Comparison of Citrus Sudden Decline from Venezuela with Citrus Blight from Florida and Delinio from Brazil

F. Ochoa, M. J. G. Beretta, K. S. Derrick, R. F. Lee and E. Rangel

ABSTRACT. Sudden decline of citrus, a disease of unknown cause in Venezuela, is similar in some ways to citrus blight in Florida and citrus declinio in Brazil. Trees affected with sudden decline show blight/declinio-like decline symptoms, but affected trees usually die within 8 to 10 months after symptoms first appear. This is in contrast to trees affected with blight and declinio, which may have very severe symptoms of decline for several years but seldom die. Using serological tests, trees with sudden decline were found to contain the 12-Kda protein associated with citrus blight and declinio. The 12-Kda protein was not found in trees affected with root decline associated with *Fusarium* species or infested with *Tylenchulus semipenetrans*.

Citrus sudden decline or "decaimiento repentino de los cítricos" was detected in Venezuela in 1985 (8, 11). The disorder was observed on Valencia sweet orange on Volkamer lemon rootstock in the tropical northwestern region of Venezuela around 300 m above sea level. The disease represents a serious threat to Venezuelan citriculture, which was recovering from great losses caused by citrus tristeza virus. This disease destroyed six million trees on sour orange rootstock in the 1980s (6, F. Ochoa, unpublished). "Decaimiento repentino", blight, declinio diseases are similar in symptoms (1, 2, 4, 11, 14, 17): reduced water uptake by affected plants, the presence of amorphous plugs in the xylem, and high zinc content in the trunk wood of affected trees. Trees affected with Venezuelan citrus sudden decline, while showing symptoms of blight-declinio, usually die within 8-10 months after the onset of symptoms. This is in contrast to blight-declinio with which trees seldom die. In Florida, Cuba and Venezuela, chronic decline citrus trees have fewer fibrous roots and, at times, wood necrosis of the main roots occurs from which *Fusarium* was isolated (9, 14, 16). However, more than one pathogen may occur in the same grove or even the same tree which makes the diagnosis of the cause of decline difficult. In addition Venezuela's tropical environment may affect disease expression (12).

Histological studies, using light and scanning electron microscopy, showed the formation of numerous amorphous plugs when cuttings of Volkamer lemon were inoculated with filtrates from *Fusarium* cultures. The appearance of these plugs ranged from a few days up to one year later. These plugs were morphologically similar under a light microscope to those observed in field trees affected with sudden decline. Moreover, high zinc levels in the trunk and some zinc deficiency symptoms with flaccidity of leaves occurred in the same plants (15). Studies have showed that the nematode *Tylenchulus semipenetrans* Cobb and the fungus *Fusarium solani* (Mart.) Appel & Wr. Snyd. & Hans, occurring together or independently, induce plug formation in the xylem but not high zinc concentrations in the wood. These trees had canopy symptoms similar to blighted trees (12).

Recently, a 12 kDa protein was associated with citrus blight in Florida, and an antisera specific for this protein was produced (4). This antiserum reacted positively with extracts from trees affected with blight in Florida and declinio in Brazil using dot immunobinding assays and western blot analysis (1, 5).

The purpose of this study was to assay this antiserum for diagnosis of citrus sudden decline in Venezuela, establishing evidence of relationships

with Florida citrus blight and citrus declinio from Brazil.

MATERIAL AND METHODS

Mature leaves and root vacuum extracts were collected from trees as previously described by Derrick *et al.* (4). Samples were collected from Valencia sweet orange trees on Volkamer lemon rootstock from two groves located in the Yaracuy State, Venezuela, at 350 m above sea level. In the first plot, referred to as the J. R. grove, four healthy-looking trees and six trees affected with sudden decline were sampled. The sudden decline-affected trees selected had visual cachexia symptoms of bark pegging and gumming in the rootstock. In the second plot, referred to as the M. D. grove, six sudden decline-affected trees were selected which were free of visual cachexia symptoms. Plot 3 was the FUSAGRI Experiment Station variety collection, trees were sampled in a seed rootstock mother block grafted on Volkamer lemon, where eight trees were selected. Two Volkamer, one Sunki and one Taiwanica orange were affected by *Tylenchulus semipenetrans* and *Fusarium solani*. *Tylenchulus semipenetrans* populations were evaluated in soil and roots following the Cobb modified procedure (3, 10) and *F. solani* was isolated on potato-dextrose agar media, pH 5.8, from roots kept in moist chambers. Affected trees showed sudden decline-like symptoms.

Water uptake, xylem plugging, and zinc concentrations were determined using previous published procedures (2, 8, 13, 15, 17). The trees were visually rated using a scale whereby 0 = healthy and 3 = a severely declined tree according to the criteria previously described (7).

