Systemic Invasion of Plum Leaves and Shoots by *Pseudomonas syringae* pv. *syringae* Introduced into Petioles

Isabel M. M. Roos and M. J. Hattingh

Fruit and Fruit Technology Research Institute, Private Bag X5013, Stellenbosch 7600, and Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa.

We thank Claude R. Swart for assistance with scanning electron microscopy and Peter S. Knox-Davies for reading the manuscript.
Part of a Ph.D. (Agriculture) thesis submitted by the first author to the University of Stellenbosch.
Accepted for publication 18 March 1987 (submitted for electronic processing).

ABSTRACT

Roos, I. M. M., and Hattingh, M. J. 1987. Systemic invasion of plum leaves and shoots by *Pseudomonas syringae* pv. *syringae* introduced into petioles. Phytopathology 77:1253-1257.

Systemic spread of an antibiotic-resistant strain of *Pseudomonas syringae* pv. *syringae* inoculated into plum petioles was investigated by scanning electron microscopy. The pathogen colonized xylem elements of the petioles and then spread to the xylem of shoots and to the xylem and other elements of the leaf veins. It was also seen in axillary buds of invaded leaves. Lateral spread from major xylem vessels into adjacent tissue was more pronounced in leaf veins than in petioles and shoots. The presence of microcolonies on leaf surfaces indicates that the pathogen is extruded from invaded tissue, most likely through stomata. The study suggests that epiphytic populations of the pathogen are constantly replenished from systemically invaded, symptomless plum trees.

Additional key words: Prunus salicina.

Pseudomonas syringae pv. *syringae* (van Hall) is the major causal organism of bacterial canker of plum (*Prunus salicina* Lindl.) in South Africa (16). How the pathogen enters this host and migrates internally to other parts of the tree is uncertain. Leaf scar infection of cherry trees by *P. s.* pv. *morsprunorum* (Wormald) Young et al received considerable attention in the United Kingdom (4–6). *P. s.* pv. *syringae* does not enter plum trees through leaf scars (7), however, and it has been suggested that wounds are more suitable infection avenues than natural openings (11). *P. s.* pv. *syringae* also colonizes the outer bud scales of cherries after fall, leading to mass destruction of dormant buds (1,2).

Recent scanning electron microscope (SEM) studies with *P. s.* pv. *morsprunorum* on cherry (19) and *Xanthomonas campestris* pv. *pruni* (Smith) Dye on plum trees (9,10) have indicated that these pathogens move systemically from infected leaves through the petioles. The present investigation considers systemic invasion of plum trees by *P. s.* pv. *syringae* via leaf petioles.

MATERIALS AND METHODS

Inoculum. A spontaneous mutant (R832) of *P. s.* pv. *syringae* resistant to 50 μg/ml of rifampicin and 50 μg/ml of streptomycin sulfate was obtained by conventional plating methods. Virulence of the mutant corresponded with that of the wild-type strain 832 isolated locally from a cankered Harry Pickstone plum tree. Overnight growth on nutrient-yeast extract-glycerol agar slants incubated at 26 C was suspended in sterile distilled water and adjusted to 10^4 colony-forming units per milliliter as described previously (19).

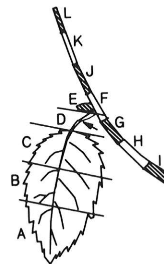
Inoculation of test trees. Three-year-old potted El Dorado plum trees with vigorous shoots (50–80 cm) on the previous season's growth were inoculated in a greenhouse (25 C) in spring (September). Single petioles halfway along the length of four shoots per tree were tagged on each of five trees. Two petioles per tree were inoculated and the other two served as controls. A 0.5-μl droplet of inoculum or sterile water was deposited midway on the abaxial side of the appropriate petiole. A 26-gauge needle was inserted through the droplet into the underlying tissue, after which the droplet moved into the petiole.

