Survey of Blight-Affected Citrus Groves for Xylem-Limited Bacteria Carried by Sharpshooters

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ABSTRACT

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Xylem-limited bacteria (XLB) carried by sharpshooters have been suggested as a possible cause of citrus blight. For 3 yr, collections of sharpshooters (*Oncometopia nigricans* and *Homalodisca coagulata*) from blight-affected citrus groves in nine locations in Florida were caged on indicator plants susceptible to XLB. In no instance were XLB infections detected in grape (*Vitis vinifera*), periwinkle (*Catharanthus roseus*), peach (*Prunus persica*), plum (*P. cerasifera*), or rough lemon (*Citrus jambhiri*). The XLB associated with ragweed stunt was detected in ragweed plants exposed to sharpshooters from four of nine groves in 1981, two of nine groves in 1982, and two of eight groves in 1983.

The Pierce's disease (PD) bacterium or other xylem-limited bacteria (XLB) have been suggested as possible causal agents of citrus blight on the basis of similarity of blight symptoms to those of diseases known to be caused by XLB (10), transmission of the PD bacterium from blighted citrus to grape (11), microscopic detection of XLB-like organisms in citrus (8,12), chemotherapy experiments (13,17), and response of citrus seedlings to inoculation with the PD bacterium (9). However, Koch's postulates for citrus blight have not been fulfilled with any XLB. We have demonstrated that a sharpshooter (*Oncometopia nigricans* (Walker)), a known vector of XLB, is prevalent in areas of Florida where blight incidence is high (16) and is more abundant in blighted than in healthy groves (14). In a study of one grove for 1 yr (4), we found that as many as 10% of the sharpshooters carried bacteria and that the percentage of sharpshooters with bacteria was highest in late summer and autumn. In that work, the only XLB recovered was the ragweed stunt organism (Rg XLB) (4,15).

The purpose of this study was to survey blight-affected groves to determine, by use of indicator plants, which XLB were commonly carried by sharpshooters from affected groves and thus to consistently associate an XLB with the occurrence of the disease.

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MATERIALS AND METHODS

Locations with severely blighted groves were selected near Apopka (Orange County), Astatula (Lake County), Lake Hamilton (Polk County), Waverly (Polk County), Vero Beach (Indian River County), Immokalee (Collier County), and Indiantown (Martin County). All groves were Hamlin, Pineapple, or Valencia oranges (*Citrus sinensis* (L.) Osb.) on rough lemon (*C. jambhiri* Lush.) rootstock. Because healthy groves have low populations of sharpshooters (14), we were unable to collect sufficient numbers of sharpshooters in comparably healthy groves in each area. A single location was found with few blighted citrus trees and high populations of sharpshooters near Orlando (Orange County). The site was planted to a citrus nursery and to oranges and grapefruit (*C. paradisi* Macf.) on various rootstocks.

Sharpshooter collections were made in late summer to early autumn in 1981 and 1982 and in late spring to early summer in 1983, i.e., after sharpshooters become naturally infective (1,2). At least 30 adults were collected from each site (Table 1). Whenever possible, sharpshooters were collected directly from citrus, but they were sometimes gathered from lantana (*Lantana camara* L.), ragweed (*Ambrosia artemisiifolia* L.), or water primrose (*Jussiaea peruviana* L.) growing within the grove. No sharpshooters were included from adjacent woods or fields.

The selective indicator plant method used previously (1,2,4) was employed to detect and differentiate the various XLB. Each sharpshooter collection was placed in a screened cage about 0.8 m tall × 0.6 m wide × 0.6 m deep with the following plants: Chardonnay or Mission grape (*Vitis vinifera* L.), ragweed, Little Pinkie periwinkle (*Catharanthus roseus* (L.) G. Don), and rough lemon. In addition, Nemaguard peach (*Prunus persica* (L.) Batsch.) seedlings were included in 1982 and Myrobalan plum (*P. cerasifera* Ehrh.) seedlings were included in 1983. These indicator plants were selected to detect the PD bacterium, the Rg XLB, the periwinkle wilt bacterium, the phony peach bacterium, the plum leaf scald bacterium, and any XLB that might be capable of infecting the blight-susceptible rough lemon rootstock.

In 1981, collections of *O. nigricans* and *Homalodisca coagulata* (Say) were placed in separate cages if at least 30 insects were obtained at a given location. In 1982 and 1983, collections of both species were combined in a single cage. After introduction to the cages, field-collected sharpshooters fed readily on all host plants and moved frequently between plants. Most of the insects survived at least 10 days. After 3 wk, the plants were removed from the cage, sprayed with malathion, and moved to a greenhouse free of sharpshooters for incubation.

Grapes were examined for symptoms of Pierce's disease for 6 mo. When no symptoms appeared during that time, attempts to culture the PD bacterium on the PD 2 medium (6) were made from four stems or petioles from each plant. All other plants were examined for XLB by squeezing drops of xylem fluid from four stems of each plant onto a microscope slide and staining with methylene blue (4,15). Questionable plants were confirmed by immunofluorescence (3) or by culturing bacteria on PW medium (5,7). Ragweed, rough lemon, and periwinkle were examined for bacteria 7–8 mo after removal from the cages; plums, 12 mo after removal; and peaches, 18 mo after removal. For peaches, four roots as well as four twigs from each plant were examined for XLB.

