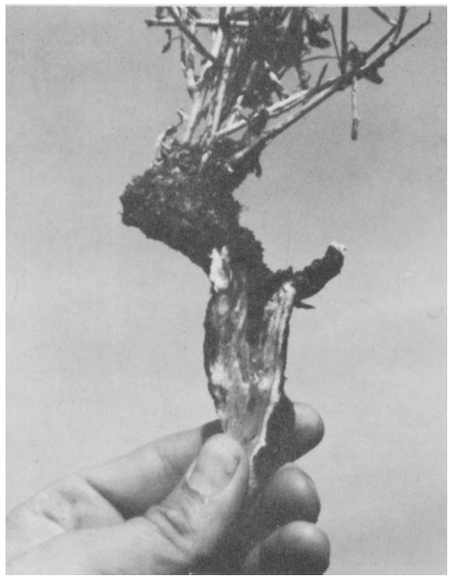

Crown damage allows entrance by disease organisms.

lowing harvest. If regrowth shoots are damaged by wheel traffic, additional root reserves will be required for shoot growth and plant survival. Plants growing on light textured soil with small root systems at the time of first harvest will be generally weakened, and many will not survive the double blow of root-inhibiting soil compaction and mechanical damage to regrowth. Fall planting will give more time for proper root development before the summer harvest season begins.

Minimizing traffic damage

Extending the stand life of alfalfa plantings in California one year beyond the present three-year average could mean savings of $30 million per year to alfalfa producers. This study demonstrates the significant effects of wheel traffic, and makes imperative the further development of techniques to minimize traffic damage. Possible ways of reducing traffic effects and extending the production life of a stand include standardizing wheel traffic patterns, establishing designated traffic lanes in the alfalfa field, corrugation or rill planting, and bed plantings with shallow furrows to be used as lanes for standardized wheel traffic. In designing alfalfa seedbeds, a grower needs to consider soil type, irrigation system, and weed populations, in addition to standardizing wheel traffic patterns.

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BACTERIUM

JAIME G. AUGER

T. A. SHALLA

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THE NEWLY DISCOVERED Pierce's disease bacterium could destroy large numbers of grapevines and render parts of California unfit for the culture of common grape varieties. The disease has already destroyed at least 75,000 acres of grapevines in four major epidemics. In certain areas, it remains endemic. Aside from California, the disease has affected states along the Gulf coast and southeastern seaboard.

Since 1884, this disease has been periodically investigated with the belief that it was caused by a virus. Recently, investigators at U.C., Davis, and at the University of Florida reported electron microscope observations of rickettsia-like bacteria in leaf vessels from infected vines. It has not been possible, however, to culture these microorganisms on artificial media and prove their pathogenicity. Since these findings raised the question of whether Pierce's disease was caused by a virus or a microorganism, renewed efforts were undertaken to determine the real cause. This study reports for the first time the isolation of a rod-shaped, gram-positive bacterium from the disease-spreading leafhopper, *Draeculacephala minerva*. This bacterium can be readily cultured in an artificial medium in the laboratory and can reproduce Pierce's disease in healthy grapevines.

Spread

Pierce's disease is efficiently spread by the leafhoppers *Carneocephala fulgida*, *Draeculacephala minerva*, and *Hordnia circellata*, and the spittle bug, *Philaenus spumaris*. Leafhoppers such as *D. minerva* were freed of the pathogenic microorganism by rearing them for more than five generations on barley plants in insect-proof cages. After the fifth generation a sufficient number of leafhoppers was obtained for experimental work.

Isolation of the pathogen

A group of noninfective leafhoppers were fed on healthy grapevines, *Vitis vinifera* cv. Mission, then they were transferred to plants with Pierce's disease. Excreta (spittle) of 10 leafhoppers was collected after they were fed at first on healthy plants, and then additional excreta samples were taken from the same vectors after they had fed on diseased plants. Each sample of excreta was streaked on an enriched bacteriological agar medium. Also, a collodion-coated electron microscope grid was floated on the same excreta samples.

Bacteria grew as small white colonies on the media streaked with the excreta of the leafhoppers which had fed on a diseased grapevine. No such colonies appeared on media streaked with excreta from leafhoppers which had fed previously only on a healthy grapevine. Numerous rod-shaped bacteria (0.5×2.0 μ) were observed with the electron microscope from these colonies and were identical to those observed in samples taken from vectors which had fed on diseased vines. No such bacteria were observed in samples from vectors which fed only on healthy vines.