Recovery of pathogen from treated trees. A single shoot containing an inoculated petiole was removed from each of four trees at 20 and again at 40 days after inoculation. Each inoculated petiole together with the attached leaf and a portion of the shoot were sectioned (2-cm segments) as illustrated in Table 1. The axillary bud of inoculated petioles was also kept, but other leaves and petioles were discarded. Corresponding samples from shoots with control petioles were prepared in the same way.

Each separate tissue sample was surface-disinfested with 70% ethanol, cut into small pieces, and shaken vigorously for 1 min in a test tube containing 10 ml of sterile distilled water. After 1–2 hr, suspensions were plated in duplicate onto Difco nutrient agar supplemented with 5% sucrose (14), 50 μg/ml of rifampicin, and 50 μg/ml of streptomycin sulfate. The presence of colonies was recorded after 3 days' incubation at 26 C.

Scanning electron microscopy. Tissue samples were obtained 20 days after inoculation as described above. Two shoots with inoculated petioles and two with control petioles were used. No

TABLE 1. Presence[a] of viable cells of antibiotic-resistant strain R832 of *Pseudomonas syringae* pv. *syringae* in plum tissue after petiole inoculation



Position of tissue examined[b]	Detection[c] in tissue from trees 1–4 at two sampling times after inoculation: 20 days, 1	20 days, 2	20 days, 3	20 days, 4	40 days, 1	40 days, 2	40 days, 3	40 days, 4
A	–	–	–	–	–	–	–	–
B	–	+	–	+	–	–	–	+
C	–	+	–	+	–	+	–	+
D	+	+	+	+	+	+	+	+
E	+	+	+	+	–	+	+	–
F	+	+	+	+	–	–	–	–
G	–	+	+	+	–	–	–	–
H	–	+	+	+	–	–	+	–
I	–	+	+	+	–	–	–	–
J	–	–	–	–	–	–	–	–
K	–	–	–	–	–	–	–	–
L	–	–	–	–	–	–	–	–

