Presymptomatic Egress of Xanthomonas pruni from Infected Peach Leaves

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Journal Series Paper, New Jersey Agricultural Experiment Station, Cook College, Rutgers–The State University of New Jersey, New Brunswick, NJ 08903.

Accepted for publication 13 January 1977.

ABSTRACT

MILES, W. G., R. H. DAINES, and J. W. RUE. 1977. Presymptomatic egress of Xanthomonas pruni from infected peach leaves. Phytopathology 67: 895-897.

Studies by scanning electron microscopy confirm the importance of stomata as infection sites as well as locations for egress of *Xanthomonas pruni* in peach leaves. Observations revealed that bacteria either as individual cells or in a mass exude from stomata as much as six days before infections are visible to the unaided eye, suggesting that bacteria may be available for new infections during much of the presymptomatic period.

Additional key words: stomata, inoculum.

Bacterial spot of stone fruits [which is caused by *Xanthomonas pruni* (Smith) Dowson] was first described by Smith in 1902 as a disease affecting the leaves and fruit of Japanese plums in Michigan. He also reported that infection occurs through the stomata (3, 4). In discussing symptom development he wrote, "when the enlarged spots have begun to sink in and become brown, the bacteria reach the surface as numerous tiny, rounded, pale-yellow, gum-like masses, which ooze from the stomata lying over the closed bacterial cavity" (3).

This investigation was undertaken to study infection, disease development, and inoculum availability as viewed with the scanning electron microscope (SEM).

MATERIALS AND METHODS

Small Rio-Oso-Gem peach trees, 1 yr after budding, were planted in sand in 11.39-liter (3-gallon) plastic pots and given Hoagland's solution 5 days per week throughout the duration of the experiment. Water without nutrients was supplied to the trees on weekends. To obtain inoculum, Rio-Oso-Gem peach leaves, collected in the field and showing newly formed bacterial leaf spots, were surface-sterilized with 95% ethanol. A few diseased spots were removed and placed in 1.0 ml of sterile water. After shaking, the liquid was streaked on 1.5% nutrient agar and, on appearance, a single yellow colony was used to inoculate sterile nutrient broth. After being shaken for 48 hr, these cultures were added in sufficient quantity to the water used in a wind-rain machine to bring the bacterial cell concentration to 2-3 $\times$ 10^6 cells/ml. Trees were exposed individually to a 40 km/hr (25 mph) windy rain in the wind-rain machine. After water-soaked areas were observed in the leaves, the trees were removed to a greenhouse bench and the water-soaked areas were marked for further studies.

Blocks approximately 2 mm $\times$ 2 mm were cut from noninoculated leaves and from the marked areas of the inoculated leaves at the following times: 15 min and then at 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 days following the inoculation. All sections were fixed in 2.5% glutaraldehyde solution for 2 to 3 hr, then washed with 0.1 M phosphate buffer (pH 7.4). Then they were dehydrated in a 20, 40, 60, 80, 95, and 100% ethanol series, followed by 20, 40, 60, 80, 95, and 100% Freon 113 (diluent was absolute ethanol). From the 100% Freon 113, the sections were critical-point dried with Freon 13 as a transitional fluid. The samples were then mounted on specimen holders and coated in an AC Sputter Coater with a layer of gold approximately 30 nm thick. The leaf surfaces, stomatal cavities, and mesophyll cells were studied under the SEM (ETEC Autoscan) for the presence of bacteria and evidence of cell degradation. The studies of the interior of the leaf were made at the edges of the leaf blocks and at locations where the epidermis fractured during preparation. The leaf surfaces, stomatal cavities, and mesophyll cells were studied for the presence of bacteria and evidence of cell degradation.

RESULTS AND DISCUSSION

A study of inoculum development and availability with time revealed the presence of bacteria on the leaf surface and within the intercellular spaces of the leaf by the 9th day following inoculation. At this time a few bacteria were observed on the cell walls and within the intercellular spaces of the mesophyll (Fig. 1), in the stomatal cavity, on the guard cells of the stomatal lip, and on the leaf surface very near the stomata. The bacteria increased rapidly in numbers during the next few days, becoming moderate to abundant by the 12th day and very abundant by the 15th day following inoculation. Bacteria never were observed on noninoculated foliage.

Throughout these studies bacteria always were found to be more abundant on the lower than on the upper leaf surface. Peach leaves have stomata only on the lower surface. The first visible leafspot symptoms appeared as water-soaked angular areas 15 days after inoculation. Before that time bacteria were observed in masses filling, and often nearly obscuring, the stomata below. These masses originated to occurred in the stomata almost without exception, but very infrequently one was observed where injury to the surface cells had occurred.

By the time bacteria were first observed on the leaf surface (9th and 12th days) they were already present in

