Association of *Pseudomonas syringae* pv. *lachrymans* and Other Bacterial Pathogens with Roots

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Journal Series Article 11-82. Salaries and research support were provided by state and federal funds granted to the Ohio Agricultural Research and Development Center.

Appreciation is expressed to William Berry, James H. Brown, John Brucia, Charles W. Vrotney, and Robert Whitmoyer for their assistance.

Accepted for publication 27 October 1982.

ABSTRACT

Leben, C. 1983. Association of *Pseudomonas syringae* pv. *lachrymans* and other bacterial pathogens with roots. Phytopathology 73:577-581.

Pseudomonas syringae pv. *lachrymans* (*Psl*) spread, with attendant multiplication, from inoculated cucumber seeds or radicles to seedling roots growing on water agar. Pathogen cells were motile in the water bordering young roots; as roots became older, areas next to them were packed with static bacterial cells. High populations were associated with hair roots. Three unidentified bacteria and a yeast from cucumber seeds spread from radicles to distal parts of cucumber roots on agar, as did *Agrobacterium tumefaciens*, *Erwinia carotovora* pv. *carotovora*, *E. stewartii*, *P. solanacearum*, *P. syringae* pv. *glycinea*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato*, *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *nigromaculans*, and *X. campestris* pv. *phaseoli*. Some microorganisms from some lots of cucumber seeds and from soil interfered with the spread of *Psl* with cucumber roots on agar. When *Psl* was inoculated on cucumber radicles and planted in an unsterilized soil mix that was watered by capillarity in greenhouse pot tests, or in growth room pot tests with another unsterilized soil mix kept at two levels of high water content, the pathogen was detected on distal roots of 44 and >50% of the seedlings, respectively. With inoculated seeds, the percentages were lower.

Additional key words: rhizoplane, rhizosphere, survival.

A number of bacterial pathogens of the plant shoot have been isolated from roots of host and nonhost plants (16). The first report was by Valleau et al (19) in 1944. They demonstrated that two leaf-spotting pathogens of tobacco (*Nicotiana tabacum* L.) survived the winter with the roots of crop and weed plants, and they provided evidence that these pathogens multiplied on tobacco roots. Valleau et al (19) speculated that the "tobacco leaf-spot phase of these pathogens is more or less accidental and is probably not essential to their perpetuation" and stated that the bacteria are "apparently adapted to a life on the surface of small rootlets of several plants, both weed and crop." With one exception (17), these provocative ideas have not been reinvestigated, and the source(s) of the various shoot pathogens that have been isolated from roots remains uncertain (8). Are they washed from diseased foliage and survive with roots, or are they capable of multiplying and surviving on roots? Can they spread on roots? Answers to these questions are necessary to better understand the ecology of shoot pathogens, particularly with respect to survival, and they may provide clues to better control practices.

The purposes of this study, mostly with cucumber (*Cucumis sativus* L.) and the shoot pathogen, *Pseudomonas syringae* pv. *lachrymans* (*Psl*), were to determine if multiplication and spread of pathogens take place in the root zone. Field or conventional greenhouse tests were unsuitable, because results would be difficult to interpret if pathogen cells were carried in moving water, as would be expected. Consequently, it was necessary to employ special methods to be assured that pathogen cells isolated from the root zone would not have been washed from the shoot and that there was no movement of water that could transfer pathogen cells from one location to another in the root zone. *Psl* was used for most of the work because it is a typical leaf-spotting pathogen that is only locally systemic when introduced directly into the vascular system (14). A short account of some of the work has been published (7).

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MATERIALS AND METHODS

There were two types of experiments: in vitro tests in which seedlings were grown on agar or in soil in petri dishes in attempts to understand mechanisms in simplified systems, and later tests in which seedlings were grown in unsterilized soil mixes in pots, to approximate conditions in the field. Either the seed or radicle was inoculated with a microorganism, and subsequently it was traced on seedling roots.