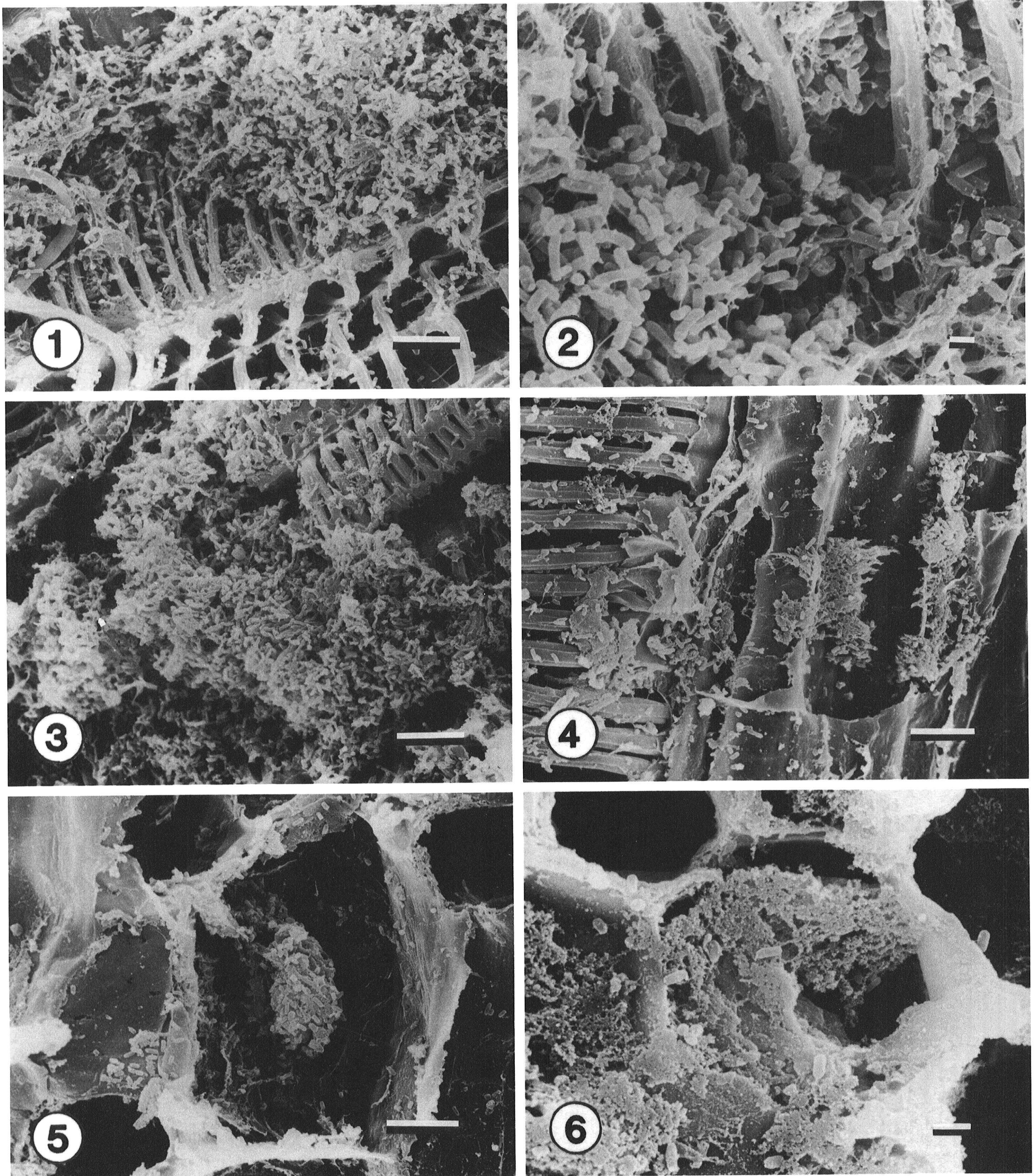
[a]Determined by plating suspensions on nutrient sucrose agar (14) supplemented with 50 μg/ml of rifampicin and 50 μg/ml of streptomycin sulfate.
[b]Arrow shows point of inoculation.
[c]+ = Pathogen detected, – = pathogen not detected.

© 1987 The American Phytopathological Society

disease symptoms were visible on any of the trees. Squares (1 cm^2) of tissue from each leaf blade segment and 1-cm lengths from each petiole and shoot segment were serially subdivided into 1-mm pieces with a new razor blade. Cross and longitudinal sections from these pieces and longitudinal sections of buds were processed for SEM as described previously (17,19). An ISI 100-A SEM (International Scientific Instruments, Santa Clara, CA) was used.