In a second experiment, two groups (10 each) of noninfective leafhoppers were fed for 48 hrs on healthy and diseased plants, respectively. Then each group of insects was immersed in 70% ethanol, transferred to 2% sodium hypochlorite, and then rinsed in sterile distilled water. They were finely ground with a sterile glass rod and the semiliquid body material was streaked on a bacteriological agar medium in Petri plates. These plates were incubated for 48 to 72 hrs at 30°C. Small white bacterial colonies identical to those seen previously appeared on the media streaked with the ground leafhoppers that had

discovered to be cause of
PIERCE'S DISEASE OF GRAPEVINES

fed on diseased vines, but no such colonies arose on the media streaked with extracts from leafhoppers that had fed on healthy vines.

Proof

A suspension of these bacteria (2×10^9 cells/ml), which had been cultured in a broth medium, was injected with a fine glass needle into noninfective leafhoppers. These leafhoppers were then transferred and allowed to feed on healthy Mission or Carignane grapevines (5 to 10 insects per plant). Another group of leafhoppers was injected the same way with sterile broth and similarly placed on healthy grapevines. All of the plants were kept individually isolated in insect-proof cages. The insects were kept on the plants for 15 days or longer, or until they died. After 6 weeks, all of the plants exposed to the leafhoppers which had been injected with the bacteria exhibited symptoms typical of Pierce's disease (center plant in photo). No symptoms developed on plants exposed to leafhoppers which were injected with sterile broth (plant to right in photo).

Samples of the inoculated plants were prepared for electron microscopy to determine whether the organism was present in the vascular tissue as it was in naturally infected vines. Organisms similar to those seen in sections of naturally infected grapevines were observed in vessels of the plants experimentally exposed to bacteria-injected leafhoppers (see electron micrograph). The organism was not observed in the vessels of plants exposed to leafhoppers which had been injected with sterile broth. The same bacterium was again only recovered from leafhoppers which had fed on experimentally infected plants. Also, as a parallel control, identical bacteria were recovered from leafhoppers fed on naturally infected plants. Again, such bacteria did not appear on media streaked with extracts of ground bodies of leaf-

