Infection Courts and Systemic Movement of ^{32}P-Labeled *Erwinia amylovora* in Apple Petioles and Stems

C. G. Suhayda and R. N. Goodman

Department of Plant Pathology, University of Missouri, Columbia 65201.
Present address of the senior author: DOE/MSU Plant Research Laboratory, East Lansing, MI 48824.
This research was supported in part by a grant from the National Science Foundation Grant PCM77-08783.
Journal Series Article 8658 of the Missouri Agricultural Experiment Station.
Accepted for publication 8 December 1980.

ABSTRACT

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.

The rate of migration of ^{32}P-labeled virulent (E9) and avirulent (E8) strains *Erwinia amylovora* was monitored in apple petiole and stem tissue. Both the capsulated E9 and capsule-deficient E8 were rapidly drawn into vascular elements of petiolar tissue and moved 5 mm within 20 min. Although most of the inoculum remained at the site of inoculation, enough cells moved into the stem from the petiole within 2–4 min to initiate systemic infection. The data from inoculation of stem tissue through cut bases with mixed inocula of virulent encapsulated E9 and avirulent capsule-deficient E8 strains suggest that the E8 strain is precluded from migrating freely in stem xylem vessels by an immobilizing phenomenon previously described. The study provides evidence that virulent strain E9 moves quickly and significant distances (34 mm/hr) in xylem vessels. The data suggest that wounding of leaves that exposes xylem vessels to the pathogen could be an important factor in the infection process in nature.

Fireblight is a vascular wilt disease caused in roseaceous plants by *Erwinia amylovora* (Burr.) Smith. Virulent strains of this pathogen (eg, strain E9) are highly mucoid and possess large extracellular polysaccharide capsules (EPS) (8,18,21,23,24), some of which are closely adherent and some are nonadherent. The E8 strain, a mutant of E9, is distinguished by its colony morphology on triphenyl tetrazolium chloride medium (TTC) (14); it is EPS deficient and avirulent (1,8). Virulence in *E. amylovora*, as in other wilt-causing species like *Pseudomonas solanacearum* (4,12,20) and *Xanthomonas campestris* (22), has been correlated with the production of EPS by the bacterium (1,3,6).

Highly virulent strains of *E. amylovora* cause rapid symptom expression in susceptible apple tissue, causing infected shoots to wilt 36–48 hr after inoculation and show reddish brown discoloration, and it increases after 72 hr. Early light microscopy studies on inoculated and naturally infected apple and pear tissue revealed extensive colonization of intercellular space by bacteria in the cortex, cambium, and pith (2,13,16). These observations led Nixon (17) and Bachman (2) to propose that bacteria migrate intercellularly through host tissue. Transmission electron microscopy of infected petiole tissue has shown that virulent bacteria enter mature xylem vessels and cause extensive subcellular disorganization of adjacent xylem parenchyma cells (8–10). Suhayda and Goodman (21) suggested that bacterial multiplication in the xylem was the primary event required for the development of the wilting symptom and for the subsequent spread of bacteria through host tissue. Results of in vivo studies have shown that strain E9 cells also may migrate through the phloem in shoot and petiole tissue of cultivar Jonathan apple (7,15), and bacteria were found well in advance of visible symptoms in the tissue (7).

In contrast, inoculation of Jonathan petiole tissue with avirulent strain E8 does not produce disease symptoms. Electromicrographs revealed that E8 cells moved 3 mm from the inoculation point in the petiole (8) and were agglutinated in xylem vessels by dark-staining granules 6 hr after inoculation (11). Twenty-four to 48 hr after inoculation, portions of the xylem vessels were totally occluded by avirulent bacteria and the agglutinating substance. Degradation and lysis of the avirulent cells in the vessels occurred after 72 hr. Avirulent cells are apparently prevented from migrating through and colonizing host tissue by a plant defense mechanism that has been termed "in vivo agglutination" (11).

EPS on the surface of the bacterium appears to play a dual role in pathogenesis by disrupting water flow in the vascular system (21) and preventing the bacteria from being recognized by the host and subsequently being agglutinated and localized (11).

The objective of this work was to determine the distribution and rate of movement of a virulent and an avirulent strain of *E. amylovora* in Jonathan apple tissue by using ^{32}P-labeled bacteria and determining whether EPS on the surface of the bacteria enhances their distribution and migration through the host.

