Citrus Variegated Chlorosis (CVC): Current Status in Commercial Orange Groves in the States of São Paulo and Minas Gerais (Southern Triângulo Mineiro)

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ABSTRACT. The intensity of citrus variegated chlorosis (CVC) was studied in commercial groves in the states of São Paulo and Minas Gerais (southern Triângulo Mineiro) from 1996 to 2001. Six surveys were conducted annually from 1996 to 2001 with the three most important varieties of sweet oranges (Pêra Rio, Valencia and Natal), using a random stratified sampling, with samples proportional to the size of the strata. The following stratified factors considered were: Variety, zone and plant age. The percentage of CVC-affected trees for each factor was estimated, and an increase in disease incidence from 22.33% in 1996 to 36.52% in 2001 was observed. The incidence in trees with symptoms restricted to leaves (grade 1) was variable, ranging from 23.23% in 1997 to 12.09% in 2001. The percentages for infection in grade 2 trees (trees with symptoms in fruits and leaves) increased from 6.41% to 24.40% in the 6-yr period. Trees of intermediate ages (3-5 yr and 6-10 yr) showed the highest intensity of disease.

In Brazil, citrus variegated chlorosis (CVC) was first noticed in 1987 in groves in Colina (São Paulo State) (3) and later in Triângulo Mineiro (Minas Gerais State) and subsequently all over São Paulo. The disease affects all commercial varieties of oranges grafted on different rootstocks, and it is caused by the xylem-restricted bacterium *Xylella fastidiosa* (1). The presence of this bacterium has been confirmed in the states of São Paulo, Minas Gerais, Paraná, Santa Catarina, Rio Grande do Sul, Goiás, Distrito Federal and Sergipe. There are also reports of the presence of CVC in Paraguay, Costa Rica and Argentina, where the disease is known as "Pecosita" and records of its presence can be found in the high Paraná area of Misiones since 1984 (1).

X. fastidiosa can be transmitted by contaminated nursery plants and sharpshooter vectors. Research results showed that the transmission efficiency of five species of sharpshooters (*Dilobopterus costalimai*, *Acrogonia* sp., *Oncometopia facialis*, *Pleisommata corniculata*, and *Bucephalogonia xanthophis*) is low, ranging from 1.3% to 11.7% (5), which partly explains the successful use of insecticides in the control of the disease.

Since CVC was first found, significant knowledge of the pathosystem has been acquired, due mainly to priority given to research in this area by various research institutions and financial agencies, namely State of São Paulo Research Foundation (FAPESP), National Council of Scientific and Technologic Development (CNPq) and Fund for Citrus Plant Protection (FUNDECITRUS).

Even with the completion of the *X. fastidiosa* genome project, a definite solution to CVC has not yet been found, although it is possible to develop a "technological package" to manage the groves so as to control the disease. The control strategy is based on procedures such as: Use of young trees from protected nurseries which are covered by plastic and laterally protected by screens, pruning of branches which show initial leaf symptoms of CVC, or the eradication of trees with advanced symptoms, and chemical control of sharpshooters.

The losses caused by CVC occur in the more advanced stages of the disease and are caused by a reduction in the development of the plant,

particularly in the fruit. In highly affected trees, protruding branches in the upper part of the tree are usually noticed. They have small leaves and fruits and show defoliation in the terminal twigs which causes, in the most serious cases, the economic death of the trees.

This study focused on evaluating the incidence and the severity of CVC in the three most grown varieties of oranges (Pêra Rio, Valencia and Natal) in the state of São Paulo and in the southern Triângulo Mineiro area of Minas Gerais, taking into account the different ages of the trees.

MATERIAL AND METHODS

Sampling. Six annual assessments were made in commercial orange farms in 1996 (June-July), 1997 (June-July), 1998 (July-August), 1999 (August-September), 2000 (June- July) and in 2001 (June-July).

Random stratified samples from the strata were collected, and the number of sample units was proportional to the size of the strata. The detailed components of the strata were: a) Varieties—data were collected from three varieties, Pêra Rio, Natal and Valencia; b) Zones—the citrus exporting area was divided into four zones, Northwest, Center and South zones in the state of São Paulo and North zone, comprising part of the state of São Paulo and the southern Triângulo Mineiro of Minas Gerais; c) Age groups—trees from 0-2 yr old (age group 0), 3-5 yr old (age group 1), from 6-10 yr old (age group 2) and older than 10 yr (age group 3).