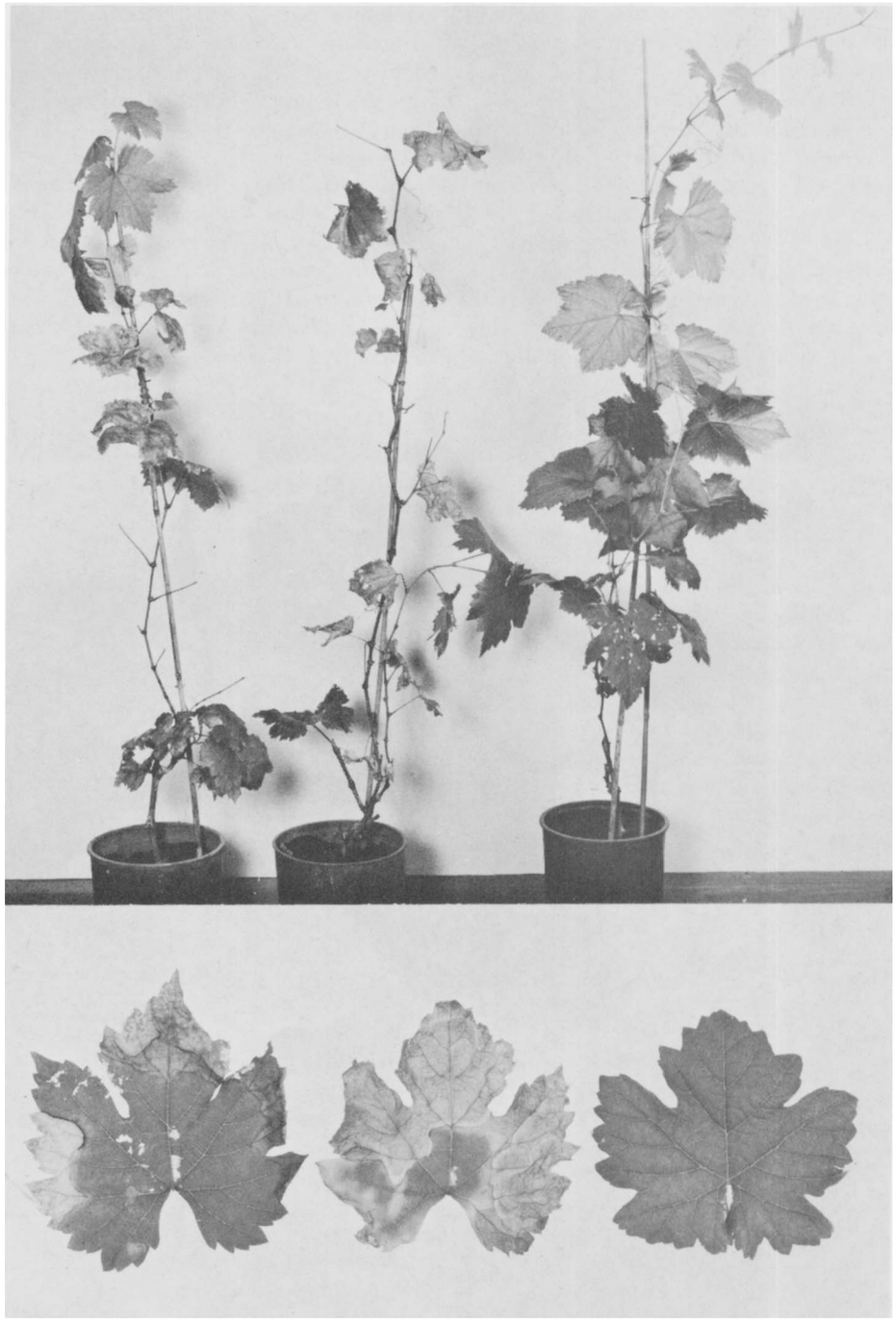

Photo 1. Experimentally inoculated plants (upper photo) and leaves (lower photo) of **Vitis vinifera** cv. Carignane. Upper and lower left, vine exposed to naturally infective vectors; upper and lower middle, exposed to bacterium-injected vectors; upper and lower right, exposed to sterile broth-injected vectors.

hopper vectors which had fed on the plants exposed previously to vectors injected with sterile broth. The reisolated bacterium had the same morphology, size, cultural, and physiological characteristics as the original isolate. The bacterium is gram-positive, rod-shaped, 0.4–0.6 μm wide, 1.0–2.0 μm long, and nonmotile. It grows well at a temperature range of 20 to 32°C with an optimum of 29 ± 1°C. On agar medium the colonies are white to white-gray in color, slightly convex, circular with entire margins, and have a smooth, shiny texture. The bacterium is a faculative anaerobe and produces acid but not gas from glucose. Tests for production of indole and methyl-red were negative.

These experiments have demonstrated that a gram-positive bacterium is the etiological agent of Pierce's disease in grapevines, and not a virus, as previously believed. The organism has been successfully cultured on artificial media. By using the leafhopper vector injected with the cultured and purified bacteria, the disease symptoms can be consistently reproduced in healthy grapevines and the same organism reisolated from clean leafhoppers fed on these plants and on naturally infected plants from the field. An attempt to isolate and to culture the bacterium from diseased plant tissues did not succeed, for reasons presently unknown. The characteristics of this bacterium, which in nature is apparently confined to its vectors and to the xylem vessels of its host plants, plus its morphological, cultural, and physiological features, suggest that the Pierce's disease agent is a distinct plant-pathogenic gram-positive bacterium heretofore unrecognized. Studies to determine the taxonomic position and characteristics of the Pierce's disease bacterium are in progress in the Department of Plant Pathology, at U.C., Davis.