MATERIALS AND METHODS

The asparagine medium of Bennett and Billings (3), which contained asparagine, 4.0; $MgSO_4$, 0.4; NaCl, 3.0; K_2PO_4, 2.0, nicotinic acid, 0.2; thiamine hydrochloride, 0.2; and glucose, 20.0 g/L, was used to culture the bacteria. For the isotope labeling experiment, ^{32}P orthophosphate in water was substituted for potassium phosphate without altering the buffering capacity of the medium.

The E8 and E9 strains were stored in lyophilized form according to the procedure of Shaffer and Goodman (19). Cultures were started by resuspending bacteria from lyophile tubes in nutrient yeast glucose broth (NYGB) and streaking a loopful of suspension on nutrient yeast glucose agar plates (20) containing 50 mg/L triphenyl tetrazolium chloride (TTC) (14) to obtain single colony isolates. Following incubation at 27 C for 48 hr a typical colony was selected and streaked on asparagine medium slants. The slants were stored at 4 C and used during a 2-wk period.

Bacteria to be labeled with ^{32}P were incubated overnight on asparagine slants at 27 C. The slants were washed with 5 ml of asparagine medium and two drops of this suspension were added to 20 ml of asparagine medium in a 250-ml Erlenmeyer flask, incubated for 16 hr on a water-bath shaker, and centrifuged at 27,000 *g* for 10 min. The bacterial pellet was resuspended in phosphate-free asparagine medium and washed twice with this medium. The concentration of bacteria was adjusted with a Beckman spectrophotometer to an 0.05 absorbance at 500 nm. This value corresponded to approximately 10^8 cells per milliliter.

The incubation mixture, which contained 300 μl of bacteria suspended in phosphate-free asparagine medium and 10 μl of ^{32}P orthophosphoric acid (New England Nuclear Co., Boston, MA 02118) (4.44×10^7 dpm), was agitated on a water-bath shaker for 3

0031-949X/81/06065605/$3.00/0

hr at 28 C. The bacteria were then captured on a 0.45 μm Amicon Microporous filter (25 mm in diameter) (Amicon Corp., Lexington, MA 02173) that was presoaked in phosphate buffered saline (PBS), pH 7.0, and washed with 20 ml of PBS. The washed filter with bacteria was placed in a borosilicate culture tube (13 ×100 mm, Kimble Glass Co., Toledo, OH 43666) to which 2 ml of phosphate-containing asparagine medium was added. The bacteria were washed from the filter by vortexing, the filter was removed and the bacteria were incubated for an additional hour on the water-bath shaker at 28 C. This chase of cold phosphate followed incubation of the bacteria with ^{32}P, which diluted and/or stabilized any free ^{32}P. The bacteria were centrifuged at 27,000 *g* for 10 min. The supernatant was discarded. The pellet was resuspended in 300–500 μl of phosphate-containing asparagine medium. Under these conditions the bacteria in the suspensions we used in our uptake and inoculation experiments had a specific activity of 0.5 CPM/bacterium or 0.5 CPM/cfu.

Tissue samples were assayed for the presence of radioactivity in a Picker liquid scintillation counter. Aquasol (New England Nuclear) was used as the scintillation cocktail at a volume of 5 ml per sample vial. These vials were counted for 10 min.

Experiments involving the elution of mixtures of E9 and E8 bacteria through small segments of apple shoot tissue were performed on the apparatus shown in Fig. 4. A 12-cc plastic syringe barrel serves as a reservoir for the elution fluid. A plastic hose connects the reservoir to a small glass tee, the lower portion of which is attached to a small piece of rubber tubing that holds the tissue segment in place. Shoot segments, 2 cm in length, were excised with a razor at a point 3–5 cm from the apex. The shoot was submerged in water during cutting, inserted into the fluid-filled apparatus, and oriented in a way that permitted the eluting fluid to flow acropetally. A 10-μl Hamilton syringe was used to introduce small volumes of bacteria into the glass tee. After sealing the injection port, a 0.01 M phosphate buffer solution, pH 7.0, was used to elute the bacterial suspension through the tissue segment. The bacteria were eluted under a head of pressure of approximately 0.4 atmospheres. Three droplet fractions eluted from the stem tissue were collected (15 min after injecting bacteria) and spread on NYGA plates containing TTC. The incorporation of TTC into the agar provided a means of distinguishing the E8 from E9 bacteria (1).

