

# Detection and Transmission of a Gram-Negative, Xylem-Limited Bacterium in Sharpshooters from a Citrus Grove in Florida

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## ABSTRACT

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 Disease* 66:590-592.

A bacterium was detected by scanning electron microscopy in the pump organs of sharpshooter leafhoppers (*Oncometopia nigricans*) collected monthly from a blighted citrus grove. The percentage of *O. nigricans* carrying bacteria reached a peak of 10% in August 1980. *O. nigricans* from the same collections transmitted a xylem-limited bacterium to ragweed (*Ambrosia artemisiifolia*) but not to grape (*Vitis vinifera*), periwinkle (*Catharanthus roseus*), rough lemon (*Citrus jambhiri*), or American elder (*Sambucus canadensis*). The bacterium reacted with fluorescein-labeled antiserum to the Pierce's disease bacterium.

Additional key words: Cicadellidae, citrus blight, rickettsialike bacteria

Citrus blight, a disease of unknown etiology, does not affect trees until they are at least 5 yr old. To date, the disease has not been graft-transmitted (11). A rickettsialike bacterium has been recovered from xylem extracts from affected citrus trees (3). Lee et al (7) used immunofluorescence to show that this bacterium was serologically related to the Pierce's disease bacterium. Attempts to visualize or culture the causal agent of blight have been unsuccessful (4). Hopkins et al (6) reported transmission of the Pierce's disease bacterium from blighted citrus to grape by the sharpshooter leafhopper *Oncometopia nigricans* (Walker), and Hopkins and Adlerz (5) induced a disease in rough lemon seedlings with Pierce's disease bacterium.

Populations of *O. nigricans* are larger in areas where the incidence of citrus blight is high than in areas where the incidence of blight is low (8). *O. nigricans* is a vector of such bacteria as the Pierce's disease bacterium (1) and the periwinkle wilt bacterium (9). Purcell et al (10), using isolation on solid media and scanning electron microscopy, found the Pierce's disease bacterium in the cibarium (pump organ) and in other portions of the foregut of sharpshooter vectors that had

fed on infected plants. We have made similar findings for *O. nigricans* and the periwinkle wilt bacterium and the Pierce's disease bacterium (*unpublished*).

In this study, we examined *O. nigricans* collected from a citrus grove with a high incidence of blight for the presence of bacteria and evaluated this leafhopper's ability to transmit bacteria to healthy plants.

## MATERIALS AND METHODS

About 200 sharpshooters were collected each month from October 1979 through October 1980 in a severely blighted citrus grove in the southeast flatwoods region of Florida near Indiantown. Diagnosis of citrus blight was based on field symptoms and zinc analysis of trunk wood (13). Most sharpshooters were collected from young citrus trees, but some were often collected from other plants such as lantana (*Lantana camara* L.), ragweed (*Ambrosia artemisiifolia* L.), water primrose (*Jussiaea peruviana* L.), and goldenrod (*Solidago* sp.).

Each month, the cibaria from 25-60 sharpshooters were examined by scanning electron microscopy. Live sharpshooters were immersed in 3% glutaraldehyde in 60 mM phosphate buffer (pH 6.8), beheaded, and fixed overnight at 4 C. The heads were postfixed in 1% osmium tetroxide for 2 hr, dehydrated in an acetone series, transferred to an acetone-Freon (1:1) mixture, and then put in 100% Freon TF (E. I. du Pont de Nemours & Co., Wilmington, DE 19898). After critical point drying in a Bomar SPC-900 critical point drier (The Bomar Co., Tacoma, WA), the cibaria and diaphragms were dissected from the head, mounted, and sputter-coated with 100 Å of gold-palladium. Dissected cibaria were

scanned for attached bacteria with a JEOL JSM 35 scanning electron microscope.

Fifty to 100 sharpshooters from each collection date were placed in a common cage containing grapes susceptible to Pierce's disease (*Vitis vinifera* L. 'Chardonnay,' 'Mission,' 'Chenin Blanc,' or 'Cabernet Sauvignon'), periwinkle (*Catharanthus roseus* (L.) G. Don), ragweed (*A. artemisiifolia*), American elder (*Sambucus canadensis* L.), and rough lemon (*Citrus jambhiri* Lush.). The insects were allowed to feed on the caged plants for at least 60 days. Insects were observed to feed on all plant species. The plants were removed from the cage and examined for symptoms and checked for bacteria two to three times in the following 4 mo. To check for the presence of bacteria in ragweed, periwinkle, rough lemon, and American elder, xylem extracts of petioles and stems were squeezed onto microscope slides, dried, stained with 0.1% methylene blue, and examined. Samples containing many small, rod-shaped bacteria were tested with fluorescein-labeled antiserum to the Pierce's disease bacterium by direct immunofluorescence (7). Attempts were made to culture rickettsialike bacteria from surface-sterilized stems and petioles of all rough lemon and grapes on PD2 medium (2).