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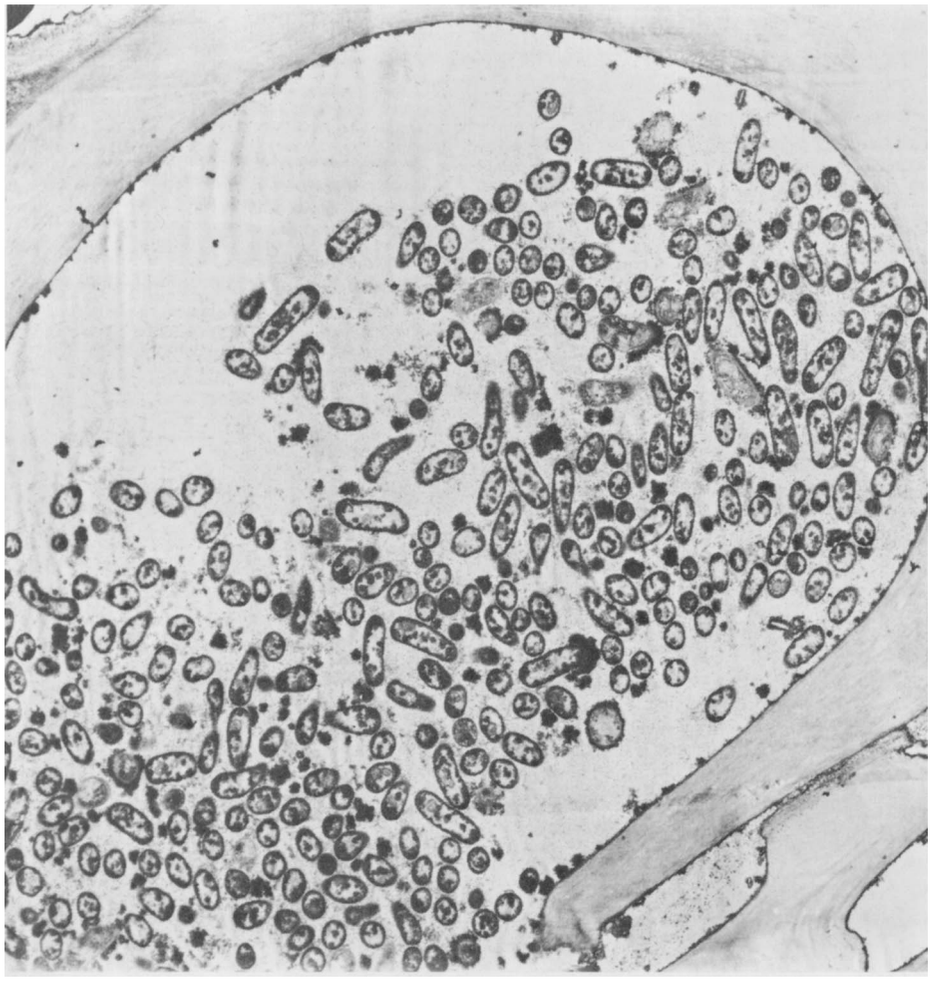

Photo 2. Electron micrograph of a transversely sectioned vessel from a leaf of a plant experimentally inoculated with the Pierce's disease bacterium. The vessel lumen is partially filled with the causal bacteria. X 5000.

WINNING THE WEST

(Continued from page 3)

The tomato story demonstrates even broader research involvement. Eighty-five per cent of this crop was being picked by Mexican nationals, until that practice was drastically restricted by Congress in 1964. Fortunately, a long-range mechanization research program had been launched in 1950, and the first machine prototypes were proving their worth.

The U.C. once-over harvester design of Coby Lorenzen is incorporated in a majority of the machines now in use. But its success depended upon plant scientists breeding a tomato plant that could set and ripen most of its fruit at one time; on food scientists who determined how to process this new tomato; and on farmers and processors working with Cooperative Extension personnel to find out how to grow and handle the crop. And it was U.C.'s G. C. Hanna whose seedstock has served as the parentage for virtually all varieties in common use internationally.

The final major achievement in the tomato project was the shift from hand-picking into 50-lb capacity field lugs, to machine harvesting into 1,000-lb bins, and finally, into bulk trailers, each holding more than 10 tons of fruit.

Tree fruits, grapes and nuts, are shaken and picked in a minute or less by machines. Raisin grapes are shaken from their vines, spread on paper on the ground to dry, then picked up by machine.

Besides eliminating the human drudgery of many farming tasks, mechanization in some crops has permitted harvesting in cooler evening or nighttime hours, benefiting both workers and crops. Harvesting also can be timed more closely to the maturity peak of the crop.

There are, and will be, arguments about whether these improvements are a boon to the public at the expense of human labor displaced from old jobs. The engineer, of course, must concern himself with all human needs. Some of his efforts are in problem solving responses, and others are the added outgrowth of problem solving. But the adoption of new technology depends upon economics. Since mechanization usually adds to the costs of processing, or increases problems of quality or quantity, new practices usually are adopted only when their absence would mean a food or fiber shortage.

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