In 1996, 1997, 1998, 1999, 2000 and 2001, respectively 7,037, 6,491, 2,968, 444, 1,020 and 1,145 trees were inspected. The number of trees inspected in the strata was proportional to the total number of trees in the strata.

To choose the sample, records from FUNDECITRUS producers were used. The North zone had 31.73% of all the trees, the Northwest zone had 11.23%, the Center zone had 31.37% and the South zone had 25.66%. These percentages were applied to the number of the samples to obtain the proportions of the sample units for each stratum.

The sample trees were located in 4,609 farming blocks. The exact identification of each tree was not available so the numerical order of each tree in the zone was used as a number for identification of the trees. The arrangement of the farms and their respective trees made it possible to identify the number of trees to be studied for each sampled farm block. To identify the row in each selected farm block, a table with random numbers was used to determine the plot, the street and the tree to be inspected.

These trees were classified according to the severity of the symptoms, based on grading for the following descriptive scale: 0—absence of symptoms; 1—tree with symptoms restricted to the leaves; 2—tree with foliage symptoms and some fruits with less than 5 cm in diameter.

To verify the precision of the diagnosis in the field, 200 plants, identified as diseased plants by control inspectors, were chosen at random in 1998. Sample leaves were collected from these trees to undergo optical microscopic tests (7) and ELISA tests to confirm the presence of the bacteria.

Calculation of the estimates. In the calculation of the estimates, the following values were considered: $y_{ij} = 0$ for absence of disease and $y_{ij} = 1$ for presence of disease (CVC level j = 0, 1 or 2) in the tree. The calculation of the percentage of the CVC contaminated trees and their respective variances for each strata *h* (variety, zone and plant age), and the general estimate for the state of São Paulo were made according to Thompson (8).

TABLE 1
INCIDENCE OF CITRUS VARIEGATED CHLOROSIS (% OF DISEASED TREES) IN THE STATE OF SÃO PAULO AND IN THE SOUTHERN TRIÂNGULO MINEIRO (MINAS GERAIS) FROM 1996 TO 2001

Year	Number of trees	Number of samples	Severity[1]					
			Grade 1		Grade 2		Total	
			$\hat{P}$[2]	$s(\hat{P})$[3]	$\hat{P}$	$s(\hat{P})$	$\hat{P}$	$s(\hat{P})$
1996	192,671,413	7,037	15.92	0.42	6.41	0.28	22.33	0.08
1997	194,125,055	6,491	23.23	0.51	9.66	0.34	32.89	0.55
1998	194,324,987	2,968	13.57	0.66	7.84	0.50	21.41	0.73
1999	194,324,987	444	20.87	2.50	15.65	1.74	36.52	2.53
2000	183,565,974	1,022	13.31	1.11	21.28	1.17	34.59	0.84
2001	190,078,996	1,145	12.09	1.04	24.43	1.15	36.52	1.35

[1]Grade 1—Trees with CVC symptoms restricted to leaves. Grade 2—Trees with CVC symptoms in fruits and leaves.
[2]Average and
[3]Standard error of the average.

RESULTS AND DISCUSSION

The optical microscopic test and the ELISA test, performed to verify the precision of the diagnosis of the disease in the field, showed positive results for 196 trees (98%), showing a good level of confidence in the identification of diseased plants performed by the field inspectors.

In the first year (1996), CVC incidence level was 22.33%, and in the following 5 yr CVC incidence levels were 32.89%, 21.41%, 36.52%, 34.59% and 36.52%, respectively (Table 1). According to these data, there was an increase in CVC incidence from 1996 to 1999, followed by a stabilization of the disease in the years 1999-2001.

The evolution of CVC in the state of São Paulo, as detected in this study, showed a lower growth rate than in previous studies made by others researchers (4, 6). It must be taken into account that these previous epidemiological studies were conducted in plots of citrus groves with young orange trees in the North and Northwest zones of the State of São Paulo, which are areas with higher populations of sharpshooters and CVC intensity. Another relevant factor is that in these experimental areas, there was no disease control. In commercial farming areas disease control comprises the introduction of healthy new plants, the pruning of branches with symptoms, the eradication of trees in the terminal phase of the disease and sharpshooter control by the use of insecticides.

There was a progressive increase in the severity of CVC in the last six surveys made by FUNDECITRUS. In 1996, 6.41% of plants surveyed were classified in grade 2, and in 2001, 24.40%. This increase in CVC severity could be explained by a tendency to have an increase in the symptoms with aging. These data show that grade 1 plants (initial stage of the disease) are now at a more advanced stage (grade 2), increasing yield damage by reducing fruit size.

