rootstock combinations. A critical situation for the citrus industry in some locations of southern Brazil is due to the appearance of severe tristeza strains with new epidemiological properties (4).

The symptomatology of "declinamiento", which occurs in Misiones, has some features in common with severe tristeza. Therefore, we felt it worthwhile to investigate the question, whether tristeza is linked with "declinamiento", as has been suspected by various experts (5, 6, 7).

One approach, which has been initiated in the Argentine-German project, is aphid transmission studies from "declinamiento"-affected and apparently healthy field trees, as well as field transmission tests. One obstacle, which interfered with these studies, is that tristeza is endemic in Misiones, as it is in all major citrus production areas of South America.

INOCULATION EXPERIMENTS

Four groups of virus-free test plants (Key lime, trifoliate orange, 'Calderón' sweet orange on trifoliate orange rootstock; and 'Koethen' sweet orange on trifoliate orange rootstock) were artificially inoculated with tristeza isolates from obviously declining and apparently healthy field trees of 'Calderón' sweet orange on trifoliate orange rootstock. Attempts to transmit

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the virus isolates were made using 30-50 individuals of the tristeza vector, *Toxoptera citricida* per test plant. The aphids, reared under screen on virus-free Key lime plants, were allowed an acquisition period of 24 hours on Key lime plants previously infected with field isolates. Subsequently, they were transferred to the test plants, where they were allowed an inoculation access period of 24 hours before being killed by insecticide. The transmission success was evaluated by reading symptoms and the enzyme-linked immunosorbent assay (ELISA) (1).

Table 1 summarizes the results. a) Almost every test plant group became partially or totally infected from both virus sources. b) Sweet orange plants on trifoliate orange rootstock did not develop symptoms during the observation period of 2 years, but CTV infection of many plants was confirmed by ELISA. c) Key lime control plants, on which aphids from the stock culture had fed, did not show symptoms, nor did the plants used for rearing aphids.

For testing the transmission efficiency of different instars of *T. citricida*, single individuals or groups of 10 or 30 aphids of each of the larval, wingless or winged stages of insect were used in separate experiments. Acquisition and inoculation feeding periods were 24 hours. The virus source plant had been inoculated with a tristeza isolate from a "declinamiento"-affected field tree. The results of this study are compiled in **table 2.** a) All 3 stages were capable of transmitting tristeza, either as single individuals or as colonies. b) Wingless aphids appear to be slightly more effective in their transmission than the winged stage. In a separate experiment, evidence was obtained that transovarial passage of these tristeza isolates did not occur.

FIELD TESTS

Tristeza transmission from "declinamiento"-diseased trees under natural conditions was investigated in a trap plant experiment. Virus-free, screenhouse-raised Key lime seedlings were placed in groups of 4 plants at two locations in each of two declining

TABLE 1
TRANSMISSION OF CITRUS TRISTEZA VIRUS FROM HEALTHY APPEARING (1-9) AND DECLINING (10 + 11) SWEET ORANGE TREES ON TRIFOLIATE ORANGE ROOTSTOCK TO 'KEY LIME', 'CALDERÓN' SWEET ORANGE AND 'KOETHEN' SWEET ORANGE TRIFOLIATE ORANGE TEST PLANTS BY *TOXOPTERA CITRICIDA*

Expt. no.	Source plant	'Key lime'		'Cald.'/P. trif.		'Koethen'/P. trif.	
		Leaf sympt.	ELISA	Leaf sympt.	ELISA	Leaf sympt.	ELISA
1	healthy	3/4*	4/4	0/4	2/4	0/4	1/4
2	healthy	4/4	4/4	0/4	3/4	0/4	2/4
3	healthy	3/4	2/4	0/4	0/4	0/4	2/4
4	healthy	4/4	4/4	0/4	3/4	0/4	4/4
5	healthy	4/4	2/4	0/4	3/4	0/4	2/4
6	healthy	3/4	3/4	0/4	4/4	0/4	2/4
7	healthy	3/4	4/4	0/4	3/4	0/4	3/4
8	healthy	3/4	4/4	0/4	1/4	0/4	2/4
9	healthy	0/4	1/4	0/4	2/4	0/4	2/4
10	declining	4/4	4/4	0/4	0/4	0/4	1/4
11	declining	4/4	4/4	0/4	—	0/4	0/4

* number positive/number tested.

TABLE 2
TRANSMISSION OF CITRUS TRISTEZA VIRUS BY DIFFERENT MORPHOLOGICAL STAGES OF *TOXOPTERA CITRICIDA* FROM INFECTED TEST PLANTS TO HEALTHY KEY LIME

Morphological stage	No. of individuals/ plant	Infection rate	Infection (%)
Larvae	1	2/12*	16.6
	10	10/12	83.3
	30	7/12	58.3
Wingless adults	1	8/12	66.6
	10	9/12	75.0
	30	11/12	91.6
Winged adults	1	3/12	25.0
	10	7/12	58.3

* number infected/number exposed.

orange groves. The 4 plants were kept on supports at an altitude of about 1.30 m at pot rim level (fig. 1). The exposure time was 3 to 5 weeks and the plants were maintained thereafter in a growth chamber at about 23°C for symptom expression. Test plants removed were replaced immediately by new ones.

