Table 1. Evaluation of cupric hydroxide formulations for controlling citrus melanose on the fruit of grapefruit trees. Melanose control 24 Oct. 1988.

Treatment	Formulation	Rate[z] per acre	% Fruit with Melanose
Champ	2.3Fl[x]	1.5	3.1 b[y]
Champion	50WP	3.0	5.9 b
Kocide 101	50WP	3.0	15.8 b
Control-Unsprayed	—	—	38.5 a

[z]Pound active metallio copper.
[x]Flowable containing 2.3 lb. copper hydroxide per gallon.
[y]Mean seperation by Duncan's New Multiple Range Test, 5% level of significance.

Table 2. Evaluation of spray treatments for controlling citrus greasy spot on the spring growth flush of grapefruit trees. Greasy spot control 24 Jan. 1989.

Treatment	Formulation	Rate[z] per acre	% Infected Leaves
Champ	2.3 Fl[x]	1.0	29.4 b[w]
Champion	50WP	2.0	24.4 b
Kocide 101	50WP	2.0	49.2 b
Control-Unsprayed	—	—	73.9 a

[z]Pound active metallio copper.
[y]Total leaves per flush present in June divided into the total of missing leaves plus leaves with one or more lesions.
[x]Flowable containing 2.3 lb. copper hydroxide per gallon.
[w]Mean seperation by Duncan's New Multiple Range Test, 5% level of significance.

If the performance of the flowable at these rates of metallic copper, continue in future trials, it may decrease the possible build-up of copper in the soil. Additional trials will be necessary to confirm.

Additional melanose and greasy spot trials are underway in 1989 as are numerous trials on other crops in Florida and other states.

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RELATIONSHIP BETWEEN XYLEM-LIMITED BACTERIA AND CITRUS BLIGHT

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Abstract. **Xylella fastidiosa Wells et al., a xylem-limited, gram negative bacterium, causes dieback-type diseases in many different trees in Florida. Fluorescence microscopy, enzyme-linked immunosorbent assay (ELISA), and culturing on PD3 medium were all used successfully to detect *X. fastidiosa* in extracts from citrus with blight. Using ELISA, the bacterium was detected in root homogenates and vacuum extracts from roots and stems. *X. fastidiosa* was detected in either root or stem extracts from blight trees in 8 of 12 months studied. The bacterium was also cultured from root and stem extracts of trees with blight, but only from a very low percentage of the samples that were positive by ELISA. Immunofluorescence was used to identify colonies of *X. fastidiosa* on the PD3 medium and these strains from citrus were shown to cause Pierce's disease in inoculated grapevine. These citrus strains were later shown to produce stunting and dieback symptoms in young citrus trees.**

Xylella fastidiosa Wells et al., a xylem-limited gram-negative bacterium, has been proposed as the possible causal agent of citrus blight (5). Blight symptoms are similar to those of other diseases known to be caused by strains of *X. fastidiosa*, including phony disease of peach, Pierce's disease of grape, oak leaf scorch, and sycamore leaf scorch (3,4,7). Additional circumstantial evidence that blight may be caused by this bacterium includes microscopic detection of *X. fastidiosa* in citrus (10), success of chemotherapy experiments (11,15), prevalence of vectors of *X. fastidiosa* in areas of Florida where blight incidence is high (14), and the utilization of one of these vectors to transmit the Pierce's disease strain from citrus trees with blight to grapevine (9). Visible symptoms, dieback and leaf drop, were produced after inoculation of young citrus trees in the greenhouse with strains of *X. fastidiosa* (6). Applications of supplemental insecticides to control sharpshooter vectors of the bacterium reduced the rate of spread of blight in one Florida grove (1).

In spite of this evidence for the involvement of *X. fastidiosa* in citrus blight, the complete blight syndrome has not been reproduced in mature trees in the grove. It has also been very difficult to demonstrate a consistent association between the bacterium and blight (13). This report presents the results of immunofluorescent and enzyme-linked immunosorbent assays (ELISA) to detect *X. fastidiosa*

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in trees with blight in a grove in Central Florida and of efforts to culture the bacterium from root and stem extracts of these trees.

Materials and Methods

A grove in Montverde, which is in the ridge area of Florida north of Orlando, was monitored for the presence of *X. fastidiosa*. This grove was in the northern edge of the citrus belt and was studied prior to the 1983 freeze. Severe blight was diagnosed in the grove by visible symptoms, high zinc content in the wood, and very poor water flow. Trees sampled were 'Valencia' sweet orange scion (*Citrus sinensis* [L.] Osb.) on rough lemon rootstock (*C. jambhiri* Lush.) with visible blight symptoms.