The incidence of CVC was higher in age group 1 trees (3-5 yr old) and age group 2 trees (6-10 yr old) (Table 2). At these ages, the incidence of the disease ranged from 27.20% to 32.22% in 1996, and from 46.32% to 47.88% in 2001, respectively. The higher intensity of CVC in the intermediate ages can be attributed to the following factors: a) young trees have more abundant growth flushes that may attract the sharpshooters which feed especially on young tis-

TABLE 2
INCIDENCE OF CITRUS VARIEGATED CHLOROSIS (% OF DISEASED TREES) AT DIFFERENT SEVERITY LEVELS IN TREES OF DIFFERENT AGES IN THE STATE OF SÃO PAULO AND IN THE SOUTHERN TRIÂNGULO MINEIRO (MINAS GERAIS)

				Severity[1]					
				Grade 1		Grade 2		Total	
Age	Year	Number of trees	Number of samples	$\hat{P}$[2]	$s(\hat{P})$[3]	$\hat{P}$	$s(\hat{P})$	$\hat{P}$	$s(\hat{P})$
0-2 years	1996	22,482,374	839	9.67	0.99	2.62	0.57	12.29	1.12
	1997	10,101,381	362	9.22	1.65	2.52	0.90	11.74	1.82
	1998	4,313,344	138	16.81	3.16	2.36	1.82	19.17	3.50
	1999	1,653,574	16	29.90	0	5.80	15.49	35.69	15.39
	2000	5,979,966	47	0.13	0	4.04	3.84	4.17	3.84
	2001	3,177,733	37	4.14	7.59	4.53	8.31	8.67	11.25
3-5 years	1996	48,852,196	1,960	22.65	0.92	9.57	0.64	32.22	1.01
	1997	44,120,864	1,614	24.83	1.07	14.02	0.81	38.85	1.14
	1998	32,494,438	669	20.77	1.79	10.83	1.42	31.61	1.99
	1999	22,482,374	56	23.18	8.59	32.91	5.58	56.08	8.90
	2000	16,916,747	98	13.20	4.02	29.46	5.26	42.66	5.39
	2001	11,830,363	136	11.77	3.91	34.55	4.95	46.32	5.37
6-10 years	1996	68,347,374	2,669	18.47	0.74	8.72	0.53	27.20	0.85
	1997	77,139,660	2,739	26.86	0.83	13.02	0.61	39.88	0.89
	1998	83,168,905	1,228	15.21	1.06	10.76	0.85	25.97	1.15
	1999	84,946,325	204	24.80	3.66	20.34	3.04	45.15	3.51
	2000	73,484,873	415	16.51	1.87	27.95	1.95	44.46	2.28
	2001	63,394,254	368	13.04	1.74	34.84	2.13	47.88	2.28
>10 years	1996	52,989,469	1,569	9.06	0.73	2.10	0.36	11.16	0.79
	1997	62,763,150	1,776	19.88	0.92	3.61	0.45	23.49	0.98
	1998	74,348,300	933	8.64	0.96	3.67	0.61	12.31	1.09
	1999	85,242,714	168	16.10	3.74	6.61	2.01	22.72	3.95
	2000	87,184,388	462	11.49	1.51	15.17	1.52	26.66	1.96
	2001	111,676,646	604	11.81	1.38	18.07	1.42	29.88	1.77

[1]Grade 1—Trees with CVC symptoms restricted to leaves. Grade 2—Trees with CVC symptoms in fruits and leaves.
[2]Average and
[3]Standard error of average.

sues, thus increasing the probability of bacteria inoculation; b) it is believed that the earlier the infection of a tree, the higher the severity of the disease since the young trees have a more active metabolism, which enhances the multiplication and translocation of the bacteria in the xylem vessels; c) the incubation period of the disease in sweet orange trees can range from 5 to 18 mo. This might be a more pertinent explanation of why trees in the intermediary age groups show a higher intensity of the disease when compared with younger trees.

From 1996 to 2001, the incidence of CVC increased, respectively, from 11.16% to 29.88%, in trees older than 10 yr.

In stratum of trees 0-2 yr old, the incidence of CVC was usually lower in most surveys, if compared to the others strata. It is possible that many young trees, in spite of being contaminated with CVC, might still be in the incubation period of the disease.

From 1996 to 2001, the incidence of CVC decreased from 12.29% to 8.67% in trees 0-2 yr old. An important factor that could explain the tendency in the decrease of CVC

intensity in younger groves was the adoption of management strategies in the last years, such as the use of healthy nursery trees, control of sharpshooters and pruning or eradication of diseased plants.

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