RESULTS

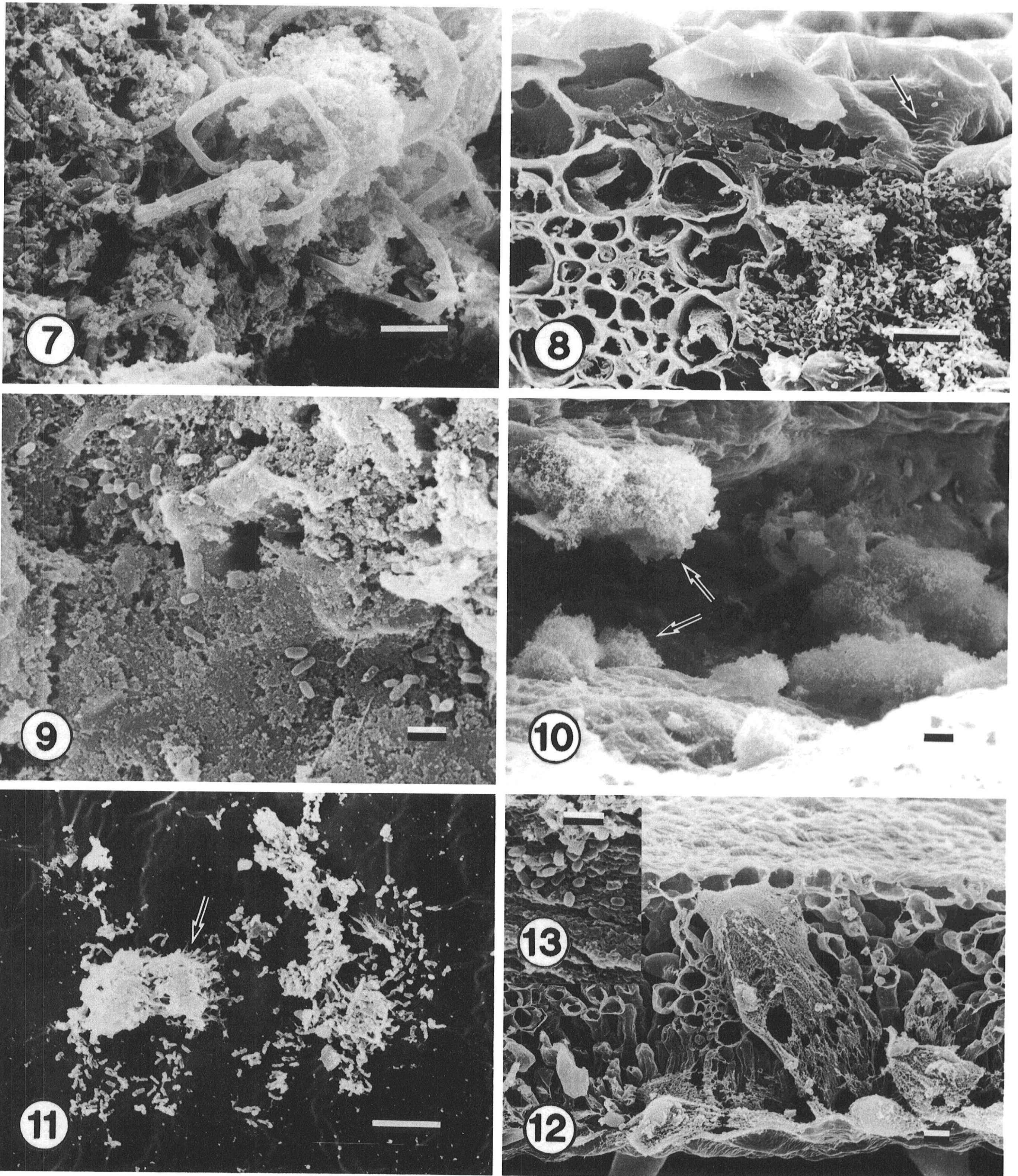
Symptoms and recovery of pathogen. Symptoms appeared on only two of the 10 leaves of inoculated petioles after 5–7 days. Minor veins and the bordering mesophyll became necrotic and a few leaf spots developed. Most shoots had no visible symptoms.



Figs. 1–6. Scanning electron micrographs of plum petiole tissue invaded by *Pseudomonas syringae* pv. *syringae*. Scale bars = 10 μm (1 and 3–5), 1 μm (2), and 2 μm (6). **1,** Bacteria in helical vessels of protoxylem. **2,** High magnification of bacteria in Fig. 1. **3,** Bacterial mass in pitted and scalariform vessels of metaxylem. **4–6,** Bacteria in xylem parenchyma (4), cortex (5), and phloem (6).

P. s. pv. *syringae* was detected in inoculated petioles (sample D) of all four trees after both 20 and 40 days (Table 1). The pathogen was recovered from leaf blade tissue (samples B and C) of two trees but not from any of the distal sections (sample A). After 20 days, the pathogen was detected in all sampled shoots that had supported petioles (sample F), in the next three lower shoot segments of three trees (samples G–I), and in all buds (sample E). Apart from the petioles, the pathogen was found less frequently in tissue 40 days after inoculation. Bacteria were not detected in any of the control samples.