Four to five leaves from each plant were extracted by grinding in a plastic bag with no buffer added, and the leaf

TABLE 1
RESULTS OF THE DOT IMMUNOBINDING ASSAY (DIBA) FOR THE 12-kDa BLIGHT-RELATED PROTEIN IN VALENCIA ORANGE ON VOLKAMER LEMON AND CLEOPATRA MANDARIN ROOTSTOCKS SYMPTOMLESS OR WITH SUDDEN DECLINE AND CACHEXIA SYMPTOMS

Rootstock	Sudden decline symptoms[z]	Visual symptoms of cachexia	DIBA rating	
			Leaves	Roots
Plot 1 (J. R. grove):				
Volkamer	2	+	+ + +	+ +
Volkamer	2	+	+ + +	+
Volkamer	2	+	+ + +	+ +
Volkamer	2	+	+ + +	+ +
Volkamer	2	+	+ + +	+ + +
Volkamer	2.5	+	+ + +	+ + +
Cleopatra	0	–	–	–
Cleopatra	0	–	–	–
Cleopatra	0	–	–	–
Cleopatra	0	–	–	–
Plot 2 (M. D. grove):				
Volkamer	3	–	+ + +	+ +
Volkamer	2.5	–	+ + +	–
Volkamer	2	–	–	+ + +
Volkamer	2.5	–	+ + +	+ + +
Volkamer	2.5	–	–	+
Volkamer	2.5	–	+ + +	+ +
Volkamer	0	–	–	–
Volkamer	0	–	–	–
Volkamer	0	–	–	–
Volkamer	0	–	–	–

[z]Canopy symptoms rated on a scale of 0 = healthy to 3 = severe decline.

TABLE 2
TREE CONDITION, WATER UPTAKE, PRESENCE OF AMORPHOUS PLUGS, PRESENCE OF 12-kDa BLIGHT-RELATED PROTEIN, AND INCIDENCE OF NEMATODE AND *FUSARIUM* ON TREES ON VOLKAMER ROOTSTOCK IN A BUDWOOD MOTHER BLOCK FROM PLOT 3

Scion variety[z]	Decline symptoms[y]	Water uptake[x]	Plugs (%)[x]	Zinc (ppm)	Nematodes[v]		*Fusarium* isolated	12-Kda protein
					Root	Soil		
Volkamer	3	1.0	2.4	5.1	6800	4200	+	–
Volkamer	3	1.1	2.0	5.6	11560	4250	+	–
Sunki	0	0.6	0.8	4.0	*0*	*0*	+	–
Sunki	3	0.6	1.9	4.2	42660	11200	+	–
Taiwanica	0	0.3	3.5	5.0	0	0	–	–
Taiwanica	2	1.4	14.1	5.9	42600	15500	+	–
Amblycarpa	0	1.7	0.2	1.6	0	0	–	–
Cleoptera	0	0.3	32.4	6.6	0	0	–	–

[z]variety collection on Volkamer lemon rootstock.
[y]ratings as described in Table 1
[x]ml/sec
[w]percent of xylem vessels with amorphous plugs
[v]per 100 g

sap was collected and used for the dot immunobinding assay (DIBA) using antisera specific to the 12-kDa blight-related protein and the procedure described by Derrick et al (4). Xylem fluids were extracted from each of 4-5 roots per tree using the vacuum extraction method previously described (4).

RESULTS

In the first plot the DIBA indicated a relationship between canopy decline symptoms and the serological reaction of the leaf and root fluid extracts with the 12-kDa blight related protein antisera (Table 1). The only apparently healthy plants at Plot 1 were trees grafted onto Cleopatra mandarin rootstocks, whereas all trees grafted on Volkamer lemon had declined. In Plot 2 which was free of cachexia symptoms (Table 1), a clearer relationship was observed between sudden decline- affected trees and a positive reaction with the 12-kDa blight related protein antisera in the DIBA assays. In Plot 3, there was no relationship between reactivity in DIBA and decline symptoms caused by nematodes and *Fusarium solani* (Table 2). Samples were collected from healthy looking trees in this Plot did not react either in DIBA.

DISCUSSION

Sudden decline of citrus in Venezuela has many of the traits associated with blight in Florida and declinio in Brazil: high zinc levels in the trunk wood, presence of amorphous-like plugs in the xylem vessels and reduced water uptake. From observations made in Venezuela, it appears that cachexia symptoms occur independently from sudden decline. Amorphous plugs, associated with blight and declinio (1, 2), have been reported in Venezuela to occur in nematode and *Fusarium* infections (12, 15), and could produce some of the symptoms associated with sudden decline. The results of the DIBA indicate the presence of the 12-kDa blight-related protein is associated with sudden decline, whereas this protein is not associated with cachexia symptoms, nematode infestations by *Tylenchulus semipenetrans* or *Fusarium solani* infections.

The effect of the tropical environment with high temperatures and moisture on the etiology of sudden decline is not known; it could put extra stress on the plants aiding in establishment of secondary infections which could cause the death of the tree. The association of the 12-kDa blight-related protein with citrus blight in Florida (4), citrus declinio in Brazil (1), and sudden decline of citrus in Venezuela indicate these decline diseases may have a common etiology. Serological differences among citrus blight, citrus tristeza virus and citrus psorosis affected trees in Florida and among citrus declinio, citrus variegated chlorosis and Murcott (tangerine) collapse affected trees in Brazil (1) give additional support. Factors (secondary infections) may exacerbate the syndrome and cause death of the susceptible combination grafted on Volkamer lemon under Venezuela's tropical environment. Cleopatra mandarin rootstock may have some tolerance to blight as reported (18, 19).