The migration and distribution of virulent E9 cells in Jonathan apple tissue were monitored using ^{32}P-labeled bacteria. The petiole inoculation procedure of Huang et al (10) was modified by placing 5 μl of the radioactive bacterial suspension (5×10^5 cells) on the cut petiole surface with a micropipette immediately after leaf excision. Following absorption of the suspension by the petiole, the shoot was removed and sectioned into consecutive 1-mm pieces and radioactivity was assayed by liquid scintillation counting (LSC). The inoculation and sectioning process required 20 min, therefore, zero time was calculated at 20 min after inoculation.

RESULTS

Data obtained from petiole inoculations by using ^{32}P-labeled E9 cells are presented in Fig. 1. Approximately 95% of the total initial inoculum was found in the first 1 mm of tissue from the point of inoculation. The remaining 5% of the radioactivity was detected in the adjacent 2 mm of tissue. Flushing the initial 5 μl of bacterial inoculum into the petiole with an additional 20 μl of asparagine

TABLE 1. The distribution of ^{32}P-labeled *Erwinia amylovora* strain E9 cells at various time intervals after inoculation in Jonathan apple petioles

Distance from inoculation point (mm)	Percentage of total cpm			
	0 hr	3 hr	6 hr	12 hr
0–2	87	91	83.5	80
2–4	4	7	4.4	17
4–6	5	2	7.8	3
6–8	3	...	3.8	...
8–10	...	...	...	...

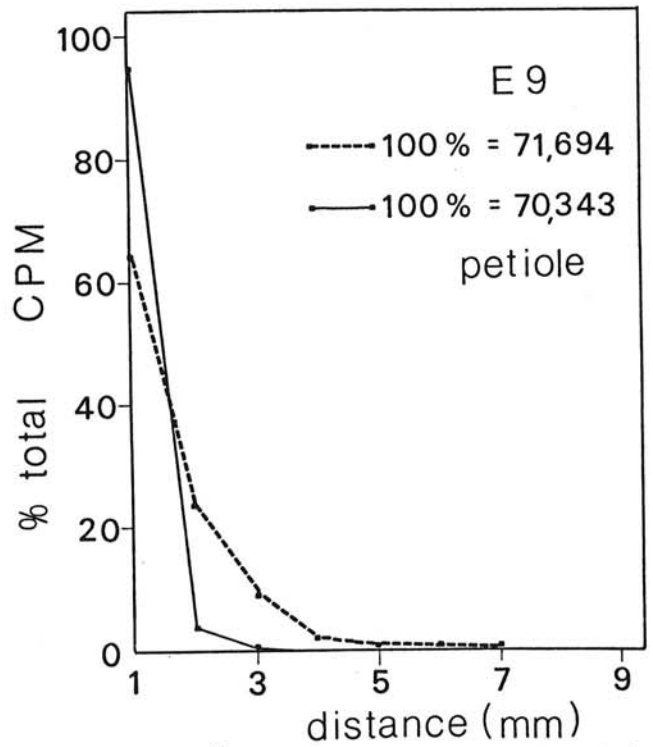

Fig. 1. Distribution of ^{32}P-labeled E9 cells of *Erwinia amylovora* in tissue segments of Jonathan apple petiole tissue following inoculation by a 5-μl suspension of bacteria in phosphate-containing asparagine medium (PCAM). The solid line represents the distribution of 5 μl of the initial inoculum. The dashed line represents the distribution of labeled bacteria following a chase with 20 μl PCAM in four 5-μl volumes. (Data in the solid line imply distribution of ^{32}P 20 min after inoculation, whereas the dashed line reflects distribution following an additional 30-min period during which the bacteria were chased by PCAM).