Scanning electron microscopy. No bacteria were seen in the xylem or other tissue in cross or longitudinal sections through



Figs. 7–13. Scanning electron micrographs of plum leaves invaded by *Pseudomonas syringae* pv. *syringae*. Scale bars = 10 μm (7, 8, and 10–12) and 2 μm (9 and 13). **7,** Mass of bacteria in disrupted xylem vessel of main vein. **8,** Bacteria in mesophyll, presumably before colonization of the adjacent minor vein. Note distortion (arrow) of epidermal cells above invaded tissue. **9,** Single bacteria in phloem. **10,** Microcolonies (arrows) on the leaf surface. **11,** Bacteria, partially embedded in fibrillar material (arrow), on surface of veins. **12 and 13,** Granular material in the distal end of the leaf, often near a vein (12), containing single and small clumps of bacteria (13).

control petioles. Twenty days after inoculation, numerous bacteria were present in xylem vessels of inoculated petioles (Figs. 1–4). Masses of bacteria occurred in helical vessels of the protoxylem (Figs. 1 and 2) and in scalariform and pitted vessels of the metaxylem (Fig. 3). Some of the vessels seemed to be virtually occluded by bacteria embedded in fine weblike material (Figs. 1–3). Single bacterial cells or sparse clumps were seen in the intercellular spaces of the xylem parenchyma (Fig. 4), in the cortex (Fig. 5), and in phloem tissue of the petiole (Fig. 6).

Bacteria were never found in tissue of control leaves. In contrast, xylem vessels of the main vein of leaves with inoculated petioles were heavily colonized (Fig. 7). Bacteria in invaded leaf blades were usually confined to the xylem elements of the main vein and a few minor veins. However, numerous bacteria were occasionally seen in the mesophyll adjacent to uninvaded minor veins (Fig. 8). Epidermal cells above the invaded mesophyll were occasionally distorted (Fig. 8), but this was not macroscopically detectable.

Bacteria were observed in the phloem (Fig. 9) and cortex of veins adjacent to heavily colonized mesophyll tissue. Large clumps of bacteria were present on the surface of the leaf blade (Fig. 10). Microcolonies seen on the main vein were embedded in fibrous material (Fig. 11).

The distal end (sample A) of the leaf contained deposits of granular material (Fig. 12) in the vascular and mesophyll tissue. Single bacterial cells or small aggregates were lodged in this material (Fig. 13). Isolated bacterial cells and granular material were seen on the bud scale leaves (Fig. 14) and in meristematic tissue of the bud. Large masses of bacteria were present in xylem tissue of the main vascular bundle of the leaf traces (Fig. 15), whereas only isolated cells occurred in the bordering xylem parenchyma.

Single bacterial cells or isolated clumps associated with fibrous material occurred in the vascular bundle of the shoot 1 cm or more below the petiole junction. A few cells were also seen in cortical tissue of these specimens.

DISCUSSION

The antibiotic-resistant strain of *P. s.* pv. *syringae* used in this investigation spread systemically from the point of inoculation on plum petioles to leaf tissue, the axillary bud, and at least 4–6 cm down the length of some shoots (Table 1). The lower recovery of bacteria 20–40 days after inoculation agrees with the known diminished activity of the pathogen in the host during summer (7).