## RESULTS

No bacteria were found with scanning electron microscopy in the cibaria of sharpshooters collected from blighted citrus from October 1979 to April 1980 (Table 1). Bacteria were detected in one of 50 sharpshooters collected 7 May 1980. We found bacteria attached to the floor of the cibarium and in the apodemal groove of the pump diaphragm (Fig. 1). These bacteria measured 2.1-3.0 × 0.24-0.40 μm and resembled a rickettsialike bacterium we previously found in *O. nigricans* that had been fed on plants with Pierce's disease or periwinkle wilt (*unpublished*). Bacteria were not found in sharpshooters collected on 28 May 1980 but were found in all subsequent months through October 1980 (Table 1). The highest percentage (10%) of sharpshooters found to have bacteria was collected 18 August 1980.

No symptoms of Pierce's disease were found on any grapes, no periwinkle wilt was found on any *Catharanthus roseus*,

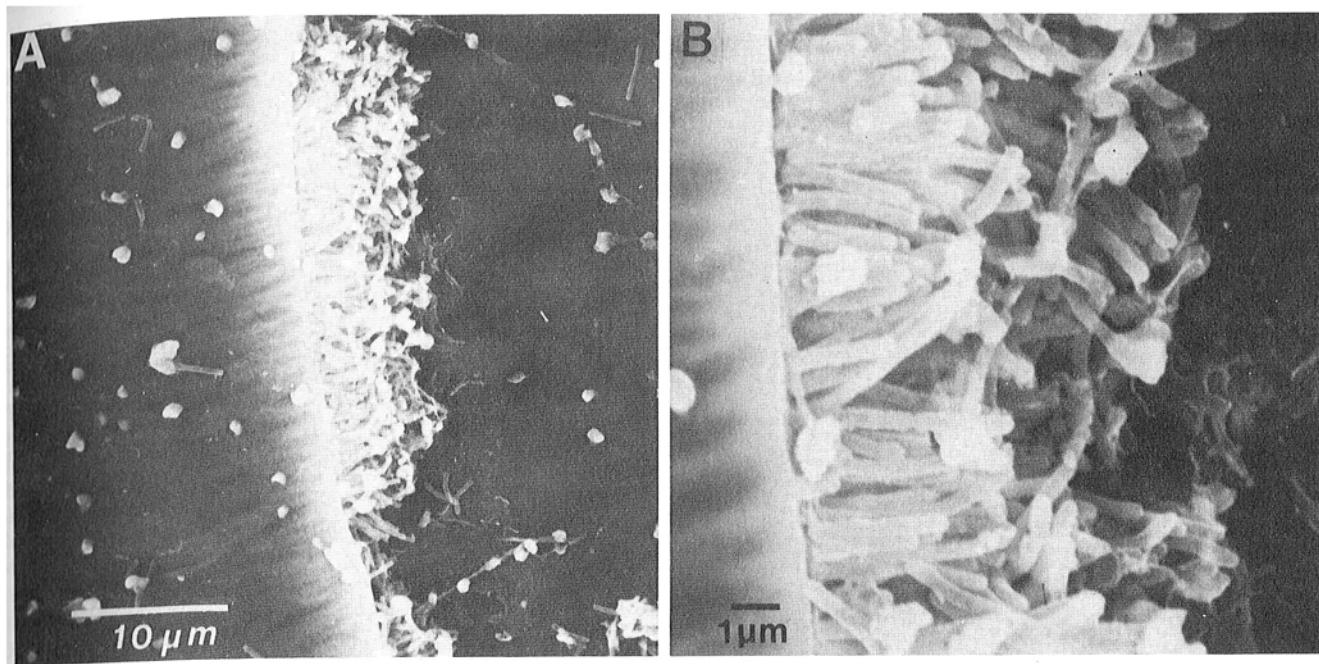


Fig. 1. Bacteria found colonizing the cibarium (pump organ) of a sharpshooter leafhopper (*Oncometopia nigricans*) collected from a blighted citrus grove,  $\times 2,200$  (A) and  $\times 7,800$  (B).