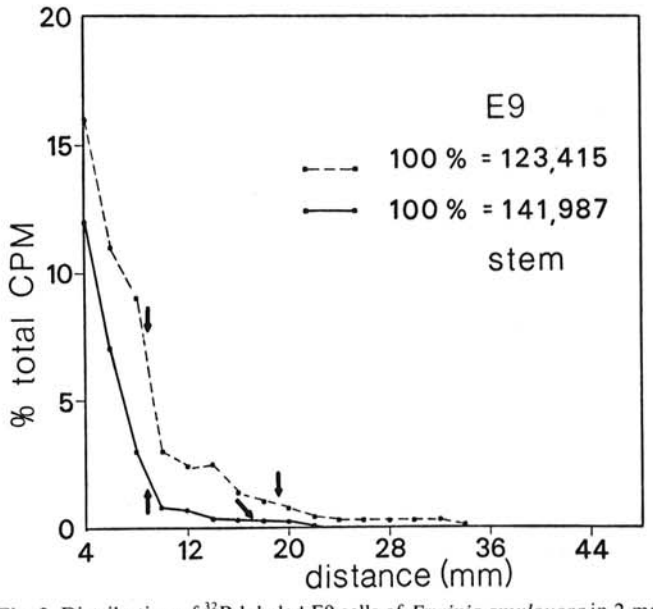

Fig. 2. Distribution of ^{32}P-labeled E9 cells of *Erwinia amylovora* in 2-mm tissue segments of Jonathan apple stem tissue following uptake of a 30-μl suspension of bacteria in phosphate-containing asparagine medium (PCAM) by stem bases of freshly cut Jonathan shoots. The solid line implies the distribution of the 30-μl bacterial suspension. The dashed line reflects the distribution of the bacterial suspension following a chase with 60 μl of PCAM in two 30-μl volumes. The arrows indicate leaf positions on the shoot. The uptake of each 30-μl volume of liquid required 20 min. Hand sectioning of the tissue required an additional 10 min.

medium, applied in 5-μl volumes, altered the distribution of the inoculum in the tissue. The apical millimeter of the petiole contained about 65% of the total radioactivity, whereas the second, third, and fourth millimeters contained 25, 10, and 2% of the total radioactivity, respectively.

Data from longer-term incubation experiments following inoculation of petioles with labeled bacteria are presented in Table 1. Petioles that were inoculated with 5 μl of a suspension of ^{32}P-labeled E9 cells and assayed 3, 6, and 12 hr after inoculation for radioactivity retained the major portion of the radioactivity in the first 2 mm of the petiole tissue. Radioactivity could be consistently detected up to 6 mm from the inoculation point throughout the 12-hr duration of the experiment. These inoculated shoots displayed typical fireblight symptoms 72 hr after inoculation. Blighted petiole tissue (showing necrosis and reddening) assayed by LSC at 72 hr revealed that the initial distribution of the E9 inoculum remained essentially unchanged.

The movement of bacteria in 5-cm-long Jonathan shoots that absorbed 30-μl suspensions of ^{32}P-labeled *E. amylovora* cells through cut stem bases was monitored in 2-mm pieces and assayed by LSC after a 20-min absorption period. The basal 2 mm of tissue of the shoot was not included as it was impossible to discriminate between radioactivity inside and outside the stem.

Uptake of labeled E9 cells by the Jonathan apple shoots is shown in Fig. 2. Twenty-six percent of the total CPM was found in the main stem, and labeled bacteria were detected up to 22 mm from the base of the shoot after 30 min. The first petiole from the base contained about 1% of the total radioactivity and no radioactivity was detected in leaf tissue. When the initial 30 μl of labeled E9 cell suspension was flushed with an additional 60 μl of asparagine medium applied in two 30-μl volumes the distribution pattern of the labeled cells in the shoot was altered. Chasing the initial bacterial suspensions resulted in an increase in the radioactivity detected in stem tissue. Under these conditions approximately 50% of the total CPM was detected in shoot tissue and radioactivity could be detected up to 35 mm from the base of the shoot. This represented a 54% increase in the distance that bacteria translocated up the stem and almost a 100% increase in uptake of radioactivity or movement of activity from the stem base. About 5% of the total CPM was found in the first petiole with a trace of radioactivity detected in the first leaf. No radioactivity was detected in the second petiole or leaf from the base.

The pattern of distribution of ^{32}P-labeled E8 cells was similar to the pattern observed for labeled E9 cells following the initial uptake of the 30 μl of bacterial suspension (Fig. 3). Labeled E8 cells were distributed over a 22-mm distance from the base of the shoot and accounted for 44% of the total CPM found in the shoot. No radioactivity was found in either leaves or petioles. However, following the 60-μl chase with asparagine medium the pattern differed significantly from that observed for E9 (Fig. 2) as only a slight increase to 58% of the total CPM was found in the main stem. Despite this 32% increase in uptake, there was no further upward distribution of E8 cells as was noted with E9 cells. A slight accumulation of radioactivity was found in the first petiole but there was no radioactivity in leaf tissue, which indicated that bacteria remained in the stem.