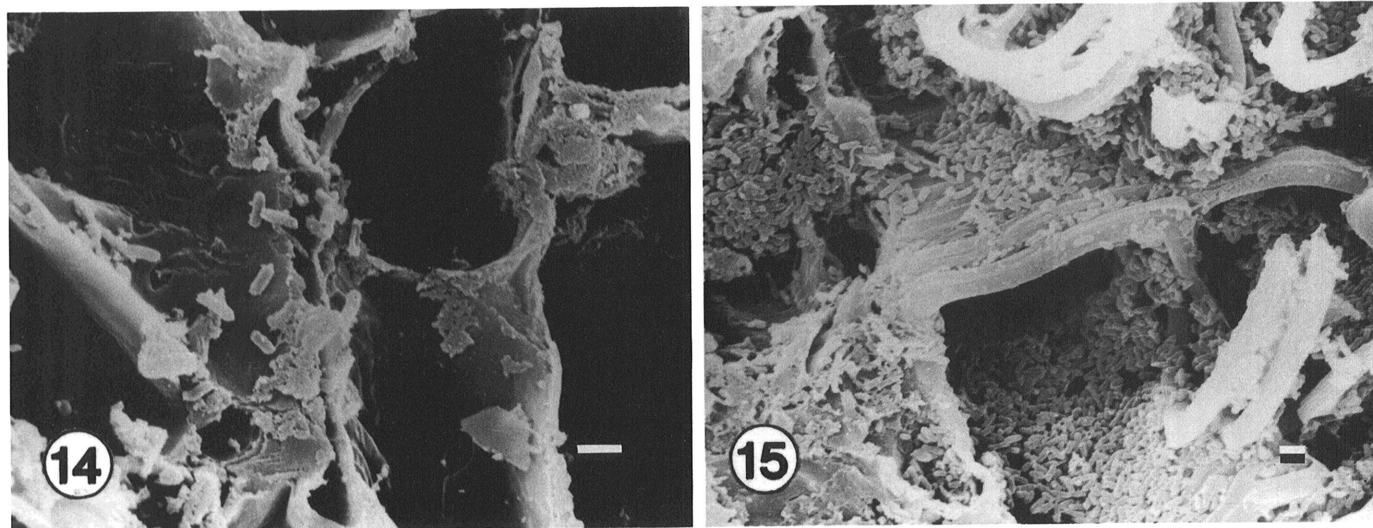
The inoculation method used in this investigation undoubtedly introduced *P. s.* pv. *syringae* to different tissues in the petiole. However, SEM of specimens sampled at increasing distances from the point of inoculation showed that the pathogen initially colonized only the xylem elements. This agrees with reports that xylem vessels are primary sites of significant proliferation of *Erwinia amylovora* (Burr.) Winslow et al in apple petioles (13,20,21). Another report (12), however, states that *E. amylovora* moves downward in inoculated stems exclusively in the phloem. Furthermore, *P. s.* pv. *morsprunorum* and *Corynebacterium flaccumfaciens* pv. *poinsettiae* (Starr & Pirone) Dye & Kemp enter phloem of cherry (19) and poinsettia (3) leaves, respectively, during the early stages of pathogenesis. Several factors, including intrinsic differences among pathogens, hosts, plant parts inoculated, and methods of inoculation, might account for these recorded differences.

P. s. pv. *syringae* spread from the xylem of plum petioles to the xylem of shoots and the veins of leaves. When present in petioles, leaf blades, and shoots, bacteria occurred most commonly in the spiral or helical vessels of the protoxylem, in scalariform and pitted vessels of the metaxylem, and in the bordering xylem parenchyma. Bacteria were seen less frequently and in lower numbers in the phloem and cortex of petioles and shoots.

Lateral spread of *P. s.* pv. *syringae* from major xylem vessels into adjacent tissue was more pronounced in leaf veins than in petioles or shoots. How the pathogen traversed xylem walls to invade the phloem and other cells in the veins and surrounding mesophyll is not known. Intercellular spread probably occurred within the mesophyll, allowing the pathogen to invade uncolonized minor veins (Fig. 8). The presence of microcolonies on leaf surfaces (Figs. 10 and 11) indicated that the pathogen had been extruded from invaded tissue, most likely through stomata.

