

Pierce's Disease of Grapevines in Mexico

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ABSTRACT

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A bacterium with rippled cell walls was isolated and cultured from the petioles of grape leaves with symptoms of Pierce's disease. The leaves were collected from Parras, Mexico. The organism was reinoculated by vacuum infiltration and needle injection into dormant and rooted green cuttings of grape cultivars. Characteristic symptoms of Pierce's disease developed within 2-3 mo; controls remained healthy. The bacterium was reisolated from the experimentally infected vines. A sonicated cell suspension of the Mexican isolate was used to produce antiserum in New Zealand white rabbits. Serologically, the Mexican isolate was the same as the Pierce's disease organism isolated from grape, almond, and alfalfa from California, Florida, and Costa Rica. However, the isolates from grapes in Mexico and Florida formed an extra band in agar gel double-diffusion tests, with homologous antisera.

Pierce's disease, first recognized by Pierce in 1892 in *Vitis vinifera* in California (13), is the most important

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factor limiting the development of European or bunch grape industries in Florida (1), Mississippi, Georgia, Alabama (8), Texas (11,12), and Costa Rica (7). The causal bacterium was recently isolated and cultured in cellfree media (2). The same bacterium causes almond leaf scorch in almonds (2,10) and alfalfa dwarf in alfalfa (6,9,14).

We report the isolation, culture, and

proof of pathogenicity of Pierce's disease bacterium from Parras, Mexico, and its serologic relationship to previous isolates from grape, almond, and alfalfa.

MATERIALS AND METHODS

Petioles were sampled from grapevines with symptoms of Pierce's disease and brought from Mexico to the University of California, Davis. Petiole pieces (0.3-0.5 cm) were surface-sterilized in 1.5% sodium hypochlorite solution for 3 min and rinsed five times in sterile double-distilled water. Juice was squeezed from petiole pieces and directly blotted on solidified JD-3 agar medium (2). All plates were aerobically incubated at 28 C. Ultrastructural studies of the bacteria were done according to the method of Davis et al (2).

Pathogenicity of the bacteria was tested by inoculating dormant stem cuttings and rooted green cuttings of grapevine cultivars Chardonnay, Flora, Mission, and Pinot Noir. Inoculation was by vacuum-infiltration or by injection. In

the vacuum-infiltration method, the upper end of each three- or four-node dormant cutting was attached to a vacuum pump, and 0.2–0.3 ml of a bacterial preparation (approximately 10^9 bacteria/ml) in sterile distilled water was drawn into each cutting. In the injection method, each of 20 rooted green stem cuttings received 0.01–0.02 ml of bacterial preparation per wound; four or five wounds were made with a 22-gauge hypodermic needle and a syringe at the second node and also near the tip of the stem. Controls included cuttings that were not inoculated or were inoculated with sterile distilled water. The dormant cuttings were rooted on a heated bench and transplanted to 1-gal plastic pots after 3 wk. All plants were kept in a greenhouse.

Antiserum against the Mexican isolate of Pierce's disease bacterium was produced in New Zealand white rabbits from which preimmune serum had been collected. The bacteria were harvested from JD-3 broth (2) cultures after 8 days of incubation at 27 C by centrifugation (13,000 rpm, 15 min), washed five times in phosphate buffer saline (PBS), 0.1 M, pH 7.0. The pellet was suspended in PBS (at a concentration of 10^9 bacteria/ml), sonicated for 30 sec at 52.5 W/cm², and used as an antigen. Each rabbit was intravenously injected with increasing doses of 1, 1.5, 2, 2.5, 3, and 3.5 ml at 3-day intervals for 3 wk. This was followed by two doses of 4 and 4.5 ml with equal volume of Freund's incomplete adjuvant at 2-day intervals; the rabbits were bled 1 wk after the final injection. Antisera were produced similarly against Pierce's disease bacterium isolated from diseased grapes and almonds from California and from grapes from Florida.

Serologic relationships of the Mexican isolate with grape, almond, and alfalfa isolates from California, Florida, and Costa Rica, respectively, were studied by agar gel double diffusion. The gels were prepared with 0.8% ion agar in PBS. All antisera (diluted 1:1 in PBS) and sonicated antigen concentrations (10^8 cells/ml) were the same throughout the study. The plates were incubated at room temperature in a humid chamber. The serologic relationships of the Mexican bacterial isolates with other Pierce's disease bacterial isolates also were studied by slide agglutination and ring precipitation (3).

RESULTS

White, smooth, circular colonies with entire margins (0.5–1.0 mm diam) were observed on the agar medium after 7–8 days of aerobic incubation at 28 C. The bacterium was Gram-negative and catalase-positive and had rippled cell walls, which are morphologic and biochemical properties characteristic of Pierce's disease bacterium (2).