Leaves, small roots, and small stems were sampled. Small roots (<4 mm diameter), petioles, and leaf veins (2.5 g) were ground in 8 ml of extraction buffer with a mortar and pestle. The buffer was 0.1 M phosphate-buffered saline at pH 7.4 plus Tween 20 (0.05%), polyvinylpyrrolidone (2%), and ovalbumin (2%). The homogenates were filtered through cheesecloth and centrifuged at 4,500 g for 15 min. The pellets were resuspended in 2.0 ml of extraction buffer. A 0.1 ml aliquot was used for direct immunofluorescence (DIF) (12) and isolation tests, and the remaining suspensions were further homogenized by adding 1 cm^3 of glass beads (0.10 – 0.19 mm diameter) to the suspensions and swirling for 2 min at top speed on a Vortex-Genie mixer. This homogenate was used for ELISA.

Larger root and stem segments (4-12 mm diameter) were vacuum extracted with buffer (disodium succinate, 1.0 g/liter; trisodium citrate, 1.0 g/liter; K_2HPO_4, 1.5 g/liter; and KH_2PO_4, 1.0 g/liter; pH 7.0). The vacuum extracts (3-4 ml per sample) were centrifuged at 4,500 g for 15 min and the pellet was resuspended in 0.8 ml of buffer. Four to five drops of the extracts were used in attempts to culture *X. fastidiosa* and for DIF. The remainder was homogenized with glass beads as described above and used for ELISA. The DIF and ELISA procedures were as previously described (8). With ELISA, extracts were considered to be positive when $A_{405\ nm}$ values were at least twice those of negative controls.

In attempts to culture *X. fastidiosa*, the homogenates and vacuum extracts were inoculated onto PD3 medium (2). One drop of inoculum was added per plate and spread over the surface of the agar with a glass rod. Plates were incubated at 28C.

Leaves from branches with dieback and leaves with zinc deficiency symptoms were collected for direct isolation attempts. Petioles and veins from these leaves were surface sterilized and aseptically cut into 0.5- to 1.0-cm sections, which were squeezed with forceps. The sap exuding from each section was blotted onto PD3 medium.

Any bacterial colony visible to the unaided eye within 3 days was considered a contaminant. Colonies visible after 3 days were transferred to nutrient agar and PD3 medium. If a bacterium grew on PD3 but did not grow or grew very weakly on nutrient agar, it was tested in an indirect immunofluorescence assay (3) with antisera to *X. fastidiosa*. Reactive strains were tested immediately for pathogenicity to grapevine (8).

Results and Discussion

Culturing on PD3 medium, ELISA, and DIF were utilized in attempts to detect *X. fastidiosa* in citrus with blight (Table 1). With root homogenates, bacteria were detected with ELISA but not with DIF. This failure with DIF probably resulted from the nonspecific background fluorescence associated with tissue debris in the homogenates making it difficult to discern the fluorescing bacterial cells. With the cleaner vacuum extracts, DIF was as effective as, or more effective than, ELISA in detecting *X. fastidiosa*. Culturing on PD3 medium was the most inefficient means of detection.

X. fastidiosa was not detected in petioles or leaf veins by any of the methods (Table 1). Using ELISA, *X. fastidiosa* was detected in root homogenates and root vacuum extracts of more than 50% of the sampled trees with blight in February 1981. It was detected less frequently in stem vacuum extracts. DIF detected bacteria in 50% of both the root and stem extracts from blight trees. Symptomless trees near the blight trees were also found to contain *X. fastidiosa*. The bacterium was detected nearly as frequently in root homogenates and vacuum extracts from symptomless trees as from trees with blight. In this test, bacteria were not detected in stems of symptomless trees. One strain of *X. fastidiosa* was successfully isolated from stem extracts of a blight tree and one strain from root extracts of a symptomless tree.

Assuming that *X. fastidiosa* causes citrus blight, the detection of bacteria in symptomless trees indicates that the causal agent can be detected prior to symptom development. Another possible explanation for the presence of *X. fastidiosa* in symptomless trees is that citrus is a symptomless host of the bacterium and it has no role in citrus blight.

Table 1. Detection of *Xylella fastidiosa* in citrus in a grove with severe blight.