Stems of 10 ragweed plants were collected directly from all groves surveyed except those at Waverly and Immokalee in 1981 and examined for bacteria as described.

RESULTS AND DISCUSSION

Grape, periwinkle, and rough lemon plants exposed to 29 lots of sharpshooters in 1981, 1982, and 1983 did not become infected with XLB (Table 1). Peaches exposed to nine lots of insects in 1982 and plums exposed to eight lots of insects in 1983 likewise remained free of XLB.

Ragweed plants became infected with

Table 1. Infection of indicator plants with xylem-limited bacteria as determined by microscopic examination of xylem extracts[a]

Location	19 Aug.-9 Oct. 1981[b]			8-30 Sept. 1982			4 May-14 Jul. 1983		
	No. of insects[c]		No. of plants infected[d]	No. of insects		No. of plants infected	No. of insects		No. of plants infected
	O.n.	H.c.	(Ra)	O.n.	H.c.	(Ra)	O.n.	H.c.	(Ra)
Apopka	0	125	0/2	15	23	0/2	6	87	0/1
	63	0	0/2	...	...	...	...	...	...
Astatula	0	157	0/2	28	77	0/2	12	58	0/1
	33	0	0/2	...	...	...	...	...	...
Orlando	0	47	0/2	117	11	0/2	80	4	0/1
	150	0	0/2	...	...	...	...	...	...
Lake Hamilton	48	15	2/2	68	5	1/2	24	12	0/2
Waverly	4	104	0/2	11	21	0/1	...	...	...
Vero Beach	100	13	2/2	121	0	0/2	213	0	0/1
Immokalee	63	6	1/2	39	20	1/2	117	37	0/2
Indiantown I	57	0	0/1	122	1	0/2	92	18	2/2
Indiantown II	103	0	1/2	112	3	0/2	214	12	1/1

[a] Plants were caged with the indicated number of sharpshooters (*Oncometopia nigricans* and/or *Homalodisca coagulata*) captured from blight-affected citrus groves in Florida.
[b] Dates when insects were collected from citrus groves.
[c] No. of *O. nigricans* (O.n.) and *H. coagulata* (H.c.) caged on indicator plants. Insects were allowed to feed on plants for about 3 wk.
[d] No. of ragweed (Ra) plants infected with xylem-limited bacteria/no. used in cage. Xylem-limited bacteria were never detected on periwinkle, grape, rough lemon, peach, or plum. The number of plants per cage was: periwinkles 1–2, grape 2, rough lemon 3, peach 1, and plum 1.

XLB when exposed to sharpshooters from four of nine groves in 1981, two of nine groves in 1982, and two of eight groves in 1983 (Table 1). The Rg XLB was detected mostly in southern and eastern Florida. Infected ragweed plants were obtained with collections from Vero Beach, Immokalee, and Indiantown I in 1 of 3 yr and in Indiantown II in 2 of 3 yr. The only central Florida location where the Rg XLB was found was Lake Hamilton, where it was detected in 2 of 3 yr.

The Rg XLB was detected in field-collected ragweed in 1981 in two of 10 plants at Lake Hamilton and in one of 10 plants at Vero Beach but not at the other locations examined.

Sharpshooters and related xylem-feeding insects are the only known natural means of spread of the PD bacterium and other XLB (1,2). Pierce's disease is endemic in wild grapes (*Vitis* spp.) and other hosts in Florida (1,2,10). Adlerz and Hopkins (2) found that 13 of 74 lots with a total of 803 sharpshooters collected from a vineyard affected by Pierce's disease were infective, whereas only 1 of 57 lots with a total of 1,379 sharpshooters collected from various weeds was infective. Adlerz (1), however, reported detection of the PD bacterium from three of 16 sharpshooter collections from teaweed (*Sida cordifolia* L.) in a central Florida citrus grove. We failed to detect the PD bacterium in 29 collections of nearly 3,000 sharpshooters in nine locations over 3 yr. Even sharpshooters collected within the grove at Lake Hamilton, where we have repeatedly isolated the PD bacterium directly from wild grapes at the grove edge (L. W. Timmer, *unpublished*), did not transmit Pierce's disease to grape. The failure to detect the PD bacterium in citrus groves in this study and only the occasional recovery of the bacterium at locations distant from infected plants in previous work (1,2) may indicate little long-distance movement of sharpshooters despite the fact that these insects are strong fliers.

Because the XLB associated with Pierce's disease, periwinkle wilt, plum leaf scald, and phony peach were not detected by the indicator plant method, the percentage of sharpshooters carrying these bacteria within citrus groves must have been low. Thus, we were unable to demonstrate a constant association between these XLB and citrus blight. The Rg XLB was recovered from some blight-affected groves, especially where it was reported previously in southern and eastern Florida (4). Although the Rg XLB may be involved in blight, we were unable to detect it at all in some severely affected groves and failed to detect it every year in others. Also, we have failed to isolate or detect the Rg XLB in blight-affected trees and have been unable to infect citrus with this XLB using sharpshooters or mechanical inoculation (R. H. Brlansky, J. H. Graham, and L. W. Timmer, *unpublished*). Despite earlier indications that an XLB might be involved in blight (8–13,17), we have not recovered any XLB consistently from blighted groves.

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