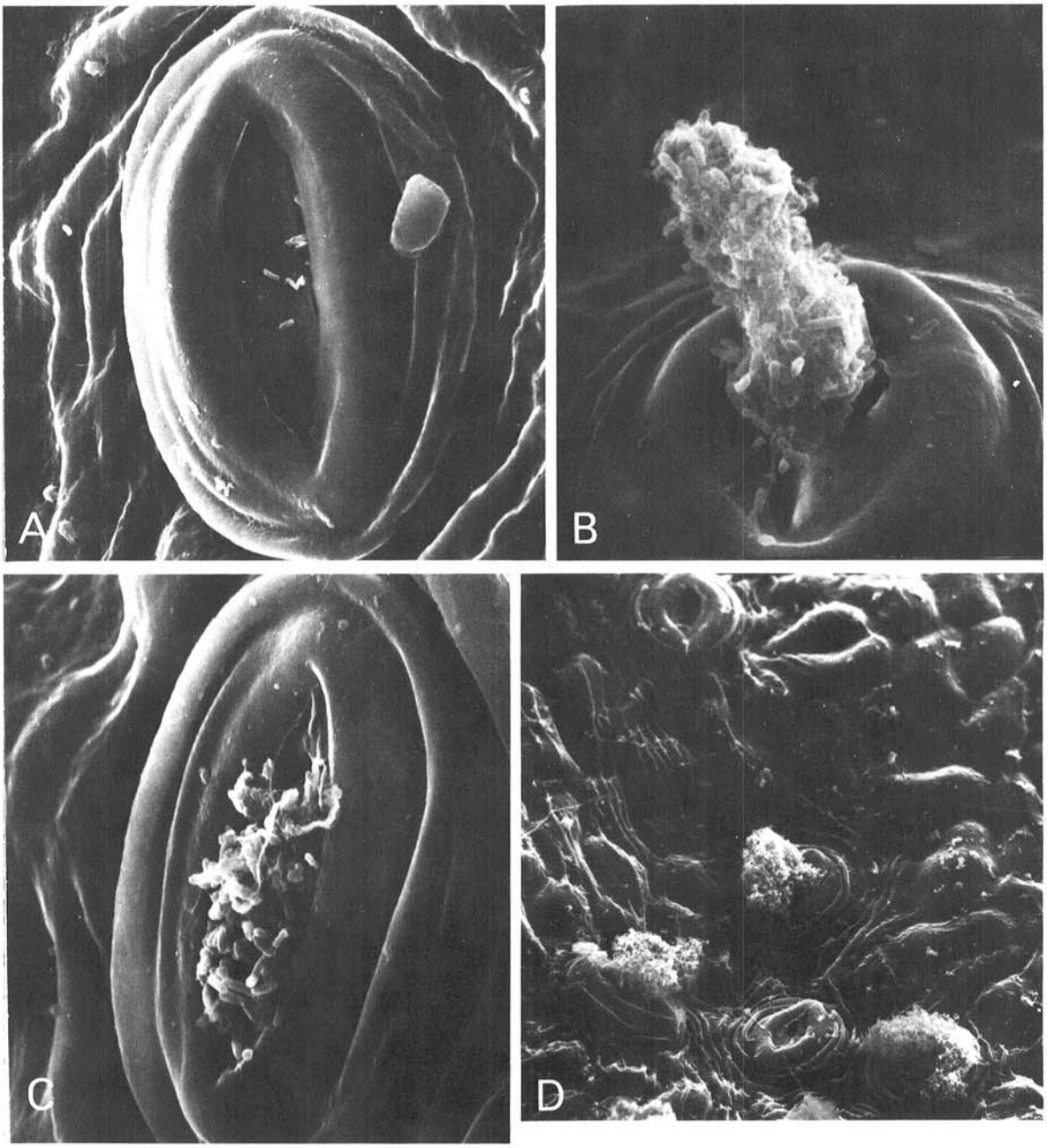

Fig. 1-(A to D). Rio-Oso-Gem peach leaves showing sites and mode of *Xanthomonas pruni* egress from infected areas. (A and B) Nine days after inoculation. **A)** ×2,500. A few *X. pruni* cells visible in the stomatal lip and the leaf surface; and **B)** ×4,300. Bacterial mass exuding from a stoma. (C and D) *X. pruni* cells exuding through the stomata 15 days after inoculation. **C)** ×5,000. Close-up of an individual stoma, and **D)** ×1,000. Showing mounds of *X. pruni* cells at the stomatal openings, with stomata in close proximity free of such exuding masses.

the spongy mesophyll and palisade areas. Whenever bacteria were found in clumps they were always enmeshed in strands of unknown composition, possibly polysaccharide slime. Even when bacteria were numerous inside the leaf, host cells generally retained their shape and appeared to be normal. Observations with the SEM never revealed host cells filled with bacteria; however, studies with the compound light microscope (1) have revealed such colonized cells. Even when such cells were filled with bacteria, the cell walls appeared intact. Although in this study the intercellular spaces seldom were found to be filled completely with bacteria; exudation of the compact masses from the stomata suggest pressure from within. Perhaps the method of tissue preparation resulted in the removal of some of the bacteria from the intercellular spaces. Pressure created from expanding masses of bacteria might disarrange, isolate, and even rupture individual or groups of host cells. Collapsed cells that appeared to have been crushed were observed.

These studies strongly support previous reports (2, 3) that the stomata provide a most important site for bacterial egress from within the leaf onto the leaf surface where, in water droplets and films, they can be disseminated for secondary infections. It is significant to note that bacteria, as individual cells or in a mass, were observed to appear on the leaf surface as long as 6 days before disease symptoms became visible to the unaided eye. Since bacterial movement may be largely by passive dispersion, one might expect that the movement out of the leaf started earlier than our studies show. Perhaps new infections, during periods of dew and water-soaking conditions, supply inoculum for additional infections very soon after infection is initiated.

Bacteria inside the leaf ranged from 2.5 to 3.2 times as long as they were wide. In addition, flagella were never observed under the conditions of these experiments even though the bacteria were observed at magnifications from ×1,000 to ×50,000. Polar flagella are common on *Xanthomonas pruni* cells grown on artificial media.

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