Aphid flight activity was assessed with a modified version of the Moericke yellow water trap (3). A refilling device prevented the traps from drying (fig. 2). One

Fig. 1. Support system used for field exposure of Key lime trap plants.

Fig. 2. Refilling device used for yellow pan aphid traps.

water trap was placed in the neighbourhood of each of the trap plant groups on supports at about the same height as the trap plants.

The trap plant experiment was conducted for 33 months between March 1978 and December 1980. Aphid trapping started in January 1979, and colony counting in September 1979.

Fig. 3 presents the records obtained in the field tests on both experimental sites. a) From the data it is evident that there is no interruption of aphid colonization in the field, and winged aphids can be captured at any time of the year. b) Aphid-borne tristeza infection can occur throughout the year, although the intensity varies seasonally. c) In the trap plant experiment, two main peaks of infection were recorded, one at the end of winter and in spring, the other in late summer and fall. However, exceptionally high infection rates outside these periods can occur, as noted during summer 1980 in Eldorado.

Out of 528 trap plants exposed to natural infection at both experimental sites, 133 showed vein clearing symptoms. Of the infected plants, 28 (19%) developed stem pitting of which 14 also showed vein corking. Reduced leaf size, cupping and leathery condition of the leaves, gum impregnation in stem pits and marked stunting of the plants were constantly associated with the other severe symptoms.

Severe symptoms appeared twice during the observation period, in winter 1978 and again in fall 1979. It is believed, that their occurrence depends on seasons with low average temperatures.

Infection frequency of the trap plants was significantly correlated with the flight activity of *T. citricida* (r = 0.64 at Eldorado, r = 0.60 at Mado) and with aphid colonization of the trap plants (r =

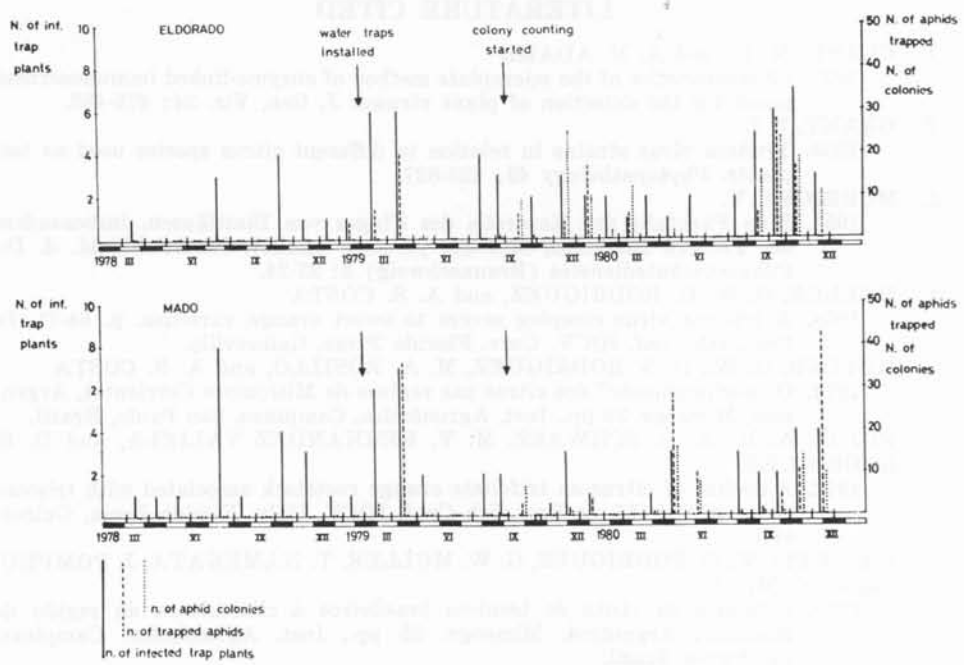

Fig. 3. Infection of Key lime trap plants with citrus tristeza virus, aphids captured in yellow water traps and colonization of trap plants by Toxoptera citricida **in Eldorado and Mado, Misiones.**

0.72 at Eldorado, r = 0.70 at Mado) at both experimental sites.

DISCUSSION

Characteristic differences between tristeza isolates originated from "declinamiento"-affected and those from healthy rated sweet orange trees with respect to their symptomatology on aphid-infected Key lime test plants have not been stated in our study. Hence, the experiments did not give an answer to the question, if tristeza is linked with the etiology of the "declinamiento"-phenomenon.

An important finding in the field trials was, that vector transmission of tristeza can happen at any time of the year, with the exception of extended periods of hot and dry weather during summer.

The high positive correlation between trap plant colonization by *T. citricidus* and the tristeza infection rate can be explained with the specific virus-vector relationship. Tristeza as a semi-persistent virus needs the aphids to be in a "settlement mood" for transmission success.

The classification of 'mild' and 'severe' virus strains in this study is only valid with respect to symptom expression on Key lime test plants. Key lime is not useful as a differential host for assessing the virulence of tristeza strains on other *Citrus* spp. Therefore, the selection of mild strains for cross protection control strategies cannot be based on Key lime reactions. For this purpose it is inevitable to test their behaviour in serial transfers on the scion-rootstock combination itself, that has to be protected.

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