Agar tests. Tests were made with seeds germinated on water agar (Bacto agar, 20 g/L) in 9- or 14-cm-diameter petri dishes held vertically under fluorescent illumination (16 hr/day) at 24 C. Two to four seeds were placed on a dish, depending on dish size (Fig. 1). Cucumber cultivar National Pickling seeds were anchored with drops of molten agar (42 C) to prevent seedlings from falling to the bottom of the dish. Anchoring was not necessary for seeds of tomato (*Lycopersicon esculentum* Mill. 'Chico III') or cabbage (*Brassica oleracea* var. *capitata* L. 'Danish Ballhead'). Early tests demonstrated that spread of *Psl* was erratic unless dishes were sealed with a laboratory film (Parafilm M; American Can Co., Greenwich, CT 06830), so dishes were sealed routinely.

The test period usually was 3–4 days. After 4 days, the cucumber seminal root had reached the lower rim of a 9-cm-diameter dish and had grown 1–2 cm along it. Cotyledons were out of seed coats and had reached the upper rim of the dish. Cotyledons and hypocotyls were green. A 3-day-old seedling is shown in Fig. 2.

Soil tests. An unsterilized, high-organic soil mix (soil, peat, and sand; 1:1:1, v/v) was used in greenhouse pot tests. Plastic pots, 10 cm in diameter, had large bottom drain holes. Pots were watered only by capillarity by placing them on individual drained beds of vermiculite, onto which water was metered periodically with a timer. Four inoculated seeds (or radicle-inoculated seedlings) were planted equidistant near the rim of a pot. Temperature was 20–28 C. Distal roots were sampled for the pathogen (see below) after 6–8 days.

In growth room pot tests, a soil mix (10 parts of a soil characterized as "loamy fine sand," 5 parts of sand, and 3 parts of $CaCO_3$, dry weight) at a given water status was placed in drainless styrofoam pots. The $CaCO_3$ was added to bring the pH to 6.0, a

level favorable for the activities of pathogenic bacteria. The soil mix was about 6 cm deep and 6 cm below the rim of the pot; there was a transparent film cover to reduce water loss. The water status, which was intended to favor *Psl* motility, was adjusted as follows. The "pot capacity" (ie, the water content after the mix was saturated and drained overnight through holes formed by repeatedly piercing the pot bottom with a needle), was 21% of the mix dry weight. An aliquot of the dry mix was brought to a "wet" condition by adding 87% of the water at pot capacity, or to a "moderately wet" condition by adding 50% of the water at pot capacity. These were approximately equivalent to water potentials of <-0.1 bar and -0.1 bar, respectively, as determined by a curve erected from porous pressure plate data. Inoculated seeds or radicle-inoculated seedlings (6–8/pot) were planted equidistant near the pot rim. Pots were held at 24 C under fluorescent illumination for 16 hr/day. Distal roots were sampled for the pathogen after 5–6 days.

A high-organic soil was used to make suspensions for inoculating soil-line dishes (Fig. 1). This soil also was used for growing cucumber seedlings in vitro. Petri dishes (14 cm in diameter) were partly filled with moist soil, sealed, and held under light, as with the agar tests. Dishes were inclined 15 degrees from the vertical so that roots grew along the dish bottom. When the soil was removed from the dish, the exposed roots were carefully sampled by segmentation or replica printing methods.

Inoculation and media. Seeds were inoculated with *Psl* cells by dipping them in a suspension (10^7–10^8 colony-forming units [cfu] per milliliter) made from tube cultures of agar medium M66 (see below) held at 24 C for 2–7 days. In most tests, inoculated seeds were dried in thin layers on paper for one or more days before use. Seeds for a few tests, including the growth room pot tests, were blotted with paper to remove excess suspension and planted within 1 hr. Seeds for the greenhouse pot tests were vacuum-infiltrated with the suspension of *Psl* (10) and dried before planting. This procedure places *Psl* cells both within and on the seeds (10).