Table 1. Detection by scanning electron microscopy (SEM) and transmission of a gram-negative bacterium in *Oncometopia nigricans* collected in a blighted citrus grove

Collection date	SEM results <sup>a</sup>	Transmission results <sup>b</sup>				
		Grape	Elder	Ragweed	Rough lemon	Periwinkle
October 1979	0/25	0/2	0/1	ND	0/3	0/4
November 1979	0/25	0/2	0/1	ND	0/3	0/1
December 1979	0/25	0/3	0/1	ND	0/3	0/1
January 1980	0/25	0/1	0/1	1/1	0/3	0/1
March 1980	0/25	0/1	0/1	1/1	0/3	0/1
April 1980	0/25	0/1	0/1	1/1	0/3	0/1
7 May 1980	1/50	0/1	0/1	1/1	0/3	0/2
28 May 1980	0/50	0/1	0/1	1/1	0/3	0/1
June 1980	2/47	0/1	0/1	1/1	0/3	0/1
July 1980	3/53	ND	ND	ND	ND	ND
4 August 1980	3/50	0/1	0/1	1/1	0/3	0/3
18 August 1980	6/60	ND	ND	ND	ND	ND
September 1980	3/55 <sup>c</sup>	0/2	ND	2/2 <sup>d</sup>	0/3	0/2
October 1980	1/57	0/3	ND	ND	0/3	0/1

<sup>a</sup> Number of sharpshooters with bacteria/total number of sharpshooters.

<sup>b</sup> Number of transmissions/number of plants tested. ND = not determined.

<sup>c</sup> Ten sharpshooters collected from ragweed were not included in this number; nine of the 10 contained bacteria.

<sup>d</sup> Fifteen sharpshooters collected from ragweed were not included in this number; when these were placed on seven ragweed seedlings, bacteria were transmitted to all seven plants.

and no symptoms occurred on rough lemon or American elder. No rickettsialike bacteria were detected by light microscopy using methylene blue and/or fluorescent antisera specific for the Pierce's disease bacterium in periwinkle, American elder, or rough lemon, nor were bacteria cultured from grape or rough lemon. Large numbers of bacteria were detected in ragweed fed upon by *O. nigricans*, but no specific symptoms were associated with infection. These bacteria fluoresced after incubation with fluorescein-labeled Pierce's disease bacterium antiserum. Bacteria were transmitted to ragweed beginning in January 1980 and continuing through October (Table 1). Ragweed plants that were not exposed to

sharpshooters were not found to contain bacteria by either immunofluorescence or methylene blue staining of xylem extracts.

On 25 September, sharpshooters were collected directly from mature ragweed growing in the grove. Using scanning electron microscopy, we found that nine of 10 sharpshooters contained bacteria. Fifteen sharpshooters from the same collection were fed on healthy ragweed seedlings for 20 days and transmitted bacteria to all seven plants.

#### DISCUSSION

A high percentage of sharpshooters in which bacteria were detected by scanning electron microscopy transmitted a

rickettsialike bacterium to ragweed; sharpshooters in which bacteria were not detected rarely did so (Table 1). The negative scanning electron microscopy results during January and March may have been the result of small sample sizes or a low percentage of sharpshooters carrying bacteria; however, when the sample size was increased to 50 sharpshooters, bacteria were detected in 1.7–10.0% of the sharpshooters.

Detection and transmission of a xylem-limited bacterium by sharpshooters collected from a blighted grove coincided with the distribution of *O. nigricans* in citrus groves (12). *O. nigricans* populations peaked in late spring and early summer (12). Bacteria were detected in sharpshooters and transmitted to ragweed at that time and into late summer. This also fits well with the peak of natural infectivity of sharpshooters on grapes and the buildup of the Pierce's disease bacterium in grapes (1). The lack of bacteria in scanning electron microscopy studies may not exclude sharpshooters from being infective or inoculative, as Purcell et al (10) showed with the sharpshooter *Graphocephala atropunctata* and the Pierce's disease bacterium.

In feeding studies, sharpshooters failed to transmit the ragweed-associated bacterium to grape, periwinkle, and rough lemon. The bacterium is probably not the Pierce's disease bacterium or the periwinkle wilt bacterium, because no symptoms developed in grape or periwinkle and no rickettsialike bacteria could be recovered from these plants. Failure to transmit the bacterium to rough lemon seedlings may not be enough evidence to exclude citrus as a host. Other than the presence of the

ragweed-infecting bacterium in a severe blight area, no evidence at present indicates any association of the bacterium with blight. Further attempts to culture and identify this bacterium are under way.

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