ACKNOWLEDGMENT

The second author is a fellow of Conselho Nacional de Pesquisa-Brazil (CNPq). This research was supported by the International Cooperation among Conselho Nacional de Pesquisa (CNPq)- Brazil; Consejo Nacional de Investigaciones Cientificas y Tecnológicas (CONICIT)- Venezuela and Citrus Research and Education Center (CREC), University of Florida.

LITERATURE CITED

1. Beretta, M. J. G., V. Rossetti, A. R. R. Teixeira and O. Sempionato 1991. Positive diagnostic tests for declinio on plants root-graft inoculated in Brazil, p. 256-260. *In*: Proc. 11th Conf. IOCV. IOCV, Riverside.

2. Brlansky, R. H., L. W. Timmer, R. F. Lee, and J. H. Graham
1984. Relationship of xylem plugging to reduced water uptake and symptom development in citrus trees with blight and blightlike declines. Phytopathology 74: 1325-1328.
3. Crozzoli, R. and D. Rivas
1988. Uso de toallas faciales de produccion nacional como alterrnativa al filtro de algodón en la limpieza de muestras nematológicas. Fitopatol. Venezol. 1(1): 32-33.
4. Derrick, K. S., R. F. Lee, R. H. Brlansky, L. W. Timmer, B. G. Hewitt and G. A. Barthe
1990. Proteins associated with citrus blight. Plant Disease 74: 168-170.
5. Derrick, K. S., R. F. Lee, M. J. G. Beretta, B. G. Hewitt and G. A. Barthe
1991. Serological comparisons of citrus decline diseases from Florida and Brazil. Fitopatologia 26: 48.
6. Fundación Servicio para el Agricultor (FUSAGRI)
1983. Resultados de la encuesta realizada para analizar la situación de la tristeza de los cítricos en Venezuela. Programa Cítricas. Informe Trimestral. 21 pp.
7. Lee, R. F., L. J. Marais and R. H. Brlansky
1984. A survey for citrus blight in South Africa, p. 270-278. *In*: Proc. 9th Conf. IOCV. IOCV, Riverside.
8. Lee, R. F., L. J. Marais, L. W. Timmer, and J. H. Graham
1984. Syringe injection of water into the trunk: a rapid diagnostic test for citrus blight. Plant Disease 68: 511-513.
9. Nemec, S., R. Baker and H. Everett
1980. Pathogenicity of *Fusarium solani* to citrus roots and its possible role in blight etiology. Proc. Fla. State Hort. Soc. 93: 36-41.
10. Meredith, J.
1969. Manual de prácticas de nematología agrícola. Univ. Central de Venezuela. Facultad de Agronomía. Maracay, Venezuela.
11. Ochoa, F., A. Vegas, N. de Albarracín, R. Mendt, J. Beretta, R. Lee and R. Brlansky
1988. Diagnóstico de un decaimiento repentino de árboles cítricos en Venezuela. Fitopatol. Venezol. 1 (1): 17-21.
12. Rangel, E., F. Ochoa, N. de Sanabria de A. and R. Crozzoli
1992. Relationship between *Tylenchulus semipenetrans* and *Fusarium* sp. with some symptoms of Venezuelan citrus sudden decline. Proc. Int. Soc. Citriculture, Catania, Italy (in press).
13. Van Vuuren, S. P., N. M. Grech and R. P. Collins
1991. Reaction of Gou Tou orange to the citrus nematode, *Phytophthora* and citrus tristeza virus, p. 128-134. *In*: Proc. 11th Conf. IOCV. IOCV, Riverside.
14. Vegas, A., F. Ochoa, J. Beretta, N. Albarracín, R. Mendt, R. Lee and R. Brlansky
1988. A preliminary diagnosis of a sudden decline in Valencia orange trees on Volkamer lemon rootstock in Venezuela, p. 393-395 *In*: Proc. 10th Conf. IOCV. IOCV, Riverside.
15. Vegas, A., F. Ochoa, N. Albarracín, A. Arcia, T. Barreto, G. Romero, R. Gutierrez, G. Trujillo and R. Mendt
1991. On the etiology of the citrus sudden decline in Venezuela. p. 297-302. *In*: Proc. 11th Conf. IOCV. IOCV, Riverside.
16. Valle, N. del
1987. Citrus blight in Cuba, a review, p. 274-284. *In*: Proc. 1st Conf. Int. Symp. of Citrus Canker, Declinio/Blight and similar diseases. Sao Paulo, Brazil.
17. Wutscher, H. K., M. Cohen, and R. H. Young
1977. Zinc and water-soluble phenolic levels in the wood for the diagnosis of citrus blight. Plant Dis. Rep. 61: 572-576.
18. Young, R. H., L. G. Albrigo, D. P. H. Tucker, and G. Williams
1980. Incidence of citrus blight on Carrizo citrange and some other rootstocks. Proc. Fla. State Hort. Soc. 93: 14-17.