Radicles were inoculated by passage through an inoculum line (Fig. 1). For tests involving growth in soil, seedlings were germinated on the agar for 2 days. When radicles were 1.5–2.0 cm long and had passed through the inoculum line, seedlings were removed from the agar and planted at once.

A wild-type *Psl* isolate (PI 785) was used for most studies with this pathogen. The rifampicin-resistant isolate of *Psl* was derived from PI 785 as a spontaneous one-step mutant on medium M72 containing 50 mg/L of rifampicin.

Detection medium for *P. syringae* pv. *glycinea* and occasionally for *Psl* was a tetrazolium agar containing 1 g/L of boric acid (medium M71 [6]). Medium M72, which has the same ingredients plus boric acid at 2 g/L, was used for *Psl*, *P. syringae* pv. *syringae*, and *P. syringae* pv. *tomato*. Both contained the fungicide, actidione (50 mg/L). The medium for the rifampicin-resistant isolate of *Psl* was M72 plus 50 mg/L of rifampicin. Cultures were incubated 2–3 days at 24 C. Medium M66, nutrient sucrose agar (Difco nutrient agar, 23 g; sucrose, 10 g/L), alone or with actidione (M66A), were used for detecting the other organisms.

Detection of pathogens associated with roots on agar. The aim was to detect a pathogen by sampling parts of the root distant from the point of inoculation. Detection routinely was by isolation and identification on detection medium.

Replica printing was the most commonly used sampling method. A velvet-covered pad, slightly smaller than a petri dish (Fig. 3), was pressed momentarily against roots growing on agar and then against detection medium in a petri dish. Dishes were incubated and microorganism placement on roots was determined by the position of colonies on the detection medium (Fig. 2). This is the preferred method for tracing organisms on roots because it is simple, and the nap ends of the velvet, which comprise about 3% of the total area of the printer, sample uniformly distributed, undisturbed small locations. Also, where appropriate, a printer may be applied to a root and successive printings made to different detection media.

Other methods used to detect microorganisms were: i) Root segmentation. Roots in situ were cut into segments and individually placed on detection medium, taking care to cut and remove segments so that organisms were not transferred among them. ii) Deep agar. Roots were allowed to grow through a layer of agar 1 cm deep in high-sided (8 cm) petri dishes, the agar was removed, and the roots exposed on the lower agar surface were printed on the detection medium. iii) Microscopic observation. Roots on agar were examined microscopically without coverslips at ×250 or

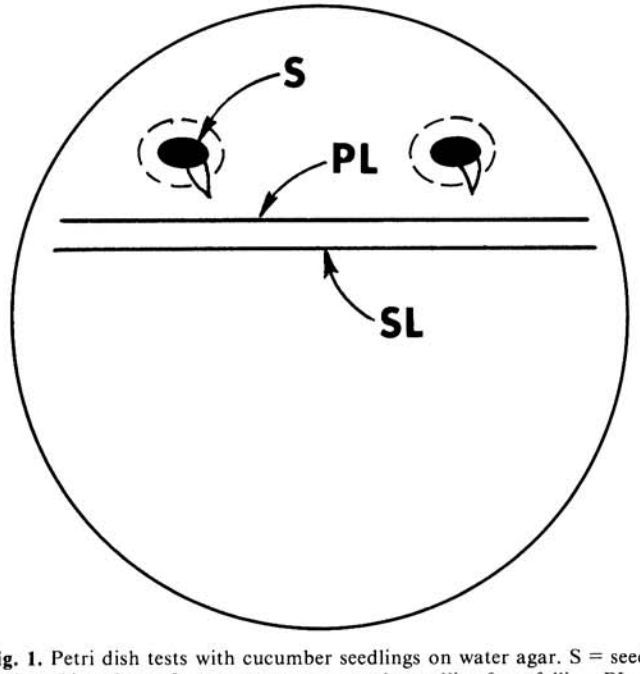

Fig. 1. Petri dish tests with cucumber seedlings on water agar. S = seed anchored in a drop of water agar to prevent the seedling from falling. PL = pathogen line, made by streaking a loop of a suspension of pathogen cells (used in "line tests" and for radicle inoculation). SL = soil line, made by streaking a loop of unsterilized soil suspension below the pathogen line (used in "soil-line tests"). The seminal root passed among the pathogen cells and then through the soil line (when used) as it grew to the dish bottom. The radicle length depicted here occurs about 1 day after seed was planted.

