The Effect of Citrus Tree Age on Citrus Variegated Chlorosis

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ABSTRACT. Citrus variegated chlorosis (CVC) is a disease of citrus trees caused by *Xylella fastidiosa*. It was detected in Misiones in 1984. Symptoms include chlorotic leaves with necrotic centers and the very small fruits. In commercial citrus groves, a higher incidence of CVC is observed on 7- to 11-yr-old sweet orange trees budded on vigourous rootstocks. Visual surveys and serological tests for CVC were done in 1993 and 1997 on a citrus experimental plot established in 1981. A total of 239 trees of the different citrus species were surveyed for CVC symptoms and assayed using dot immunobinding assay (DIBA). CVC symptoms were observed in all sweet orange cultivars in 1993, however, only Valencia, Natal, San Miguel, Hamlin sweet orange, and Buckeye navel showed symptoms in 1997. Furthermore, a lower percentage of sweet orange samples was serologically positive in 1997 than in 1993. All lemon and tangerine cultivars were negative for CVC symptoms in both surveys. However, Eureka lemon was CVC positive by DIBA in both surveys. Only the 10% of the samples were DIBA positive in 1997. The grapefruit-like fruits of Dalan-Dan which showed strong symptoms of CVC, and were DIBA positive during the 1993 survey, but were DIBA negative and showed no symptoms in the 1997 survey.

Citrus variegated chlorosis (CVC) is caused by the bacterium *Xylella fastidiosa* (3, 7, 11). CVC has been detected in Misiones Province since 1983; and later in Corrientes Province. Its symptoms are very similar to CVC of Brazil (2); they show a yellowish or variegation in the upper face of the leaves with coincident small brown spots in the back of the leaves. Small fruits also are produced. In commercial groves, CVC is mainly observed on 7- to 11-yr-old sweet orange trees on vigorous rootstocks. Little visual symptoms are found in other citrus species (1).

Several serological and molecular assays allow for a more efficient diagnosis of such pathogens (5, 8). The objective of this study was to evaluate the incidence and severity of CVC on different citrus cultivars and species from a field collection based on visual symptoms and dot immunobinding assay (DIBA).

MATERIALS AND METHODS

The incidence and severity of CVC on different citrus species and cultivars of citrus were evaluated on trees of a citrus collection in the Experimental Station Plot of INTA Montecarlo, Misiones. The collection block; established in 1981 has 239 citrus trees of 81 different cultivars. The trees are spaced at 7m × 5 m in an east west orientation. The tree collection consists of 25 cultivars of tangerines, 40 of oranges and 16 of lemons. Each cultivar has from two to four trees set in the same row, and each have a random distribution within the block. Surveys were done during the winter of 1993 and 1997. The CVC incidence was determined by the visual presence of symptoms, and the symptom severity was rated on the following scale proposed by Agostini and Haberle (1): 0 = without symptoms; 1 = from 1 to 5 branches with young leaves with CVC, or new flush with the symptoms; 2 = a sector of the canopy (20%) with symptoms of CVC and among 5 to 10 sprouts distributed in the tree; 3 = all the yearly flushes with symptoms and a sector of the tree (20%) with symptomatic leaves and fruits with small size; 4 = symptomatic leaves and small fruit size all around the tree and very few normal sprout; and 5 = reduced tree size with symptomatic leaves and small fruit size all around the tree.

Serological testing. Leaves from symptomatic and non symptomatic trees were taken from every tree in the block. A total of five leaves were taken from symptomatic trees. From non symptomatic trees, 20 leaves were used with one sample with five leaves from each quadrant of the tree. The samples were kept in plastic bags until their transfer to the laboratory. DIBA was done using antiserum UF-26 for CVC of Brazil according to the technique of Lee and Beretta (6, 12). Positive controls were Valencia sweet orange leaves from a commercial field plot with severe symptoms of CVC. Negative controls consisted of leaves of sweet orange trees growing in an isolated pest free greenhouse. Two replications were made for the DIBA test and the samples were considered positive even when only one of the replication produced a colorimetric reaction.

RESULTS

During the first survey, in winter of 1993, all the sweet orange trees were positive by DIBA and showed severe symptoms of CVC in the field (Table 1). Four years later, only the Hamlin, and Valencia 36 sweet oranges had CVC symptoms. During the second serological assay, a lower percentage of positive samples were founded than in the first survey. Sweet orange varieties such as Comun and Cadenera were positive by serology in 100% of the samples but lacked visual symptoms of CVC.

