

VARIATION IN THE SEVERITY OF CITRUS TRISTEZA VIRUS ISOLATES FROM GROVES WITH QUICK DECLINE

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Abstract. Citrus tristeza virus (CTV) isolates were collected from healthy appearing and quick decline trees on sour orange rootstock from several locations in Florida. The isolates were biologically indexed on: Mexican lime [*Citrus aurantifolia* (Christm.)] for severity of vein-clearing and stem pitting, Valencia sweet orange [*C. sinensis* (L.) Osbeck] on sour orange [*C. aurantium* L.] for stunting and decline, grapefruit [*C. paradisi* Macf.] seedlings for stem pitting and seedling yellows, and 'Madam Vinous' sweet orange for stem pitting. None of the isolates caused stem pitting on grapefruit or sweet orange nor caused a seedling yellows reaction. Only isolates from 3 of 8 locations induced decline when assayed on 'Valencia'/sour orange indicator plants. Several CTV isolates collected from quick decline trees in groves with fluctuating water tables did not cause quick decline on the indicator plants and were considered to be relatively mild isolates by biological indexing. Probably the stress placed in the root system of citrus trees on sour orange rootstock in areas with fluctuating water tables allows some mild isolates the advantage needed to cause quick decline.

Citrus tristeza virus was first confirmed by indexing on Mexican lime to be present in Florida in Orange, Lake, and Highland Counties early in the 1950's (8). In 1953, Cohen and Knorr (4) reported widespread presence of tristeza in Florida with detection of the virus in 27 counties of Florida. Since that time, there have been sporadic outbreaks of tristeza decline occurring on trees on sour orange rootstock. Some of the first outbreaks of tristeza decline occurred in the Fort Pierce area in 1956, with other outbreaks with tree losses occurring in Orange and Polk Counties during the 1960's (3, 12). An outbreak of tristeza decline was reported in 1975 in western Orange and southern Lake Counties in Central Florida (7). In the early 1980's, tristeza decline was found in several locations in

the central and south Ridge areas (9). By the mid 1980's, it became apparent that a full scale epidemic of tristeza quick decline was occurring in the Indian River citrus area and in the flatwoods of Southwest Florida (3).

Citrus tristeza isolates collected from several locations in the Florida citrus areas were collected and biologically indexed to determine the severity of the isolates collected and to compare the severity of isolates obtained from healthy trees and decline trees in each location. The results of the indexing are reported herein.

Materials and Methods

Several locations were selected in Florida where mature trees on sour orange rootstock were suffering from tristeza quick decline or in one case, where young trees on sour orange rootstock were dwarfed due to tristeza. Budwood was collected from declining and healthy appearing trees in individual groves at 8 different locations. Mexican lime seedlings were inoculated to establish the virus isolates. Each Mexican lime seedling was inoculated with a bark piece cut from each of 3 or 4 budsticks taken from each field tree. The location and other information for each grove is given in Table 1.

CTV infection of index and field plants was confirmed by enzyme-linked immunosorbent assay (ELISA). The double antibody sandwich ELISA procedure as described by Bar-Joseph et al. (1) was used. Antisera were prepared against whole, unfixed CTV isolate T-36, a severe Florida CTV isolate previously described (6). The IgG fraction was collected using a protein A affinity column (11). The substrate, p-nitrophenyl phosphate, concentration was 1 mg/ml in ethanolamine substrate buffer (1). The plates were read after 30 min on a Bio-Tek EIA plate reader at 405 nm. Healthy controls ranged from 0.02 to 0.08 OD units, OD values twice the value of healthy control values for each plate were considered to be CTV positive.

The biological activity of each CTV isolate was evaluated by graft inoculating 3 plants each of Mexican lime seedlings, 'Valencia' sweet orange budded onto sour orange, 'Duncan' grapefruit seedlings, and 'Madam Vinous' sweet orange seedlings. ELISA was used to verify CTV infection of the indicator plants. The plants were maintained free of aphids. Once the plants were all systemically infected, they were pruned back to a height of 12 inches. After 9 months, the individual indicator plants were rated from 0 to 5 using the following rating system. All comparisons were made with a group of 12 healthy plants for each indicator as follows: Mexican lime plants, 0 = healthy, 1 = mild vein clearing, no stunting, 2 = mild vein clearing, mild stunting, 3 = moderate vein clearing, moderate stunting, mild leaf cupping, mild chlorosis, 4 = severe vein clearing, severe stunting, moderate chlorosis, 5 = severe

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Table 1. Results of biological index of citrus tristeza virus isolates recovered from declining (or stunted) and apparently healthy citrus trees from several different locations in Florida.

