Occurrence in Johnsongrass of Rickettsia-like Bacteria Related to the Phony Peach Disease Organism

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ABSTRACT

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Rickettsia-like bacteria (RLB) were consistently observed in KOH extracts of johnsongrass stems collected in peach orchards with phony disease. Electron microscopic examination of xylem in leaves of johnsongrass revealed RLB morphologically similar to those associated with phony peach and other xylem-limited, leafhopper-vectored diseases. Johnsongrass RLB were antigenically related to RLB associated with phony peach and Pierce's disease. RLB in johnsongrass are closely related to the phony disease organism, and johnsongrass is a possible natural reservoir of RLB associated with phony peach disease.

Additional key words: *Prunus persica, Sorghum halepense,* xylem-limited bacteria

Phony disease of peach (PPD) (*Prunus persica*) and Pierce's disease of grapevines (PD) are caused by xylem-limited, rickettsia-like bacteria (RLB) (8,9,13). The PD bacterium has been cultured (2), but attempts to culture RLB associated with PPD have failed. PPD occurs in the southeastern United States, where a widespread epidemic currently exists. Efforts to control PPD have relied on removal of diseased peach trees and eradication of wild Chickasaw plum (*P. angustifolia*), an inoculum reservoir, to prevent transmission of the causal agent to peach trees by insect vectors. However, whether weeds in orchards may also serve as inoculum sources for infection of peach trees is not known. Newly developed techniques (6,7) have made it possible to test orchard weeds for the presence of RLB and determine their relationship to PPD-associated RLB.

This report presents evidence that a bacterium closely related to PPD-associated RLB is present in johnsongrass (*Sorghum halepense*), a weed commonly found in peach orchards throughout the Southeast.

MATERIALS AND METHODS

Sites in which johnsongrass was found growing in experimental and commercial peach orchards in Peach and Houston counties, Georgia, were selected for the study. The phony disease was present in nearly all orchards in these counties, which constitute the major peach-growing area of the state.

Stems of johnsongrass were cut at the ground line, and the lower 15 cm was brought to the laboratory for examination. Tissues were examined for RLB by the method of French et al (6), with slight modifications. Internodal sections were cut to 7.5-cm length, and 1 ml of 0.1 M KOH was drawn through by vacuum infiltration within 4 hr of sampling. In some cases, 1-ml KOH extracts from two or three internodal pieces per site were combined and examined as a composite sample for that site. Two drops from the extract were placed on a microscope slide and dried for subsequent examination.

Slides were examined at ×400 with a microscope (Carl Zeiss, Inc., NY) equipped with phase contrast optics and an objective lens with a 20-mm field of vision. The entire slide was examined, and the numbers of RLB in the three fields with the highest counts were recorded. Only particles with light transmittance characteristics and rodlike shape and dimensions typical of RLB were counted.

The serologic relationship of the bacterium in johnsongrass with PPD and PD bacteria was studied by immunofluorescence. Antiserum prepared against the PPD bacterium was obtained from N. W. Schaad, Department of Plant Pathology, University of Georgia, Experiment. Immunoglobulins were separated from bulk sera with a DEAE Sephadex A-50 column, as recommended by Dedmon et al (4). Protein concentration was monitored by optical density at 280 nm. Antibacterial immune γ-globulins were conjugated with fluorescein isothiocyanate (FITC) through dialysis membranes (4). Conjugated antibody was then separated from free FITC and highly charged protein by the method of Auger and Shalla (1). The conjugate had the highest staining when diluted at 1:10. Plant tissues were prepared and stained according to the method of Auger and Shalla (1). The stained materials were observed with a Zeiss GFL microscope equipped with a HB 200-W mercury vapor lamp and filters. PD-infected grapevine stem tissues and PPD-infected root tissues were also included in the test. Cells from pure cultures of *Agrobacterium tumefaciens* and *Pseudomonas syringae* (obtained from J. E. DeVay, Department of Plant Pathology, University of California, Davis) and healthy peach and grape tissues were used as controls.

Attempts were made to cultivate RLB using media developed by Davis et al (2) for the Pierce's disease organism and also on several other media formulated by us. Johnsongrass stems collected from 19 sites were used. Media contained in petri dishes were inoculated by expressing the xylem fluid from 2-cm-long internodal sections onto the agar surface. Plates were incubated for a minimum of 12 days at 25–28 C and observed for colonies of RLB.

Electron microscopic examinations were made on ultrathin sections of johnsongrass leaf blades. Pieces of leaf tissue 2 × 2 mm were cut from lamina of leaves, processed (8), and embedded in Spurr's medium (14). Ultrathin sections were cut with a diamond knife, mounted on copper grids, stained (8), and examined with an electron microscope.

RESULTS

RLB were detected in vascular extracts from johnsongrass stems from November 1977 through May 1979 (Table 1). Some RLB were found in at least one stem from each of the three sites tested on all but one sample date. Numbers of RLB per microscopic field ranged from one to 35, but counts of one to five RLB per field were most common. Stems were also collected from 19 additional sites in experimental and commercial orchards in May 1979. Extracts from 18 of the 19

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samples contained RLB, with numbers ranging from one to six per microscopic field. No disease symptoms were associated with the presence of RLB in the johnsongrass. All attempts to cultivate RLB from johnsongrass failed.

Electron microscopic observations revealed that RLB occurred primarily in large vessels of johnsongrass. Only 1% or less of vessels contained RLB, and only low numbers of cells were detected in such vessels.