In the retransmission trials, typical

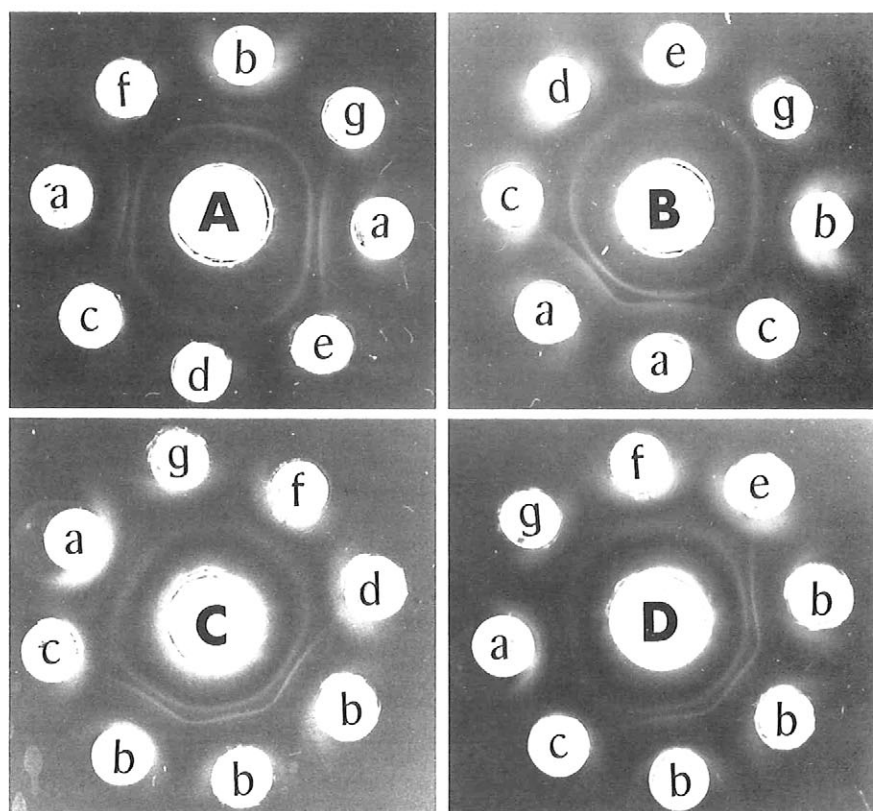


Fig. 1. Serologic relationships of Pierce's disease bacteria isolated from alfalfa, almond, and grape. Central wells contain antisera (titer 2048) against bacteria isolated from grape from (A and B) Mexico and (C and D) Florida. Peripheral wells were used for antigens to Pierce's disease bacterium isolated from: grape from a = Mexico, b = Florida, c = Napa Valley, California, and d = Costa Rica; almond from e = Contra Costa County, California, and f = Los Angeles County, California; and alfalfa from g = San Joaquin Valley, California.

symptoms of Pierce's disease developed in 40 of 45 grape dormant cuttings (inoculated by vacuum infiltration) and in 16 of 20 green-rooted cuttings (needle injection) after incubation for 2–3 mo. All 35 control plants remained healthy. Colonies characteristic of the Pierce's disease bacterium were reisolated from all 30 symptomatic plants but from none of 13 control plants.

In agar double-diffusion tests (Fig. 1) the Mexican isolate of the Pierce's disease bacterium reacted with its own antiserum and with antisera developed against the grape and almond isolates from California or Florida. Reciprocal reactant combinations also were reactive. The alfalfa isolate reacted with all the antisera in this study. In each reaction one or two bands were observed. However, the Mexican and Florida grape isolates of Pierce's disease bacterium produced an extra band in homologous reactions, which was absent in the tests with the California or Costa Rican isolates. This distinct band was not observed when the Mexican and Florida grape isolates were tested against antiserum that was produced using glutaraldehyde-fixed bacteria (2,7).

No serologic differences were observed among the isolates of Pierce's disease bacterium in slide agglutination (titer 2048–4096) or ring precipitation (titer

256–512) tests. The homologous and heterologous titers were almost the same.

DISCUSSION

Our study confirms the diagnosis (4) and electron microscopic evidence (5) of Pierce's disease of grapevines in Mexico. Originally the disease was observed only in the United States, but it also occurs in Costa Rica (7) and Mexico, and we suggest that it may occur in other places in Central and South America.

The serologic studies confirm the previous findings that the same pathogen causes Pierce's disease in grapes (2,10), almond leaf scorch in almonds (2,10), and alfalfa dwarf in alfalfa (9,14). The presence of an extra band in the agar gel diffusion tests with the Mexican and Florida isolates may indicate different bacterial strains. Our study also indicates that the antiserum produced by using sonicated cells may be used to distinguish differences between some isolates, which antisera prepared against the bacteria after fixation in glutaraldehyde did not (7).

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