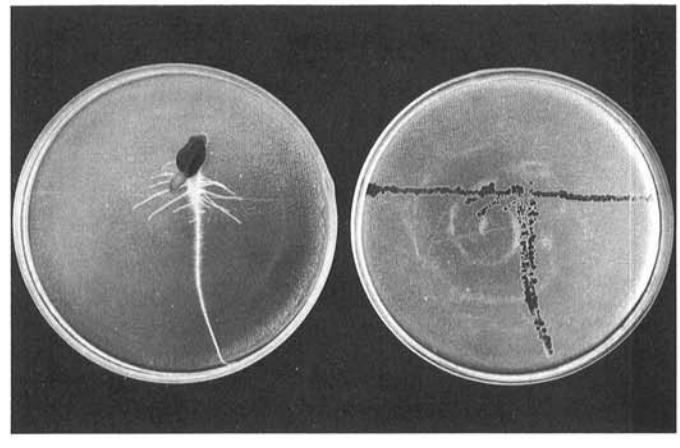

Fig. 2. Spread of *Pseudomonas syringae* pv. *lachrymans* from a horizontal inoculum line to roots of a 3-day-old cucumber seedling in a line test. The seminal root grew through the inoculum line 2 days after planting, as in Fig. 1. A replica printer (Fig. 3) was pressed momentarily against the seedling on the agar (left) and then momentarily against the detection medium in a petri dish. After incubation, the position of the pathogen colonies on the detection medium (right) indicated the position of the pathogen when the print was made (a dye in the medium produced characteristic pink [here black] pathogen colonies). After printing, the dish with the seedling (left) was stored at 5 C to stop growth until this photograph was made.

×1,000. iv) Dilution plating. Tenfold, spread-plate dilutions on M72 were used to determine cfu of *Psl* associated with roots. Seeds were planted and the inoculum line made as indicated in Fig. 1. To determine cfu that served as inoculum for one seminal root after 2 days, five 2-mm sections of the inoculum line distant from roots were ground separately for 15–30 sec in mortars and pestles with water and assayed (2 mm was the width of a root passing through the line at 2 days). At the end of an experiment, the seminal root and the few lateral roots from it were cut 2 mm below the inoculum line. The roots below the cut and the agar beneath them were ground and assayed.

Detection of *Pseudomonas syringae* pv. *lachrymans* associated with roots in soil pot tests. Since the aim of these tests was to detect the pathogen at a distance from the point of inoculation, only the distal half of a root system was sampled.

In greenhouse pot tests, roots of a plant were removed gently from the pot with a spatula and shaken to remove most of the soil. The distal half (~3–5 cm of the seminal and associated lateral roots) was excised, washed vigorously in several changes of water (nearly all of the soil was removed), drained, and macerated with a spatula in about 1 ml of sterile water in a petri dish. The macerate was "run-streaked" on M72. A run-streak was made by placing two drops of a macerate on the agar surface near the rim of the petri dish, holding the dish vertically, and allowing the liquid to run down the agar to the lower rim. Successive needle passes then were made down the plate through the run. Run-streaking permitted detection of pathogen colonies among colonies of other organisms better than conventional streaking.

In growth room pot tests, distal roots were sampled for *Psl* somewhat differently. The soil ball was tapped gently from the pot, and the lower half was separated from the upper half with a knife. The seminal and associated roots from an individual plant were teased gently from the bottom half of the ball and washed and streaked as described above.