Data from the stem uptake experiments indicated that both E8 and E9 cells are readily taken up by shoots. However, E9 cells were translocated further in the stems than were E8 cells. To investigate the possibility that E8 cells might be adsorbed or retained by stem tissue, we used the device shown in Fig. 4. An inoculum containing a total of about 2,000 E8 and E9 cells in 3:1 ratio was used. These were eluted through the stem with a 0.01 M phosphate buffer solution, pH 7.0. The bacteria-containing eluate was collected 15–20 min after injection in three drop fractions on TTC agar

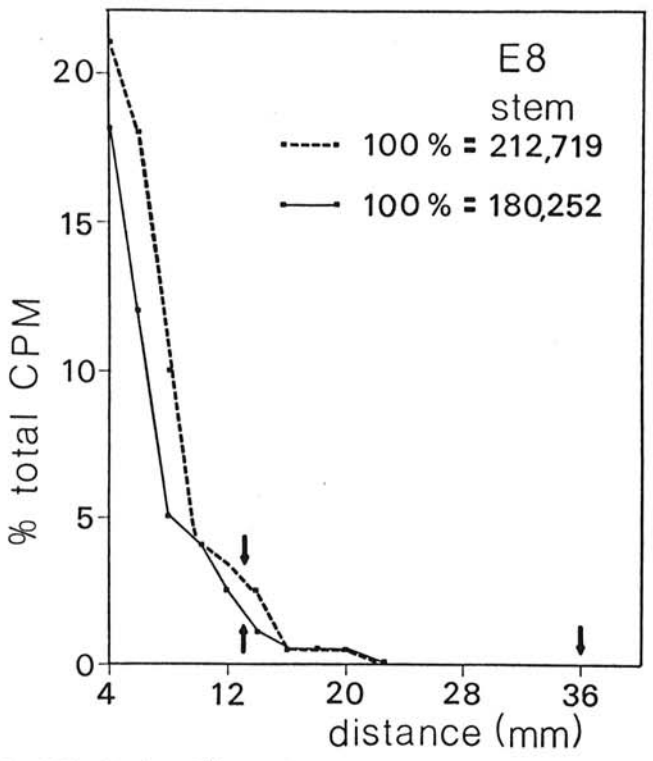

Fig. 3. Distribution of ^{32}P-labeled E8 cells of *Erwinia amylovora* in 2-mm tissue segments of Jonathan apple stem tissue following uptake of 30 μl of bacterial suspension in phosphate-containing asparagine medium (PCAM). The solid line represents the distribution of the 30-μl bacterial suspension. The dashed line represents the distribution of the bacterial suspension following a chase with 60 μl of PCAM in two 30-μl volumes. The arrows indicate leaf positions on the shoot. The uptake of each 30-μl volume of liquid required 20 min. Hand sectioning of the tissue required an additional 10 min.