Granular deposits (Fig. 12) found in distal specimens of invaded leaves might have been gums or gels secreted by the host in response to infection (23) rather than by the pathogen itself. This is supported by the failure to detect the few bacteria seen in the deposit by plating of suspensions prepared from corresponding distal leaf samples (Table 1). In contrast, a connecting fibrillar network of bacterial origin apparently anchored microcolonies to the leaf surface (Fig. 11). This agrees to some extent with the situation reported in xylem vessels of grape petioles invaded by the pathogen causing Pierce's disease (22). The fibrils, especially in xylem vessels (Fig. 2), also resembled the cellulose fibrils produced by *Agrobacterium tumefaciens* (Smith & Townsend) Conn during attachment to carrot cells (15).

Under favorable field conditions, even a few cells of *E. amylovora* entering vascular elements can lead to systemic infection of apple trees (8,20). This might also apply to the development of bacterial canker of plum in South Africa where *P. s.* pv. *syringae* is present on the surfaces of symptomless leaves throughout the growing season (18).



Figs. 14 and 15. Scanning electron micrographs of plum bud (14) and shoot tissue (15) invaded by *Pseudomonas syringae* pv. *syringae*. Scale bars = 2 μm. **14,** Single bacterial cells in bud scales. **15,** Bacteria in xylem vessels of the leaf trace.

LITERATURE CITED

1. Cameron, H. R. 1962. Mode of infection of sweet cherry by *Pseudomonas syringae*. Phytopathology 52:917-921.
2. Cameron, H. R. 1970. *Pseudomonas* content of cherry trees. Phytopathology 60:1343-1346.
3. Creager, D. B., and Matherly, E. P. 1962. Bacterial blight of poinsettia: Histopathological studies. Phytopathology 52:103-110.
4. Crosse, J. E. 1955. Bacterial canker of stone-fruits. I. Field observations on the avenues of autumnal infection of cherry. J. Hortic. Sci. 30:131-142.
5. Crosse, J. E. 1956. Bacterial canker of stone-fruits. II. Leaf scar infection of cherry. J. Hortic. Sci. 31:212-224.
6. Crosse, J. E. 1957. Bacterial canker of stone-fruits. III. Inoculum concentration and time of inoculation in relation to leaf-scar infection of cherry. Ann. Appl. Biol. 45:19-35.
7. Crosse, J. E. 1966. Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees. Annu. Rev. Phytopathol. 4:291-310.
8. Crosse, J. E., Goodman, R. N., and Shaffer, W. H., Jr. 1972. Leaf damage as a predisposing factor in the infection of apple shoots by *Erwinia amylovora*. Phytopathology 62:176-182.
9. Du Plessis, H. J. 1984. Scanning electron microscopy of *Xanthomonas campestris* pv. *pruni* in plum petioles and buds. Phytopathol. Z. 109:277-284.
10. Du Plessis, H. J. 1986. Systemic migration and establishment of *Xanthomonas campestris* pv. *pruni* in plum leaves and twigs. J. Phytopathol. 116:221-227.
11. Endert, E., and Ritchie, D. F. 1984. Overwintering and survival of *Pseudomonas syringae* pv. *syringae* and symptom development in peach trees. Plant Dis. 68:468-470.
12. Gowda, S. S., and Goodman, R. N. 1970. Movement and persistence of *Erwinia amylovora* in shoot, stem and root of apple. Plant Dis. Rep. 54:576-580.
13. Huang, Pi-Yu, and Goodman, R. N. 1976. Ultrastructural modifications in apple stems induced by *Erwinia amylovora* and the fire blight toxin. Phytopathology 66:269-276.
14. Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
15. Matthyse, A. G., Holmes, K. V., and Gurlitz, R. H. G. 1981. Elaboration of cellulose fibrils by *Agrobacterium tumefaciens* during attachment to carrot cells. J. Bacteriol. 145:583-595.
16. Roos, I. M. M., and Hattingh, M. J. 1983. Fluorescent pseudomonads associated with bacterial canker of stone fruit in South Africa. Plant Dis. 67:1267-1269.
17. Roos, I. M. M., and Hattingh, M. J. 1983. Scanning electron microscopy of *Pseudomonas syringae* pv. *morsprunorum* on sweet cherry leaves. Phytopathol. Z. 108:18-25.
18. Roos, I. M. M., and Hattingh, M. J. 1986. Resident populations of *Pseudomonas syringae* on stone fruit tree leaves in South Africa. Phytophylactica 18:55-58.
19. Roos, I. M. M., and Hattingh, M. J. 1987. Systemic invasion of cherry leaves and petioles by *Pseudomonas syringae* pv. *morsprunorum*. Phytopathology 77:1246-1252.
20. Suhayda, C. G., and Goodman, R. N. 1981. Infection courts and systemic movement of ^{32}P-labeled *Erwinia amylovora* in apple petioles and stems. Phytopathology 71:656-660.
21. Suhayda, C. G., and Goodman, R. N. 1981. Early proliferation and migration and subsequent xylem occlusion by *Erwinia amylovora* and the fate of its extracellular polysaccharide (EPS) in apple shoots. Phytopathology 71:697-707.
22. Tyson, G. E., Stojanovic, B. J., Kuklinksi, R. F., DiVittorio, T. J., and Sullivan, M. L. 1985. Scanning electron microscopy of Pierce's disease bacterium in petiolar xylem of grape leaves. Phytopathology 75:264-269.
23. VanderMolen, G. E., Beckman, C. H., and Rodehorst, E. 1977. Vascular gelation: A general response phenomenon following infection. Physiol. Plant Pathol. 11:95-100.

