Seasonal Fluctuation in the Occurrence of *Xylella fastidiosa* in Root and Stem Extracts from Citrus with Blight

D. L. HOPKINS, Central Florida Research and Education Center, University of Florida, Leesburg 34748; F. W. BISTLINE and L. W. RUSSO, Coca-Cola Foods, P.O. Box 368, Plymouth, FL 32768; and C. M. THOMPSON, Central Florida Research and Education Center, University of Florida, Leesburg 34748

ABSTRACT

Hopkins, D. L., Bistline, F. W., Russo, L. W., and Thompson, C. M. 1991. Seasonal fluctuation in the occurrence of *Xylella fastidiosa* in root and stem extracts from citrus with blight. Plant Dis. 75:145-147.

In two citrus groves in southern Florida where blight is severe, mature trees with blight symptoms were sampled and tested for the presence of *Xylella fastidiosa* by means of enzyme-linked immunosorbent assay (ELISA). Root and stem extracts were obtained monthly from 12 randomly selected trees with blight from each grove. In both groves, there were two yearly peak periods of detection of *X. fastidiosa*, midsummer (June–August) and midwinter (December–February). Bacteria were not detected in tree extracts in April, May, or October. The seasonal pattern and frequency of detection were similar for both root and stem extracts. Strains of *X. fastidiosa* could be cultured from fewer than 10% of the samples that were positive by ELISA. The frequency of detection of *X. fastidiosa* by ELISA varied with the scion/rootstock combinations, with detection most frequent in Pineapple sweet orange on rough lemon rootstock and least frequent in Pineapple on Cleopatra mandarin.

Additional keywords: xylem-limited bacteria

Citrus blight, a vascular wilt-type disease, has been present in Florida citrus groves for at least 100 yr and results in extensive annual losses of up to 500,000 citrus trees per year in Florida (18). Citrus blight has been transmitted by root grafts, implicating a systemic pathogen (17). The etiology of the disease has not been proved, however.

There is considerable circumstantial evidence that blight may be caused by *Xylella fastidiosa* Wells et al, a xylem-limited, gram-negative bacterium that causes dieback-type diseases on many different hosts in Florida (8). Tetracycline antibiotics, which suppress Pierce's disease symptoms in grapevines, have suppressed blight symptoms (16). Microscopy has been used to detect *X. fastidiosa* in citrus with blight (11), and the Pierce's disease strain of *X. fastidiosa* has been transmitted from trees with blight to grapevine using a common sharpshooter vector of the bacterium (9). The Pierce's disease strain has also been cultured directly from vacuum extracts of citrus stems and roots (D. L. Hopkins, *unpublished*). Visible and diagnostic symptoms of blight developed after inoculation of young citrus trees in the greenhouse with strains of *X. fastidiosa* (6). Although this evidence strongly suggests the involvement of *X. fastidiosa* in the blight syndrome, the etiology of the disease has not been completely described. Demonstration of a consistent association between *X. fastidiosa* and blight is very difficult (14). The bacterium can be detected or cultured only from a very low percentage of trees with blight (D. L. Hopkins, *unpublished*) and from a low percentage of sharpshooter vectors in blighted groves (3,14). In the grove, blight normally does not develop in trees younger than 5–8 yr old, and symptom development has been induced by inoculation only in young trees in pots in the greenhouse (6).

In grapevines, the development of peak populations of *X. fastidiosa* was seasonal (10). In *Vitis labrusca* L. bunch grapes, the Pierce's disease bacterium did not accumulate until late May or early June, and in the more resistant *V. rotundifolia* Michx., bacterial populations peaked in late July to mid-August. The objective of this study was to examine the seasonal occurrence of *X. fastidiosa* in citrus trees with blight and to determine if the bacterium was consistently associated with blight.