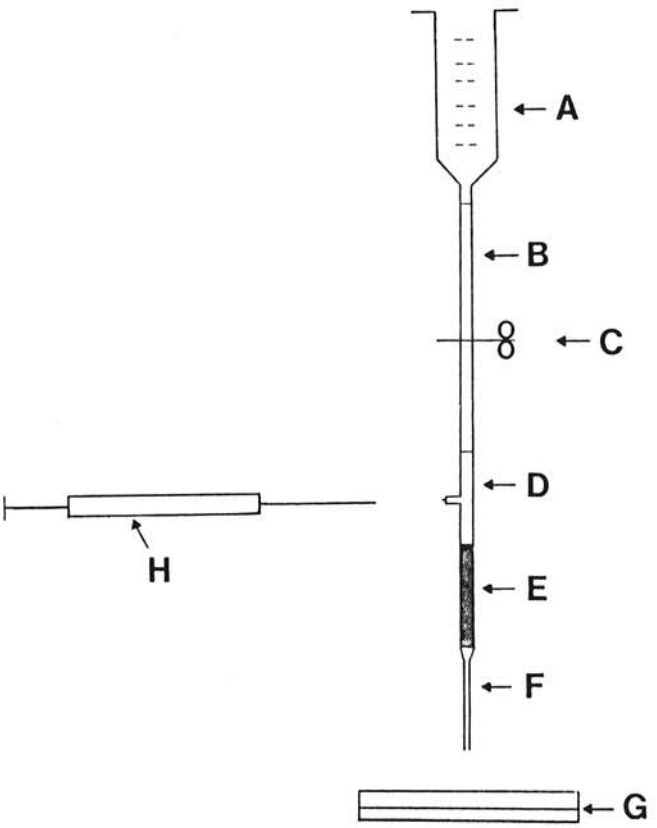

Fig. 4. A 2-cm stem segment (E) pictured in the apparatus used to elute bacteria through Jonathan apple stem tissue. Bacteria were introduced into a port in the glass tee (D) with a 10-μl syringe (G). The opening was sealed and the clamp (C) restricting the plastic tubing (B) was removed. The wash solution flowed from a reservoir (A) through the glass tee and the stem segment carrying with it the bacteria, which were recovered on a TTC plate (F). Three drop fractions were collected per plate (G).

plates. Comparable results were obtained from three repetitions of the experiment with results from a representative experiment shown in Fig. 5. Both E8 and E9 cells had maximum elution peaks in fraction 5, which was collected 16–17 min after the start of the experiment. The ratio of E8 to E9 cells eluted from the stem remained unchanged from the starting mixture. A 50% recovery of cells injected into the tissue segments was possible under optimum conditions. Hence, the experiment did not reveal either the adsorption or retention of the E8 strain that had been anticipated.

DISCUSSION

Inoculation of Jonathan apple petioles with ^{32}P-labeled E9 cells showed that bacteria rapidly enter petiole tissue. However, more than 90% of the initial inoculum remained in the apical 2 mm of the petiole during the course of the 72-hr experiments. When additional medium was supplied as a chase, the initial inoculum was washed further into the petiole with bacteria being distributed up to 7 mm from the inoculation point. Evidently the additional liquid enabled transport of some of the bacteria through mature vascular elements. Although most remained trapped on the cut surface of the petiole, the fact that they were distributed further into the tissue suggests that additional bacteria had entered vascular tissue and these were capable of being flushed through xylem vessels. When an antibiotic resistance marker in the virulent E9 cells was used to study the distribution and population dynamics of bacterial cells inoculated into Jonathan apple petiole tissue (S. Ouchi, A. Nishimura, and R. N. Goodman, *unpublished*), the distribution pattern in the petiole was similar to what we obtained using ^{32}P-labeled E9 cells. Although the initial inoculum remains static in the petiole, it is apparent that some bacteria must migrate from the petiole to the stem since systemic infection was observed 72 hr after inoculation. There are two likely explanations for this observation. First, systemic infection was probably the result of the migration of a relatively small number of bacteria away from the inoculation site. Bacteria labeled with ^{32}P have their highest specific activity at the end of the 3-hr labeling period. Division of bacteria in plant tissue following inoculation decreased the specific activity per bacterial cell, making the small numbers of bacteria difficult to detect. Second, bacteria applied to the petiole migrate in a downward direction through petiolar vessels. Whether this is against the transpiration stream or with transpirational pull of the leaves at the apex is not known. The downward migration of bacteria also may have occurred through the phloem as proposed by Lewis and Goodman (15) and Gowda and Goodman (7). However, the observation of large numbers of bacteria only in the xylem vessels during the initial 48 hr after petiole inoculation suggests that the primary routes of migration are the xylem vessels (21).

Our observations on petiole inoculations agree with those of Crosse et al (5) who reported that extremely low numbers of bacteria in an inoculum may, under favorable conditions, cause systemic infection in Jonathan apple if the bacteria enter vascular elements. More recent experiments in this regard (R. N. Goodman, *unpublished*) have demonstrated that bacteria move extremely rapidly in vascular tissue following inoculation. In these experiments E9 cells were inoculated onto petiole cut surfaces and at time intervals thereafter the petiole was severed at the stem-petiole interface. Systemic infection developed in 30% of the shoots where the petioles had been removed 2–4 min after inoculation. The petioles that were inoculated were 6–10 mm in length. This experiment was repeated three times with essentially the same results.