Recognition of pathogen colonies. Colonies of *Psl* are recognized reliably on detection medium M72 with a dissecting microscope, as they are distinguishable in color and morphology from colonies of other organisms when examined at ×10 with tangential light directed at the bottom of the culture dish (see color photograph [8]). Pathogenicity tests were made with cells from representative, putative *Psl* colonies to be certain of identity (cells on a moist cotton swab were rubbed on seedling cucumber leaves previously dusted with carborundum; characteristic water-soaked lesions developed in 5–8 days). Colonies of *P. syringae* pv. *glycinea*, *P. syringae* pv. *syringae*, and *P. syringae* pv. *tomato* were similar to those of *Psl* on M72 and also were reliably recognized. In the line tests (Fig. 1), colonies of each pathogen on replica print cultures of roots could be compared visually in the same dish with colonies derived directly from the pathogen line (Fig. 2). Similarly, the origin of colonies of organisms other than pathogens on roots could be traced to the seed, or in soil-line tests (Fig. 1), to the soil line.

RESULTS

***Pseudomonas syringae* pv. *lachrymans* and cucumber seedlings on agar.** After inoculation of seeds or radicles, this pathogen was found associated with all parts of the seedling root system (Fig. 2). With seed inoculation, this was demonstrated by all detection methods: replica printing (69 plants in 13 tests), root segment (12 plants in two tests), deep agar (12 plates of 10 seedlings each in two tests), and microscopic observations (discussed below). Since roots of a few seedlings from inoculated seeds did not carry the pathogen for some reason, radicle inoculation by the line method was devised (Fig. 1). With this method, the pathogen was invariably associated with roots, so it and the simple replica sampling method were used for most of the later studies. Thus, the spread of *Psl* to distal roots was demonstrated with an additional 62 plants in nine tests.

To determine the location of *Psl* on roots, inoculated seeds were planted, as in Fig. 1, on boiled dialysis membrane, which had been placed on the water agar. Roots grew on the membrane. Seedlings were removed and bacteria on the membrane were stained with carbol-fuchsin. Bacterial cells in lightly stained material characteristically were observed in rows next to roots (Fig. 4). Some cells were dividing. Further evidence for bacterial multiplication was obtained with dilution assays of roots of radicle-inoculated seedlings on agar. When the roots of 10 seedlings were assayed 3 days after planting (Fig. 2), there were 1.6×10^7 cfu per root (s.e. = 0.25×10^7), compared with 8×10^5 cfu (s.e. = 2.4×10^5) in the inoculum (cells in contact with the seminal root when it passed over the inoculum line). After 4 days, the figures were 2.1×10^7 cfu (s.e. = 0.4×10^7) and 6.3×10^5 cfu (s.e. = 0.7×10^5), respectively. This represents a 20-fold increase in cfu 1 day after the root had passed through the inoculum line and a 30-fold increase after 2 days. These values are conservative, as they do not include roots above the inoculum line, those between the excision point and the root base, a region with many hair and lateral roots (Fig. 2) and where large numbers of bacteria could be seen with the microscope.

On agar, it was clear that *Psl* moved by swimming, since motile cells were observed readily in the small film of water that bordered roots. That cells were moved with the seminal root terminus as it grew was evident, as some bacteria could be seen swimming near the tip. However, populations of vigorously motile cells were much larger near young hair cells, probably because more nutrients were available. The position of the water explained rows of cells next to the hair roots on dialysis membrane (Fig. 4). As roots became older, motility slowed and populations increased. Motility ceased altogether near old roots, and the water film was entirely occupied by masses of bacteria. This was particularly notable near old hair roots. Observations on motility were made routinely in later work, and they supplemented observations with replica prints.