MATERIALS AND METHODS

Beginning in May 1987 and continuing through April 1989, mature citrus trees in two groves that had severe citrus blight were monitored monthly for the presence of *X. fastidiosa*. The two groves were located in southern Florida—one in Fort Pierce, in the southeast coast flatland area, and the other in DeSoto City, in the South Ridge area in the center of the peninsula. In the flatland grove, 25-yr-old Pineapple sweet orange (*Citrus sinensis* (L.) Osbeck) on Cleopatra mandarin (*C. reticulata* Blanco) rootstock and 11-yr-old Valencia sweet orange on rough lemon (*C. jambhiri* Lush.) rootstock were tested. In the ridge grove, 35-yr-old Valencia and Pineapple sweet orange on rough lemon rootstock

Florida Agricultural Experiment Station Journal Series Paper R-00636.

Accepted for publication 5 July 1990 (submitted for electronic processing).

and 7-yr-old Valencia sweet orange on *C. volkameriana* Ten. & Pasq. rootstock were monitored.

Monthly, 12 trees with citrus blight symptoms were selected randomly and sampled in each grove. Trees were selected for sampling on the basis of visible blight symptoms, including wilt, canopy thinning, zinc deficiency (manifested by small narrow leaves with chlorotic tissue between the main lateral veins), twig dieback, and the presence of trunk sprouts. Trees with similar symptoms in these two groves were positive in tests for zinc accumulation in the trunk wood and decreased water uptake, which are diagnostic for blight. Three stem and three root samples were taken randomly from different locations around the circumference of each tree. Samples were immediately placed in an insulated ice chest, transported to the laboratory, and kept in a refrigerator until extraction, which was done within 24 hr.

Root and stem segments (4–12 mm in diameter and 2–3 cm long) were vacuum-infiltrated with succinate-citrate-phosphate buffer (1.0 g/L disodium succinate, 1.0 g/L trisodium citrate, 1.5 g/L K_2HPO_4, and 1.0 g/L KH_2PO_4, pH 7.0) to extract bacteria. The vacuum extracts (3–4 ml per sample) were concentrated by centrifugation at 4,500 *g* for 15 min and resuspended in 0.8 ml of buffer. One drop of the extract was plated on PW medium (4) modified by the addition, per liter, of 40 μg of penicillin G, 20 μg of sulfamethoxazole, 2 mg of nalidixic acid, 2 mg of trimethoprim, 1 mg of neomycin, and 50 mg of bacitracin. The inoculated plates were incubated at 28 C for 10–14 days. Slow-growing bacterial colonies that became visible after three or more days were streaked onto nutrient dextrose agar and PW media. Bacteria that grew on PW medium but not on nutrient dextrose agar were evaluated in an indirect immunofluorescence test with antisera to the Pierce's disease strain of *X. fastidiosa*. Reactive strains were considered to be *X. fastidiosa*.

The remaining vacuum extract suspension was used in an enzyme-linked immunosorbent assay (ELISA) for *X. fastidiosa*. The antiserum preparation was as previously described (5) and was against a strain causing Pierce's disease of grapevine. For ELISA, γ-globulin was purified and conjugated with alkaline phosphatase. The ELISA procedure was slightly modified from a previous description (13). Flat-bottom microtiter plates were coated with γ-globulin by incubation at 37 C for 2–4 hr. The vacuum extracts were homogenized by adding 1 cm^3 of glass beads (0.17–0.18 mm in diameter) and swirling the extracts for 2 min at top speed on a Vortex-Genie mixer. These extracts were incubated overnight in wells at 6 C. The enzyme substrate (*p*-nitrophenyl phosphate, 1 mg/ml) was allowed to react for 30 min. Plates were read at A_{405nm} using a Bio-Tek Instruments Microplate Autoreader. For positive controls, a 10^5 cfu/ml suspension of a Pierce's disease strain in buffer was homogenized with glass beads. Extracts that gave a mean absorbance reading exceeding two times the mean absorbance of negative controls were considered positive.

RESULTS

There appeared to be two periods a year when *X. fastidiosa* was present in levels detectable by ELISA in symptomatic trees (Fig. 1). One peak occurred in midsummer (June–August) and the other in midwinter (December–February). All trees tested negative for *X. fastidiosa* in April, May, and October. The percentage of trees that tested positive for *X. fastidiosa* was quite variable in the peak periods. For example, in the flatland grove, 58% of the trees were positive in July 1987 and January 1988 but only 17% were positive in July 1988 and 8% in January. In the ridge grove, 75% of the trees were positive in December 1987 and June 1988, but fewer than 20% were positive in midsummer 1987.