Stem base uptake experiments with ^{32}P labeled bacteria showed that the rapid distribution and transport of bacteria over considerable distances through vascular tissue was possible and this movement was aided by the transpiration stream. Labeled E9 bacteria moved acropetally through the shoot and were distributed an average of 22 mm from the base after 20 min. Bacteria remained almost exclusively in the stem. Additional liquid applied as a chase enhanced the transport of bacteria further up the stem where they were detected an average of 34 mm from the base after 1 hr. This represented a 54% increase in the distance bacteria were translocated up the shoot.

Uptake of 30 μl free ^{32}P orthophosphate by shoots resulted in the distribution of radioactivity over the entire 5-cm length in 20 min. Of the 1.7×10^{-6} CPM taken up, 75% was detected in the three expanded leaves and 5% was found in the apical whorl of leaves. The remaining 20% was in stem tissue. These data suggest that the radioactivity distribution presented in the bacteria-uptake experiments (Figs. 2 and 3) reflect label predominantly associated with the bacteria per se.

Uptake experiments with labeled E8 cells showed that they too were initially translocated an average distance of 20 mm from the stem base. The liquid chase served to distribute more bacteria into the stem from the basal 2-mm portion; however, after 1 hr no significant increase in upward movement of E8 cells beyond the initial 20 mm was observed. We have interpreted this to suggest that EPS-deficient E8 cells interact with components of the vascular tissue in a way that prevents their further upward distribution in the shoot after 1 hr. These findings support the data of Huang et al (11) who reported that avirulent E8 cells were being agglutinated near the inoculation point in the petiole xylem elements within 6 hr. They also suggest that capsulated bacteria are able to migrate further through host tissue than capsule-deficient strains.

Attempts to detect the selective localization or retention of the EPS deficient E8 strain by Jonathan apple tissue were unsuccessful. The ratio of virulent to avirulent cells did not change when a mixture of cells was eluted through small stem segments. It is possible that localization of E8 cells by the tissue was interfered with by the 0.4 atmospheres pressure that is required for the elution process. It is more likely, however, that the relatively short time interval that the bacteria were in the tissue (15–20 min) was insufficient to detect development of the agglutination phenomenon (11).

From these studies it is possible to infer that leaf tearing that exposes vessel elements to *E. amylovora*, for example in a wind driven rain or hail storm, could be a major factor in causing fireblight infections in the field. It would require but a minute tear to expose vessel elements to a few bacterial cells. Crosse et al (5) reported that an inoculum dose of about 50 *E. amylovora* cells could cause 50% of Jonathan apple shoots so inoculated to develop systemic infections. Our data indicate that long-distance transport of bacteria from the wound site is swift. The migration rate has been calculated to be $\cong$ 2,000 μm/min.

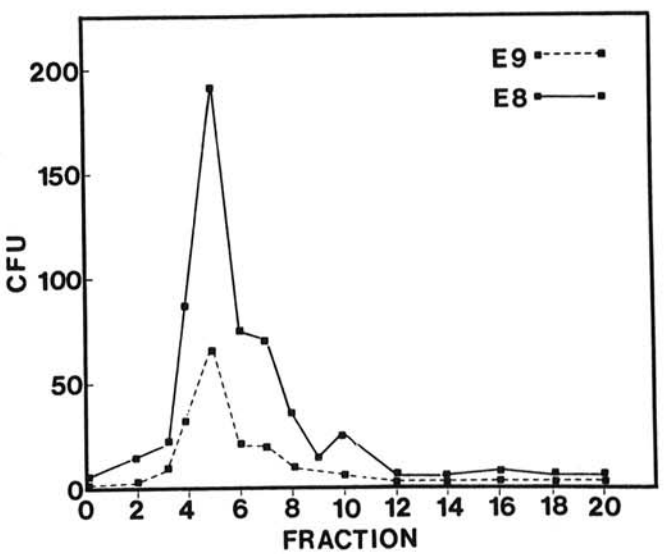

Fig. 5. Elution profile obtained from the passage of a mixture of bacterial cells of the E8 and E9 strain at a ratio of 3:1 through 2-cm Jonathan apple tissue segments in the apparatus shown in Fig. 4. Three drop fractions were collected on TTC plates 15 min after injection of the bacteria. Colonies were counted after a 48-hr incubation period.