The pseudo grapefruit, Dalan Dan, were symptomatic and DIBA positive in the winter of 1993 but

TABLE 1
THE INCIDENCE OF CITRUS VARIEGATED CHLOROSIS IN TWO SURVEYS ON SEVERAL CITRUS CULTIVARS AT THE CAMPO EXPERIMENTAL LAHARRAGUE, MONTECARLO

	1993 Survey			1997 Survey		
		DIBA			DIBA	
Citrus sp. and cultivars	Symptoms	Results	% positive	Symptoms	Results	% positive
Sweet orange						
Bahianina	YES	+[z]	100	NO	+	17
Wash. Navel	YES	+	100	NO	—[y]	0
Hamlin	YES	+	100	YES	+	100
Pineapple	YES	+	100	NO	+	25
Valencia 36	YES	+	100	YES	—	0
Val. Mexico	YES	+	100	NO	—	0
Pera Olimpia	YES	+	100	NO	—	0
Comun	YES	+	100	NO	+	100
Cadenera	YES	+	100	NO	+	100
Natal	W/D	W/D[x]	W/D	YES	+	17
San Miguel	W/D	W/D	W/D	YES	+	17
Buckeye	W/D	W/D	W/D	YES	+	33
Grapefruits						
Dalan Dan	YES	+	100	NO	—	0
Lemons						
Eureka	NO	+	100	No	+	2
Tangerines						
Okitsu	NO	—	0	NO	—	0
Murcott	NO	—	0	NO	—	0
Ellendale	NO	—	0	NO	—	0

[z]+ Positive samples.
[y]— Negative samples.
[x]W/D no data.

TABLE 2
SEROLOGICAL (DIBA) TESTS AND SEVERITY RATING OF VARIOUS LEMONS AND GRAPEFRUITS CULTIVARS AT THE CAMPO EXPERIMENTAL LAHARRAGUE, MONTE-CARLO DURING WINTER OF 1997 SURVEY

Scion cultivars	No. trees tested	DIBA positive samples/ total	Severity rating[z]
Lemons			
Eureka	19	1/48	0
Génova	10	0/13	0
Verna	5	3/13	0
Monniachelo	2	1/5	0
Primofiori	2	1/5	0
Fino	3	0/4	0
Frost Lisbón	4	0/4	0
Frost Lisboa	2	0/10	0
Pseudo-grapefruits			
C. pseudoparadisi	4	0/7	0.25
C. natsudaidai	4	0/4	0
Positive Control	1	6/6	5
Negative Control	1	0/6	0
Buffer	—	—	0

[z]Severity rating: 0 = without symptoms; 1 = from 1 to 5 branches with young leaves with CVC, or new flush with the symptoms; 2 = a sector of the canopy (20%) with symptoms of CVC and among 5 to 10 sprouts distributed in the tree; 3 = all the yearly flushes with symptoms and a sector of the tree (20%) with symptomatic leaves and fruits with small size; 4 = symptomatic leaves and small fruit size all around the tree and very few normal sprout; and 5 = reduced tree size with symptomatic leaves and small fruit size all around the tree.

were negative in the 1997 survey. The commercial Eureka lemon trees did not show symptoms at any time but were DIBA positive in both surveys. The second survey produced a very low number of positive samples (Table 2). None of the lemon varieties showed any symptoms that could be related to CVC, but some samples from the Eureka, Verna, Moniachello, and Primofiori lemon trees were positive serologically in the 1997 survey.

All the 21 different tangerines or their hybrids were always negative for both tested parameters (Table 3). Most of the navel trees were non symptomatic, except the cultivar Buckeye which also was DIBA positive. Some navels such as Prolific and Warren had some positive samples by serology but did not show CVC symptoms (Table 3).