The organisms possessed a general morphology and ultrastructure very similar to the bacteria found in tissues affected by PPD, PD, and almond leaf scorch (ALS) (10,12,13). Based on the few cells we saw, the measurements were 0.35–0.45 μm in diameter and 1.1–1.7 μm in length. Rod-shaped and ovoid forms representing longitudinal and transectional views of the organisms were observed (Fig. 1). The bacteria had a multilayered cell wall that was often rippled. The cytoplasm of cells was rich in ribosomes and often contained DNA-like strands and a nuclear region. Capsular material was present on some cells; however, little or no electron-dense (gumlike) matrix was seen in the lumens of the invaded vessels.

After treatment with fluorescent PPD-associated RLB antiserum, bright fluorescence was observed in samples from johnsongrass, PPD-infected peach, and PD-infected grape. No fluorescence was observed with *A. tumefaciens, P. syringae,* healthy peach, or healthy grape tissues.

DISCUSSION

This is the first report describing the occurrence of RLB in johnsongrass. Previous studies have shown associations between johnsongrass and diseases caused by xylem-limited RLB. Freitag (5) reported that in California the causal agent of PD was artificially transmitted to johnsongrass but naturally infected johnsongrass was not found. Turner (*unpublished*, Special Report FVPP-4, USDA, Fort Valley, GA) showed a direct correlation between the presence of johnsongrass and other weeds and the incidence of PPD in Georgia peach orchards. This association was attributed to the buildup of vector populations on the weeds, and no mention was made of johnsongrass being a potential source of inoculum.

Davis et al (3) recently reported that RLB associated with PPD were antigenically related to RLB causing PD and ALS. Our results demonstrated that johnsongrass RLB were also antigenically related to RLB of PPD and PD. Mircetich et al (11) suggested that ALS and PD were caused by the same bacterium. They also reported that ALS bacteria failed to induce symptoms in peach, and they did not detect RLB in peach rootstocks supporting diseased almond tops.

The high incidence of RLB in johnsongrass collected in orchards with phony peach disease further supports the concept of a possible association between RLB in johnsongrass and PPD. Attempts will be made to artificially transmit RLB from johnsongrass to peach, but approximately 2–3 yr are required for symptom development in peach even if transmissions are successful. Until transmission studies are completed or a more specific procedure for identifying RLB can be developed, we can only suggest that the johnsongrass RLB may be important in the spread of PPD in the Southeast.

Table 1. Occurrence of rickettsia-like bacteria (RLB) in extracts from internodes of johnsongrass stems

Date sampled	No. stems with RLB/total stems tested[a]		
	Site 1	Site 2	Site 3
1977 8 November	5/7	3/7	5/7
1978 17 May	1/3	2/3	1/3
18 September	3/3	3/3	1/3
17 November	3/3	2/3	0/3
1979 24 April	1/3	1/3	1/3
22 May	2/2	2/2	2/2
30 May	3/3	3/3	3/3
Overall average	18/24 = 75%	16/24 = 67%	13/24 = 54%

[a]One ml of 0.1 M KOH was drawn through each stem by vacuum infiltration and extracts were observed for RLB using phase contrast microscopy.

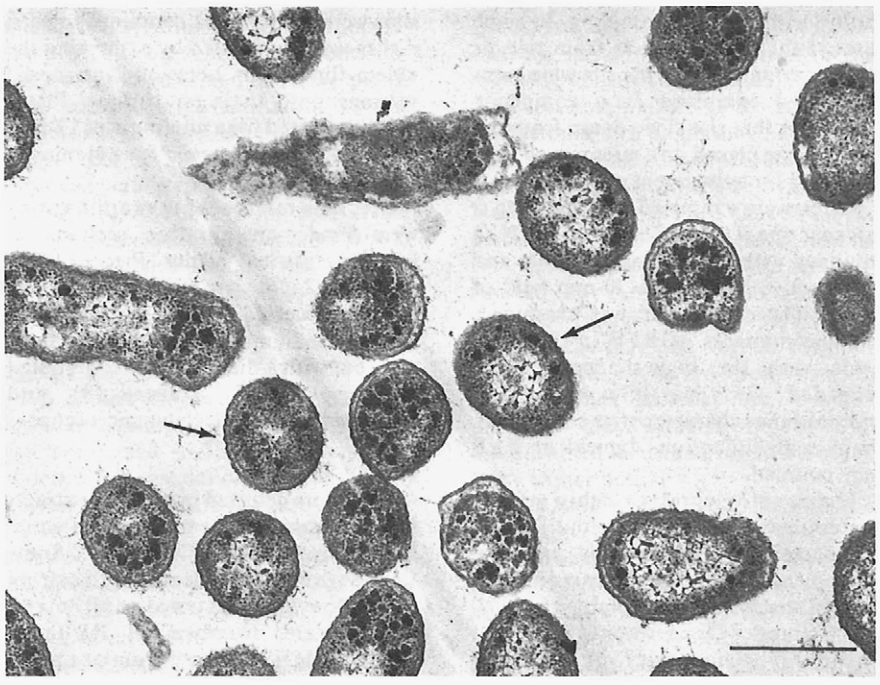

Fig. 1. Electron micrograph of rickettsia-like bacteria (RLB) found in the lumen of a vessel in a leaf of johnsongrass (*Sorghum halepense*). Bacterial cells contain numerous ribosomes and DNA-like strands and have multilayered, rippled cell walls (arrows). Condensation (clumping) of ribosomes in the cytoplasm is also evident. Bar = 0.5 μm.

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