Citrus sample	Number of trees assayed	Trees positive by:[z] ELISA	DIF	Culture
	Blight trees			
Petiole and leaf vein homogenate	10	0	0	0
Root homogenate	12	7	0	0
Root vacuum extract	6	3	3	0
Stem vacuum extract	6	1	3	1
	Symptomless trees			
Root homogenates	9	4	0	0
Root vacuum extract	3	1	2	1
Stem vacuum extract	3	0	0	0

[z]ELISA = enzyme-linked immunosorbent assay, DIF = direct immunofluorescence. Culturing was done on PD3 medium. This experiment was conducted in February 1981.

Root and stem extracts from trees with blight were assayed monthly for *X. fastidiosa* using ELISA. Bacteria were detected in either root or stem extracts in 8 of the 12 months of the assays (Table 2). Over the year, similar numbers of stem and root samples were positive for *X. fastidiosa*. Bacteria were not detected in extracts from any of the trees in April, May, or June. In the Montverde grove, bacteria were present in detectable numbers in blight trees from mid-summer through the fall and winter seasons, but not during spring. *X. fastidiosa* was not detected in more than 50% of the sampled trees in a month and often was detected only in a single tree. The reason for this low detection rate is not known. Perhaps, the bacteria are unevenly distributed in the tree and random sampling of roots and stems results in this low rate of detection. Only 3 root or stem segments, 2-3 cm long, were sampled per tree. Low bacterial populations in these segments may be below the limits of detection for ELISA (10^5 cells/ml).

While ELISA has been quite effective in detecting *X. fastidiosa* in citrus with blight, the bacterium can be cultured only from a very low percentage (<10%) of the samples that were positive by ELISA (unpublished). *X. fastidiosa* was never cultured from citrus leaves, either by plating homogenates or by direct blotting of exuded sap from petioles or veins onto PD3 medium (Table 1). Attempts to culture the bacterium from root homogenates were also unsuccessful. In other studies, tissue homogenates have generally been inhibitory to the growth of strains of *X. fastidiosa*. In this test, cultures of *X. fastidiosa* were obtained from 1 of 4 vacuum extracts of blight trees that were positive by ELISA and the one vacuum extract of a symptomless tree that was positive. This is a much higher success rate than is usual. However, pure colonies of *X. fastidiosa* are almost never obtained directly from citrus extracts and were not obtained in this experiment. Some slow-growing colonies similar in appearance to those of the Pierce's disease bacterium were obtained on the PD3 medium from vacuum extracts from citrus. Indirect immunofluorescence was used to determine that these colonies were primarily bacteria that did not react with the antisera but sometimes a few clusters of bacterial rods from the colonies did react. When these slow-growing, partially reactive colonies were used to inoculate indicator grapevines, severe Pierce's disease symptoms developed and the Pierce's disease strain of *X. fastidiosa* was reisolated in pure culture from the grapevine. The difficulty in isolating *X. fastidiosa* from citrus extracts appears to be primarily due to *faster growing* contaminants that inhibit its growth.

Table 2. Detection of *Xylella fastidiosa* in vacuum extracts of citrus trees with blight symptoms using enzyme-linked immunosorbent assay (ELISA).

Month (1981-82)	Number of positive trees[z]/total trees	
	Root vacuum extract	Stem vacuum extract
June	0/4	0/4
July	1/8	—
August	—	2/4
September	0/10	1/10
October	2/8	3/8
November	0/8	0/8
December	1/8	1/8
January	2/4	1/4
February	1/4	0/4
March	1/8	0/8
April	0/8	0/8
May	0/8	0/8

[z]Extracts with a mean absorbance reading that exceeds two times the mean absorbance of negative controls were considered positive.

In summary, *X. fastidiosa* was shown to occur in roots and stems of citrus trees with blight using ELISA, DIF, and culturing on PD3 medium to detect the bacterium. In some experiments the bacteria were detectable in 50%, or more, of the sampled trees. The bacterium was detected in all seasons of the year except late spring-early summer. Bacteria that were cultured from these trees produced Pierce's disease in grapevine and, therefore, are the Pierce's disease strain of *X. fastidiosa*. These strains were later shown to produce stunting and dieback symptoms in citrus rootstock seedlings and in 'Pineapple' orange trees on rough lemon rootstock, and in a few cases produced the diagnostic symptoms of blight (6). While this adds to the strong circumstantial evidence supporting the hypothesis that citrus blight is caused by *X. fastidiosa*, the crucial evidence that is lacking is the reproduction of the complete blight syndrome by controlled inoculations of mature citrus trees.

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