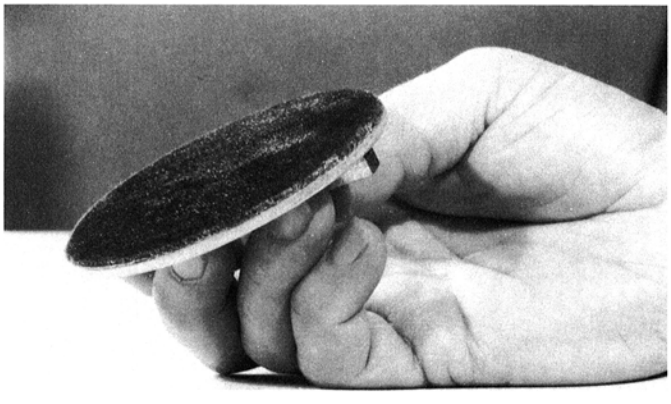

Fig. 3. A velvet-covered replica printer. A sterile printer was pressed momentarily against a seedling on agar and then momentarily against detection medium (see Fig. 2).

Fig. 4. Stained *Pseudomonas syringae* pv. *lachrymans* cells adjacent to hair roots of a cucumber seedling growing on dialysis tubing on water agar. The seed was inoculated.

Tests in which one or two 0.5–1.0 cm bands of agar were removed from the path of the radicle after it had moved through the *Psl* line (Fig. 1) demonstrated in two tests with 10 plants sampled by replica printing that the pathogen had spread on the root across the air gaps. This was also demonstrated by culturing segments of roots below gaps. *Psl* was found on segments of most lateral roots that grew in the air and were not in contact with the agar (30 roots from 14 plants were examined). These results indicated that water was on the root, probably in depressions between epidermal cells, and that *Psl* was motile in it.

Hypocotyls and cotyledons as well as roots often were sampled during replica printing of older seedlings. *Psl* from the inoculated seeds or radicles spread to the shoot as well as to the root, as was reported previously (4). Occasionally there were water-soaked lesions on cotyledons.

Not all lots of cucumber seeds were suitable for agar tests, because some nonpathogenic organisms associated with seeds spread to the root and interfered with the spread of *Psl*. Bacteria were the most common organisms; three were selected for further study (see below). Seed lots that did not carry these organisms were identified by replica printing seedlings on agar to a nonselective medium (M66) and also by examining roots with the microscope.

Agar tests with *Pseudomonas syringae* pv. *lachrymans* and tomato and cabbage seedlings. In two radicle inoculation tests and one seed inoculation test with *Psl*, the pathogen moved to all root parts of 12 tomato and 12 cabbage seedlings growing on agar, as determined by replica printing and as described with cucumber. Similarly, pathogen cells in the water adjacent to roots were motile. Roots did not appear to be harmed. Seed lots in these experiments did not contain microorganisms that interfered with the spread of *Psl*.

Agar tests with other pathogens and cucumber seedlings. Seed inoculation tests with *P. syringae* pv. *glycinea* and *P. syringae* pv. *tomato* demonstrated that these pathogens as well as *Psl* spread to all distal parts of cucumber roots, as detected by replica printing. Results were verified in three line tests with 12 plants. Parallel line tests also demonstrated that *P. syringae* pv. *syringae* (an isolate from bean [9]) spread similarly. As with *Psl*, the three pathogens were motile in the water film adjacent to roots.

Line tests also were used with other pathogens. There were four tests, each consisting of four plants. Detection was by replica printing on media M66 or M66A. The following spread to distal portions of the root: *Agrobacterium tumefaciens*, *Erwinia carotovora* pv. *carotovora*, *E. stewartii*, *P. solanacearum*, *P. syringae* pv. *phaseolicola*, *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *nigromaculans*, and *X. campestris* pv. *phaseoli*. The mechanism of spread was not studied. *Corynebacterium michiganense* pv. *michiganense* and *C. michiganense* pv. *nebraskense* were not associated with distal roots.

Root inhibition by pathogens. It was observed in line tests that hair root development on the seminal roots of cucumber seedlings was inhibited by *Psl*. Seedlings were 4–7 days old. Inhibition occurred in the mid-portion of the root: hair roots were shorter and fewer than those on uninoculated seedlings. *P. syringae* pv. *glycinea*, *P. syringae* pv. *syringae*, and *P. syringae* pv. *tomato* also caused the same type of hair root inhibition of cucumber seedlings.