DISCUSSION

The pseudo grapefruit Dalan Dan, several lemons and most sweet oranges were positive by DIBA for CVC, however, no symptoms were observed in any of the lemon trees in either survey. Based on the present data, a tolerance order for varieties can be given. All the sweet orange surveyed in 1993 has showed symptoms for CVC, but only few of them were symptomatic during the 1997 survey; also the yield and the fruit size on these cultivars were normal. Sweet orange thus appears to be the most susceptible of the citrus species in this block. The lowest number of symptomatic trees and lowest percentage of positive samples by DIBA during the second survey suggested some tolerance of the sweet orange trees to CVC after they are 11 yr of age. A lower incidence of CVC also was observed in São Paulo, Brazil in sweet orange trees older than 8 yr of age (14). The lemon trees were positive by DIBA in a large number of samples but never showed symptoms of CVC, thus, they could be considered as tolerant. Finally, the

TABLE 3
SEROLOGICAL (DIBA) TESTS AND SEVERITY RATING OF VARIOUS TANGERINES AND SWEET ORANGES CULTIVARS AT THE CAMPO EXPERIMENTAL LAHARRAGUE, MONTECARLO DURING WINTER OF 1997 SURVEY

Scion cultivars	No. trees tested	DIBA positive samples/ total	Severity rating[z]
Tangerines			
Nova	3	0/4	0
Willow Leaf	3	0/2	0
Mexerica do Río	1	0/2	0
Mariscal Lopez	3	0/4	0
Campeona	2	0/2	0
Kinnow	4	0/4	0
Beauty	4	0/4	0
Encore	3	0/2	0
Malvasio	2	0/3	0
Mijouchi	2	0/5	0
Satsuma	2	0/6	0
Satsuma Owari	4	0/5	0
Improved xComun	7	0/5	0
Clauselina	1	0/7	0
Fremond	2	0/5	0
Dancy	3	0/4	0
Tangerina sp.	3	0/8	0
Ellendale Savio	4	0/4	0
Sweet orange			
Buckeye	4	3/9	0.25
Warren Navel	2	1/4	0
Gillette Navel	4	0/5	0
Prolific Navel	2	2/16	0
Parson Brown	2	0/4	0
Hamlin	8	2/9	0.12
Pera	4	0/6	0
Joao Llunes	4	0/4	0
Calderón	8	1/21	0.25
Valencia Stein	4	0/2	1
Lue Gim Gong	4	1/5	0
Natal	11	2/16	0.27
Valencia Seedless	4	2/6	0
Valencia Frost	3	0/1	0.67
Valencia Olinda	4	0/7	0.5
Valencia Late	8	0/4	0.25
Valencia	13	4/18	0.38
Folha Murcha	4	1/5	0.25

[z]Severity rating: 0 = without symptoms; 1 = from 1 to 5 branches with young leaves with CVC, or new flush with the symptoms; 2 = a sector of the canopy (20%) with symptoms of CVC and among 5 to 10 sprouts distributed in the tree; 3 = all the yearly flushes with symptoms and a sector of the tree (20%) with symptomatic leaves and fruits with small size; 4 = symptomatic leaves and small fruit size all around the tree and very few normal sprout; and 5 = reduced tree size with symptomatic leaves and small fruit size all around the tree.

tangerines and tangerine hybrids appear resistant to CVC.

Because of the irregular distribution of the bacteria in their host, many of the non symptomatic trees could be hosts of *Xylella* but most samples were negative by serology. During the winter season, the symptoms appear to be more evident (4). The concentration of *Xylella* in other diseases is considered to be higher during the winter (9, 10, 13). In our

survey in winter of 1997, few symptoms were observed on the trees from the field. The large number of samples taken from non symptomatic trees should have enhanced the possibility to get positive results by serology if the pathogen was present in those trees.

Bacteria in xylem could lead to production of plugs in vessels that do not allow the normal water uptake, thus resulting in the small fruit size on the symptomatic trees. Systematic studies of water conductivity on sweet orange trees on various rootstocks has showed that the first centimeter of the wood from the cambium is the more active in the water uptake and, thus, could be the explanation of the tolerance of sweet orange trees on other rootstocks than trifoliate orange to blight, another xylem disfunction disorder in Misiones (15). The fast growth of Eureka lemon trees in our conditions, also could be the reason why they are non symptomatic for CVC even though that they were positive by DIBA during both surveys. The larger new wood of older sweet orange trees than in younger trees could explain the symptom reversion for CVC in trees older than 11-yr-old.

At present, the tangerines have not been reported as being affected by CVC even in the region where a high incidence of the disease is present. The negative serology results and symptomatology in the field during both surveys could suggested that tangerines may be an alternative for sweet orange production in the regions with a high incidence of CVC. The degree of susceptibility of these cultivars must be further analyzed by more specific techniques (8). However, the selection of resistant or tolerant trees to CVC from several cultivars looks to be a good strategy for disease control of the disease, until trees resistant to CVC is available.

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