The percentage of sampled trees that tested positive for *X. fastidiosa* in root extracts was determined monthly, and, again, bacteria were most frequently detected in midsummer and midwinter. A similar cyclic pattern was observed in stem extracts, but the occurrence of peak periods of bacterial detection was much more erratic in the stems than in the roots. For example, in 1987 bacteria were not detected in any stem extracts from trees in the ridge grove during June, July, or August but were detected in approximately 30% of the stem extracts from trees in the flatland grove. In contrast, in the summer of 1988 bacteria were not detected in stem extracts from the flatland grove but as many as 50% of the stem extracts from trees in the ridge grove were positive for *X. fastidiosa*.

Over the entire 2-yr study, bacteria were detected in root and stem extracts in nearly equal numbers. In the flatland grove, 37 trees tested positive for *X. fastidiosa*: 14 in root extracts only, 11 in stem extracts only, and 12 in both root and stem extracts. In the ridge grove, 53 trees tested positive: 20 in root extracts only, 16 in stem extracts only, and 17 in both root and stem extracts.

Strains of *X. fastidiosa* were cultured from extracts of roots and stems of blighted trees in the two groves. However, *X. fastidiosa* could be cultured only from a very low percentage of the samples that were positive by ELISA (<10%). This low percentage appeared to be due to fast-growing contaminants that occur in the samples (especially root extracts) and inhibit growth of *X. fastidiosa*.

Large differences were observed in the frequency of detection of *X. fastidiosa* in various scion cultivar and rootstock combinations (Table 1). Tremendous variability was also noted within a scion/rootstock combination. For example, during June–August 1987, none of 10 sampled Pineapple/rough lemon trees were positive by ELISA, whereas 13 of 16 were positive during June–August 1988. In three of the four peak periods of bacterial detection during the 2-yr study, *X. fastidiosa* was detected most frequently in Pineapple/rough lemon trees and least frequently in Pineapple/Cleopatra mandarin.

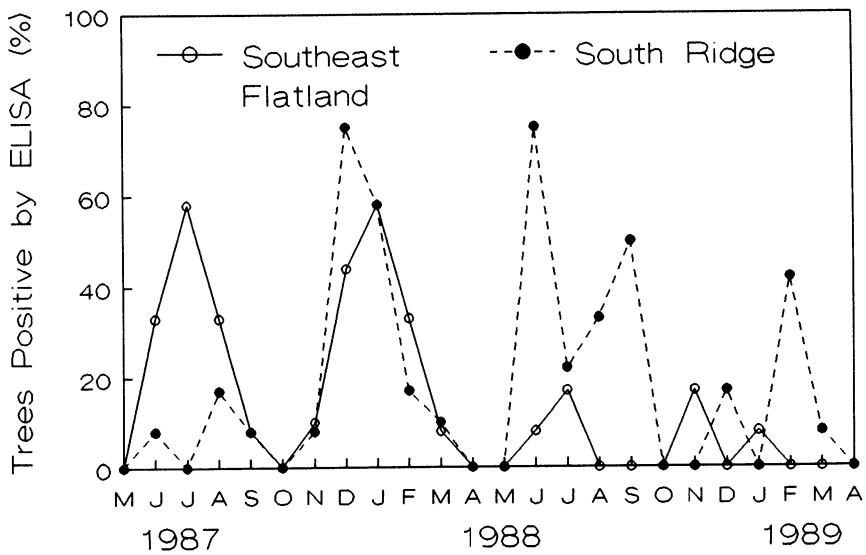

Fig. 1. Seasonal fluctuations in the detection of *Xylella fastidiosa* in stem and root extracts of citrus trees with blight in two groves. ELISA was used to detect the bacteria, and each data point represents 12 randomly sampled blighted trees.