Agar tests with nonpathogenic organisms and cucumber seedlings. Some lots of cucumber seeds naturally carried nonpathogenic organisms that moved from seed to root as described above. Four isolates with distinctive colonies were isolated and studied in line tests. All four spread to distal portions of the root, as determined by replica printing. Cells were stained with carbol-fuchsin and examined. Three isolates were small bacterial rods and the fourth was a yeast. Other bacteria and yeasts were isolated from roots of different lots of seeds but were not studied further. Thus, many kinds of nonpathogenic microorganisms appear to be carried with seeds and are able to spread to roots on agar.

Tests with soil. In two tests made with sterilized (autoclaved) moist soil in large petri dishes, *Psl* spread from inoculated seeds to the distal parts of roots of all seven seedlings examined. When the soil was not sterilized, *Psl* was found only on distal portions of roots of four of the 14 seedlings examined in two tests. Other organisms were plentiful on the detection medium and some inhibited *Psl*. This in vitro method was used only for exploratory studies with unsterilized soil because fungi often overgrew the shoot.

In greenhouse pot tests, with unsterilized soil mix watered from below by capillarity, *Psl* on seeds rarely spread to distal parts of the root (Table 1). However, *Psl* spread from inoculated radicles was common; it occurred with 44% of seedlings inoculated with the wild-type isolate and 22% of seedlings inoculated with the rifampicin-resistant isolate (Table 1).

Similar results with *Psl* were obtained with a different unsterilized soil mix in growth room pot tests in which the water status of the soil was high (Table 2). Seeds or radicles were inoculated with the wild-type isolate. There was more spread of the pathogen from the seed to distal roots in moderately wet soil (11%) than in wet soil (0%), but as in the greenhouse pot tests, spread from the radicles was much greater at both moisture levels (>50%).

Prior to making these pot tests, a greenhouse pot test similar to the ones described above examined methods for isolating the pathogen from distal roots. *Psl* was detected more often in run streaks when roots were washed free of attached soil, as in the pot tests described above, than when roots and attached soil were used directly.

Psl also spread to distal portions of roots of cucumber seedlings growing on agar in soil-line tests (Fig. 1), but the distribution clearly could be altered by various other microorganisms that originated from the soil line. Thus, with most of the 16 seedlings in two tests using the replica printing detection method, *Psl* did not reach the seminal root tip and was confined to regions closer to the root base. Other organisms were associated with roots; some of

TABLE 1. Spread of *Pseudomonas syringae* pv. *lachrymans* from inoculated cucumber seeds or radicles to distal seedling roots. Greenhouse pot tests with a high-organic matter nonsterilized soil mix[a]

Type of pathogen	Seeds inoculated[b]		Radicles inoculated[c]	
	Seedlings assayed[d]	Distal roots with pathogen[e]	Seedlings assayed[d]	Distal roots with pathogen[e]
Wild-type (P1 785)	159	1	132	58
Rifampicin-resistant	83	0	102	22

[a] Plants were watered from below by capillarity.
[b] Three tests at different times.
[c] Two tests at different times.
[d] The distal half of the seminal and attached lateral roots of each seedling was washed, ground in water, and the suspension streaked on a selective medium to detect the pathogen.
[e] Bacterial cells from representative colonies from each seedling were tested for pathogenicity. All were positive.

TABLE 2. Spread of *Pseudomonas syringae* pv. *lachrymans* (P1 785) from inoculated cucumber seeds or radicles to distal seedling roots. Growth room pot tests with a nonsterilized loamy fine sand soil mix[a]

Part inoculated	Soil mix water status[b]	Seedlings assayed[c]	Distal roots with pathogen[d]
Seed	Moderately wet	44	4
	Wet	25	0
Radicle	Moderately wet	30	17
	Wet	30	21

[a] Results from three tests at different times.
[b] "Wet" = <−0.1 bar; "moderately wet" = 0.1 bar. See text.
[c] The distal half of the seminal and attached lateral roots of each seedling was washed, ground in water, and the suspension streaked on a selective medium to detect the pathogen.
[d] Bacterial cells from representative colonies from each seedling were tested for pathogenicity. All were positive.

them grew intermixed with the pathogen on the detection medium and others apparently excluded the pathogen from a portion of the root. Exclusion was demonstrated by printing the same seedling on two detection media: on M72, which allowed the pathogen to grow and not most other organisms, and on M66, which was nonselective and allowed the pathogen as well as the other organisms to form colonies.