Isolate number	Condition of original tree	Index results ^z			
		Mex. lime	SWT/SO	Duncan	Mdm. Vin.
Location 1: 4-yr-old Hamlin/SO ^x , deep soil on ridge, near Wachula					
CW 1	severe stunt	3	2	NT ^y	NT
CW 2	mild stunt	3	2	NT	NT
CW 3	normal	0	0	NT	NT
Location 2: 20-yr-old Hamlin/SO, deep soil on ridge, near Frostproof					
CH 97	quick decline ^y	4	5	NT	NT
CH 98	normal	1	0	NT	NT
CH 99	quick decline	4	5	NT	NT
Location 3: 40-yr old Queen/SO ^m high water table, near St. Cloud					
CK 10	quick decline	1	0	0	0
CK 4	quick decline	3	2	0	0
CK 1	quick decline	1	0	0	0
CK 8	normal	1	0	0	0
CK 9	normal	1	0	0	0
Location 4: 13-yr-old Hamlin/SO, deep soil on ridge, near Davenport					
CD 2	slow decline	3	1	0	0
CD 3	slow decline	3	1	1 (ST) ^y	0
CD 4	decline	3	3	2 (ST)	0
CD 5	decline	3	3	2 (ST)	0
CD 7	decline	3	1	1 (ST)	0
CD 10	normal	0	0	0	0
Location 5: 12-yr-old Hamlin/SO, high water table, bedded grove, near LaBelle					
CL 1	normal	0	0	0	0
CL 2	normal	0	0	0	0
CL 3	normal	0	0	0	0
CL 4	quick decline	2	1	1 (ST)	0
CL 5	quick decline	2	1	1 (ST)	0
CL 6	quick decline	2	1	1 (ST)	0
Location 6: 12-yr-old Valencia/SO, bedded grove, near Immokalee					
CT 1a	quick decline	1	0	2 (ST)	0
CT 2a	quick decline	1	0	0	0
CT 3a	normal	1	0	0	0
CT 4a	normal	0	0	0	0
CT 5a	quick decline	2	0	0	0
CT 6a	quick decline	1	1	0	0
Location 7: 8-yr-old Valencia/SO, bedded grove, near Immokalee					
CT 8b	quick decline	4	4	1 (ST)	0
CT 9b	quick decline	4	4	1 (ST)	0
CT 10b	declining	4	4	3 (ST)	0
CT 11b	normal	0	0	0	0
Location 8: 12-yr-old Hamlin/SO, high water around irrigation pond near Frostproof					
CR 1	normal	1	0	0	0
CR 2	normal	1	0	0	0
CR 3	normal	1	0	0	0
CR 4	quick decline	1	0	0	0
CR 5	quick decline	1	0	0	0
CR 6	quick decline	1	0	0	0

¹Ratings on a scale whereby 0 = healthy to 5 = most severe.

²NT = not tested; ST = stunted.

^xHamlin = Hamlin sweet orange; SO = sour orange; Queen = Queen sweet orange; Valencia = Valencia sweet orange.

vein clearing, small leaves with severe cupping and vein corking, severe chlorosis, severe stunting. 'Duncan' grape-

fruit were rated + or - for seedling yellows, rated + or - for stem pitting, rated for stunting on a scale of 0 = no stunting, 1 = very mild stunting, 2 = mild stunting, 3 = mild to moderate stunting, 4 = moderate, and 5 = severe stunting. 'Madam Vinous' sweet orange indicator plants were rated + or - for stem pitting. The 'Valencia' on sour orange indicators were maintained for 2 yr before making the final evaluation. 'Valencia' on sour orange were rated as follows: 0 = healthy, 1 = slight stunting, no chlorosis, 2 = moderate stunting, no chlorosis, 3 = stunting, little or no chlorosis, 4 = severe stunting, chlorosis, 5 = decline of scion, severe chlorosis.

Results

The results of the biological index are summarized in Table 1. Seedling yellows was not detected on any of the grapefruit or sour orange seedling indicator plants. No stem pitting on either grapefruit or sweet orange indicator plants was found. There was some stunting on grapefruit seedlings inoculated with some isolates (Table 1).