DISCUSSION

The presence of *X. fastidiosa* in citrus trees with blight symptoms was consistently detected by means of ELISA in this 2-yr study. Bacterial populations

Table 1. Comparison of scion/rootstock combinations for differences in detectable populations of *Xylella fastidiosa* during four peak periods of bacterial occurrence

Scion/rootstock	Percent trees positive for *X. fastidiosa*[a]				
	Summer 1987	Winter 1987–88	Summer 1988	Winter 1988–89	Mean
Valencia sweet orange/rough lemon	34	54	16	11	29
Valencia sweet orange/*Citrus volkameriana*	8	29	43	13	23
Pineapple sweet orange/rough lemon	0	62	81	21	41
Pineapple sweet orange/Cleopatra mandarin	33	14	8	0	14

[a]Values represent percentage of blighted trees tested for *X. fastidiosa* that were positive by ELISA of root and/or stem extracts.

were detectable in tree extracts in a regular, cyclic, seasonal pattern. The seasonal occurrence pattern in citrus with blight differed from the seasonal pattern previously described for Pierce's disease of grapevine (10). In grapevine, *X. fastidiosa* was not detectable in the spring until late May or early June; populations of the bacteria peaked in July, August, or September and remained detectable until late fall or early winter when defoliation occurred. In citrus, there was a summer peak similar to that in grapevine with Pierce's disease, but *X. fastidiosa* populations declined in the fall and another period of bacterial accumulation in the trees was observed in December, January, and February. Whereas this seasonal pattern of association of *X. fastidiosa* with blight was fairly consistent, there were seasons when bacteria were not detectable in samples from one of the groves during a time when populations normally were at a peak.

The reason for the twice yearly peak period of detection of *X. fastidiosa* is not obvious. With Pierce's disease of grapevine, *X. fastidiosa* did not develop in actively growing juvenile tissue and there appeared to be a relationship between time of fruit maturation and time of maximum bacterial populations (10). In most hosts, symptoms of diseases caused by *X. fastidiosa* are not visible until fruit maturation or late autumn when the hosts are undergoing senescence (7). A similar explanation could be made for the midwinter period of detection of the bacteria in citrus with blight, but it does not seem to explain the summer peak.

Susceptibility to blight in the grove in Florida varies with the rootstock (19). In this study, *X. fastidiosa* was detected most frequently in trees on rough lemon rootstock, which is also reported to be the most susceptible to blight, and was detected least frequently in trees on Cleopatra mandarin, which is reported to be one of the more tolerant rootstocks. Transmission of blight by approach-grafting roots of healthy trees to the roots of blight-affected trees demonstrated that the infectious agent causing blight occurs in the rootstock (17). In this study, we consistently detected *X. fastidiosa* in root extracts during midsummer and midwinter.

This study demonstrated that populations of *X. fastidiosa* are greatly increased in citrus stem tissue during two peak periods of the year, June–August and December–February. During these periods, larger numbers of bacteria would be available for acquisition by sharpshooter leafhopper vectors of *X. fastidiosa*. The Pierce's disease strain of *X. fastidiosa* has been transmitted from blight-affected citrus to grapevine, using a sharpshooter vector, *Oncometopia nigricans* (Walker) (9). *Homalodisca coagulata* (Say) and *O. nigricans*, two sharpshooter vectors, are commonly found feeding on citrus (1). *O. nigricans* was prevalent in areas of Florida where blight incidence is high (15) and was more abundant in groves with high incidence of blight than in groves with low incidence (12). In Valencia scion on rough lemon rootstock in a South Ridge grove, the spread of citrus blight was reduced when supplemental insecticides were used to control sharpshooter populations (2). In an early study, peaks in populations of *O. nigricans* in the flatland area occurred during midsummer, with some sharpshooter activity through the winter (15). This indicates that, at least in the flatland area, peaks in vector populations and in *X. fastidiosa* populations in the trees occur at the same time. To prevent spread of *X. fastidiosa* in citrus, the most important time to apply insecticide to control vectors appears to be during May or June and during November or December, just before bacteria occur in the stems.