DISCUSSION

In this work it was demonstrated that the cucumber shoot pathogen, *Psl*, which had been inoculated on cucumber seeds or radicles, was detected on distal parts of seedling roots growing in two types of unsterilized soil. This also was demonstrated with sterile soil. As the pathogen was not transferred with a flow of water in these tests, I suggest that arrival at distal positions was accomplished by the active spread of the organism. More information will be needed to be certain of this, but the opinion is based on results of seedlings growing on agar. The original inoculum was not passively distributed by root tips as they grew. Rather, the pathogen was seen to be vigorously motile near root tips and other roots. An active multiplication was demonstrated. The rhizoplane may be an important location for these activities. With seedlings on agar, the pathogen spread on the surface of roots that were in air and not in contact with agar, and in soil, *Psl* was more easily detected with roots that were washed than with roots that were not washed. Motility near roots probably can take place readily in soil because roots are ensheathed with water or a watery gel most of the time (5); in the present tests soils were very moist. These model tests were with seedlings and with soil mixes in pots, so they may hold tenuous ecological reality in nature. On the other hand, results add to the findings of Valleau et al (19) and more recent workers (17) and offer one explanation for the isolation of so many bacterial shoot pathogens from roots. These organisms may indeed at times be actively growing on or near roots of host plants. This may be true for nonhost plants as well. The survival of pathogen cells overwintering with roots may have epidemiological consequences by initiating a pathogenic or a resident phase of the pathogen life cycle on an emerging seedling the next spring. I have discussed survival of pathogens elsewhere (8).

A prominent difference between seedling roots on agar and those growing in unsterilized soils in these tests was that, aside from the inoculated organism, there were no other organisms associated with roots on agar. The spread of *Psl* to distal portions of roots in unsterilized soils must have been at great odds, owing to exploitive (11) and interference (21) competition exerted by microorganisms naturally carried in the soils. These complex interactions probably explain why spread to distal roots in unsterilized soils was more prevalent when radicles rather than seeds were inoculated: the pathogen was better able to compete once it was established with roots. Or, stated differently, competitive forces were more intense during the first day or two of seedling development. Competition undoubtedly also influenced motility, as *Psl*, which is attracted to nutrients (3; *unpublished*), would move toward these chemicals, but also would be repelled by chemicals produced by many of the competing organisms. Competitive interactions were seen easily in soil-line tests, in which the pathogen and soil organisms were present on roots of seedlings growing on agar. Seeds of some lots also carried competing microorganisms. The complicated biotic and abiotic constraints of bacterial pathogens in the root zone are expected to be the same as those encountered by rhizobia introduced into soil or by microorganisms added to soil or to seed to control root diseases (1,12,13,15,16).

Information about the location of bacteria near seminal roots was obtained in these tests with agar. Bacteria were observed in situ and were not subject to possible artifacts introduced by sampling techniques. Highest populations of *Psl* appeared to be near hair roots; they probably grew on nutrients exuded from the root (the stained material around bacterial cells in Fig. 4). Older hair roots were bordered with static masses of bacterial cells. Lysis of these roots may have been taking place, providing more nutrients for the pathogen. Lysing root cells have been linked with high populations of unidentified nonpathogenic bacteria associated with the rhizoplane of seminal wheat roots in soil (20). In fact, under some conditions shoot pathogens may inhibit roots, as shown in the present work and elsewhere (2,17,18). More study of populations and distribution of bacteria with roots are needed, but given the motility of so many pathogens and soil organisms and the interactions among them, new information probably will be difficult to evaluate on a micro-scale.

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