LITERATURE CITED

1. Ayers, A. R., Ayers, S. B., and Goodman, R. N. 1979. Extracellular polysaccharide of *Erwinia amylovora*: a correlation with virulence. Appl. Environ. Microbiol. 38:659-666.
2. Bachmann, F. M. 1913. The migration of *Bacillus amylovorus* in the host tissues. Phytopathology 3:3-14.
3. Bennett, R. A., and Billing, E. 1978. Capsulation and virulence in *Erwinia amylovora*. Ann. Appl. Biol., 89:41-48.
4. Buddenhagen, I., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.
5. Crosse, J. E., Goodman, R. N., and Schaffer, W. H. 1972. Leaf damage as a predisposing factor in the infection of apple shoots by *Erwinia amylovora*. Phytopathology 62:176-182.
6. Goodman, R. N., Ayers, A. R., and Politis, D. J. 1978. The relationship of extracellular polysaccharide (EPS) of *Erwinia amylovora* to virulence. Pages 483–485 in: Proc. 4th Int. Conf. Plant Pathogenic Bacteria. Angers, France.
7. 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.
8. Huang, P.-Y. 1974. Ultrastructural modification by and pathogenicity of *Erwinia amylovora* in apple tissues. Ph.D. dissertation, University of Missouri, Columbia.
9. Huang, P.-Y., and Goodman, R. N. 1973. Ultrastructure of apple shoots infected by *Erwinia amylovora*. Abstr. 1125 in: Abstract of Papers, 2nd Int. Congr. Plant Pathology, 5–12 September 1973, Minneapolis, MN.
10. Huang, P.-Y., and Goodman, R. N. 1976. Ultrastructural modifications in apple stems induced by *Erwinia amylovora* and the fire blight toxin. Phytopathology 66:269-276.
11. Huang, P.-Y., Huang, J. S., and Goodman, R. N. 1975. Resistance mechanisms of apple shoots to an avirulent strain of *Erwinia amylovora*. Physiol. Plant Pathol. 6:283-287.
12. Husain, A., and Kelman, A. 1958. Relation of slime production to mechanism of wilting and pathogenicity of *Pseudomonas solanacearum*. Phytopathology 48:155-165.
13. Jones, D. H. 1909. Bacterial blight of apple, pear and quince trees. Ontario Dep. Agric. Bull. 176. 63 pp.
14. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium chloride medium. Phytopathology 44:693-695.
15. Lewis, S. M., and Goodman, R. N. 1965. Mode of penetration and movement of fireblight bacteria in apple leaf and stem tissue. Phytopathology 55:719-723.
16. Miller, P. W. 1929. Studies of fireblight of apples in Wisconsin. J. Agric. Res. 39:579-621.
17. Nixon, E. L. 1927. The migration of *Bacillus amylovorus* in apple tissue and its effects on the host cells. Pa. Agric. Exp. Stn. Bull. 212. 16 pp.
18. Politis, D. J., and Goodman, R. N. 1980. Fine structure of extracellular polysaccharide (EPS) of *Erwinia amylovora*. Appl. Environ. Microbiol. 40:596-607.
19. Schaffer, W. H., Jr., and Goodman, R. N. 1962. Progression in vivo, rate of growth in vitro, and resistance to streptomycin, as indices of virulence of *Erwinia amylovora*. Phytopathology 52:1201-1207.
20. Sequeira, L., and Graham, T. 1977. Agglutination of avirulent strains of *Pseudomonas solanacearum* by potato lectin. Physiol. Plant Pathol. 11:43-54.
21. Suhayda, C. G., and Goodman, R. N. 1981. Early proliferation and migration of *Erwinia amylovora* and subsequent xylem occlusion. Phytopathology 71:(In press).
22. Sutton, J. C., and Williams, P. H. 1970. Comparison of extracellular polysaccharide of *Xanthomonas campestris* from culture and from infected cabbage leaves. Can. J. Bot. 48:645-651.
23. Vörös, J., and Goodman, R. N. 1965. Filamentous forms of *Erwinia amylovora*. Phytopathology 55:876-879.
24. Wallis, F. M., and Truter, S. J. 1978. Histopathology of tomato plants infected with *Pseudomonas solanacearum*, with emphasis on ultrastructure. Physiol. Plant Pathol. 13:307-317.