ACKNOWLEDGMENTS

This research was supported in part by the U.S. Department of Agriculture under CSRS special grant 85-CRSR-2-2575 managed by the Caribbean Basin Advisory Group (CBAG). We thank Richard Lobinske for technical assistance.

LITERATURE CITED

1. Adlerz, W. C. 1980. Ecological observations on two leafhoppers that transmit the Pierce's disease bacterium. Proc. Fla. State Hortic. Soc. 93:115-120.
2. Adlerz, W. C., Bistline, F. W., Russo, L. W., and Hopkins, D. L. 1989. Rate of spread of citrus blight reduced when leafhoppers (Homoptera:Cicadellidae) are controlled. J. Econ. Entomol. 82:1733-1737.
3. Brlansky, R. H., Timmer, L. W., and Lee, R. F. 1982. Detection and transmission of a gram-negative, xylem-limited bacterium in sharpshooters from a citrus grove in Florida. Plant Dis. 66:590-592.
4. Davis, M. J., French, W. J., and Schaad, N. W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 5:311-316.
5. Hopkins, D. L. 1984. Variability of virulence in grapevine among isolates of the Pierce's disease bacterium. Phytopathology 74:1395-1398.
6. Hopkins, D. L. 1988. Production of diagnostic symptoms of blight in citrus inoculated with *Xylella fastidiosa*. Plant Dis. 72:432-435.
7. Hopkins, D. L. 1989. *Xylella fastidiosa*: Xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27:271-290.
8. Hopkins, D. L., and Adlerz, W. C. 1988. Natural hosts of *Xylella fastidiosa* in Florida. Plant Dis. 72:429-431.
9. Hopkins, D. L., Adlerz, W. C., and Bistline, F. W. 1978. Pierce's disease bacteria occurs in citrus trees affected with blight (young tree decline). Plant Dis. Rep. 62:442-445.
10. Hopkins, D. L., and Thompson, C. M. 1984. Seasonal concentration of the Pierce's disease bacterium in 'Carlos' and 'Welder' muscadine grapes compared with 'Schuyler' bunch grape. HortScience 19:419-420.
11. Lee, R. F., Feldman, A. W., Raju, B. C., Nyland, G., and Goheen, A. C. 1978. Immunofluorescence for detection of rickettsialike bacteria in grape affected by Pierce's disease, almond affected by almond leaf scorch, and citrus affected by young tree decline. (Abstr.) Phytopathol. News 12:265.
12. Lee, R. F., Timmer, L. W., and Tucker, D. P. H. 1981. Populations of sharpshooters in healthy and blight-affected citrus groves in Florida. (Abstr.) Phytopathology 71:235.
13. Nomé, S. F., Raju, B. C., Goheen, A. C., Nyland, G., and Docampo, D. 1980. Enzyme-linked immunosorbent assay for Pierce's disease bacteria in plant tissues. Phytopathology 70:746-749.
14. Timmer, L. W., and Lee, R. F. 1985. Survey of blight-affected citrus groves for xylem-limited bacteria carried by sharpshooters. Plant Dis. 69:497-498.
15. Timmer, L. W., Lee, R. F., Allen, J. C., and Tucker, D. P. H. 1982. Distribution of sharpshooters in Florida citrus groves. Environ. Entomol. 11:456-460.
16. Tucker, D. P. H., Bistline, F. W., and Gonsalves, D. 1974. Observations on young tree decline-affected citrus trees treated with tetracycline. Plant Dis. Rep. 58:895-896.
17. Tucker, D. P. H., Lee, R. F., Timmer, L. W., Albrigo, L. G., and Brlansky, R. H. 1984. Experimental transmission of citrus blight. Plant Dis. 68:979-980.
18. Wheaton, T. A., chairman. 1983. Citrus blight task force report. University of Florida, Gainesville. 12 pp.
19. Young, R. H., Albrigo, L. G., Tucker, D. P. H., and Williams, G. 1980. Incidence of citrus blight on Carrizo citrange and some other rootstocks. Proc. Fla. State Hortic. Soc. 93:14-17.