Mexican lime is susceptible to CTV, often showing severe symptoms. The severity of vein-clearing symptoms on Mexican lime indicators ranged from 0 (barely detectable by visual evaluation) to 4 (severe). At 5 of the 8 locations, the CTV symptoms on the Mexican lime were rated more severe for the isolates collected from declining trees than those from the healthy appearing trees at the same location. However, at Locations 3, 6, and 8, symptoms on Mexican lime indicators were no more severe with isolates from the declining trees than those from the healthy appearing field trees. In no instance did an isolate from a tree which appeared normal in the field receive a Mexican lime rating higher than 1 (mild), whereas some isolates from quick decline affected trees were rated as high as 4.

With the rating system used for the 'Valencia' sweet orange on sour orange rootstock indicator plants, an isolate which gave a 1 or 2 rating would probably produce a stunted tree if propagated on sour orange rootstock. An isolate with a rating of 3 or greater would probably produce a tree which would develop a budunion disorder and decline eventually. A rating of 4-5 indicates the quick decline symptom was produced under the greenhouse conditions within 2 yr. At Location 1, CTV isolates collected from stunted trees received a rating of 2 on the sweet on sour orange indicator plants. CTV isolates with ratings of 3 or greater were associated with declining field trees in the Ridge citrus area from Locations 2 and 4, but not from Locations 3 or 8. From the bedded groves in the southwest flatwoods citrus area, CTV isolates with ratings of 3 or greater were associated with declining field trees only in Location 7, but not from Locations 5 and 6. The CTV isolates collected from the stunted trees at Location 1 caused moderate symptoms (3 rating) on Mexican lime and stunting but no chlorosis (2 rating) on the sweet orange on sour orange indicator plants.

Discussion

There are numerous isolates of CTV, each isolate has unique biological properties which must be determined by indexing on appropriate indicator plants (6). Mexican lime has been a commonly accepted indicator plant for detection of CTV. A severe reaction on Mexican lime does not

necessarily indicate that the CTV isolate would induce other biological activities such as seedling yellows, stem pitting, or decline on sour orange rootstocks (10). For this reason, several other indicator plants had to be used to detect a potential for causing stem pitting on grapefruit and sweet orange, seedling yellows, and decline on sour orange rootstock.

It was surprising to find that not all the CTV isolates collected from trees suffering from quick decline induced decline in the sweet orange on sour orange rootstock indicator plants. It is possible that the trees contained a mixture of CTV isolates and that only the milder isolates were transmitted to the indicator plants. We tried to avoid this possibility by taking samples from 3 or 4 different branches from the field tree in case an isolate was confined to only a part of the tree canopy. A bark piece from each budstick collected was then budded onto the Mexican lime plant used to establish the original field source of each CTV isolate. If the bark pieces were dead after 3 weeks, the Mexican lime was reinoculated from the original source. The groves selected for this study had a random pattern of tree decline characteristic of CTV induced decline instead of a more localized pattern suggestive of a water or soil problem. Eventually we concluded that the trees did not contain a severe CTV isolate and that some apparently mild CTV isolates could cause a decline if the trees were growing in a site with a fluctuating water table. Location 3 was near a lake which during periods of heavy rainfall overflowed to cause a high water table in the adjoining citrus grove. The grove at Location 8, although in the Ridge area, had a water reservoir for irrigation and when the reservoir was filled a high water table resulted and remained for several days. All the bedded groves in flatwoods locations contained typical Alfisol and Spodosol soils which often have impermeable horizons near the surface (2).

The rooting depth of citrus on these Flatwoods soils is usually limited to about 18 inches (13). In studies on the effects of the rate of water table recession on root health, Ford (5) concluded that drawdown rates of less than 2 inches per day may not be sufficient to prevent anaerobiosis during periods of high rainfall. During periods of heavy rainfall, the soil may become waterlogged and the resultant anaerobic conditions can damage or kill the citrus roots.

While there are many CTV isolates in Florida capable of inducing decline in trees on sour orange rootstock, it seems probable that the stress placed on the root system of citrus trees on sour orange rootstock in some areas with fluctuating water tables may combine with some relatively mild CTV isolates to cause trees to decline quickly. There is a need to better determine the interaction of water table fluctuations with the onset of tristeza quick decline, depth and amount of feeder roots, and the amount of starch and carbohydrates in roots and scion. Further, there is a need to develop a method for the rapid *in vitro* assessment of the biological characteristics of a CTV isolate without the time and expense inherent in the biological assay.

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