Etiology

Bacterial Leaf Blight of *Syngonium* Caused by a Pathovar of *Xanthomonas campestris*

Robert S. Dickey and Cathy H. Zumoff

Professor and research technician, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

We thank Herbert W. Israel and Susan J. Bucci for assistance with electron microscopy of bacterial cells and Robert E. Stall for identification of bacterial pigments.

Accepted for publication 20 March 1987 (submitted for electronic processing).

ABSTRACT

Dickey, R. S., and Zumoff, C. H. 1987. Bacterial leaf blight of *Syngonium* caused by a pathovar of *Xanthomonas campestris*. Phytopathology 77:1257-1262.

The bacterial leaf blight pathogen of *Syngonium podophyllum* was determined to be a xanthomonad. The pathogen was readily isolated from affected leaf surfaces, transferred on fingers from affected leaf surfaces to agar medium, and spread from infected to healthy leaves by overhead watering. It was not isolated from nodal and adjoining stem sections to which infected leaves were attached. Healthy plants were produced from stem cuttings removed from above the infected leaves of subirrigated plants. The strains of the *Syngonium* pathogen produced similar symptoms on leaves of three cultivars of *S. podophyllum* that were petiole- or spray-inoculated. The pathogen was identified as *Xanthomonas campestris* and was found to be phenotypically different from seven selected pathovar strains. Only three of the seven pathovar strains produced any reaction in *S. podophyllum*; the reactions, however, were distinctly different from those produced by the *Syngonium* strains. We propose that the pathogen be designated *Xanthomonas campestris* pv. *syngonii* pv. nov.

During the last 6 yr, *Syngonium podophyllum* Schott (nephthytis) plants affected by a leaf blight disease have been received for diagnosis. The symptoms are similar to or the same as those described by Wehlburg (19,20), who attributed the cause of the disease to a xanthomonad that was similar to *Xanthomonas campestris* pv. *vitians* (19). We have also isolated a xanthomonad from affected leaf tissues, but the strains isolated since 1980 have grown slower and been less pigmented than strains of *X. c.* pv. *vitians* from lettuce. Growers have estimated and we have confirmed that as much as one-third of a production crop can be affected by the disease. Therefore, an investigation was initiated concerning the identification and spread of the pathogen and the development of symptoms in selected cultivars of *Syngonium*.

© 1987 The American Phytopathological Society