Enhanced Colonization of Pea Taproots by a Fluorescent Pseudomonad Biocontrol Agent by Water Infiltration into Soil

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ABSTRACT

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Root colonization by an introduced strain of *Pseudomonas fluorescens* was examined to determine the importance of the root apex in passive transport and to quantify the effect of infiltrating water on distribution of the bacterium. Pea seeds coated with strain PRA25rif of *P. fluorescens* were sown in columns containing a sandy field soil at soil-water matric potentials of −1, −6, or −10 kPa. After 7 days, the largest population density of the bacterium was found on roots at −1 kPa, but the bacterium was detected on only 5% of root segments 4–5 cm below the seed, approximately 8 cm above the root apex. At −6 and −10 kPa, the bacterium could not be detected on roots beyond 3 cm from the seed, more than 16 cm from the root apex. Addition of 27.2 and 54.4 mm of water to the top of the columns 4 days after planting increased the depth from which PRA25rif was recovered. The bacterium was detected on root segments at least 9–10 cm from the seed 24 hr after water was applied. Transport of the bacterium on the root apex apparently was limited to a short period after seed germination, but the bacterium was carried long distances by percolating water.

Additional keywords: biological control, *Pisum sativum*, rhizosphere.

Rhizobacteria have been introduced on seeds and other propagative materials of many plants to increase germination, plant vigor, and plant health (7,30). It now is clear that, for many of these beneficial effects to occur, introduced bacteria must be able to change the composition of the rhizosphere microflora (27). Before this can happen, however, introduced bacteria must move from the site of inoculation and establish on and around the growing root. Root-colonizing ability, which encompasses this movement and proliferation in competition with indigenous microorganisms (21), appears to be a prerequisite for subsequent effects on plant growth or disease control. Attempts to manipulate the rhizosphere flora, however, often yield inconsistent results because little is known about the colonization process and how it is influenced by soil physical, chemical, and biological factors.

Bowen and Rovira (4) postulated three methods of bacterial dispersal in the rhizosphere: motility or passive movement in a film of water on the root surface, passive transport on the root apex, or passive transport via fungal hyphae. However, relatively few investigators have provided experimental evidence for the mechanism of root colonization by introduced rhizobacteria. Howie et al (15) questioned the role of bacterial motility in wheat root colonization by strain 2-79 of *Pseudomonas fluorescens* because motile and nonmotile mutant strains colonized roots to the same extent when grown in soil at a steady-state matric potential. They suggested that root colonization occurs by a process of passive transport of the bacterium on the root apex (phase 1), followed by multiplication of the bacterium after cells are deposited along the root (phase 2). However, research on the same strain by Parke et al (22) indicated that populations of the introduced bacterium were not recovered from the apex of inoculated wheat roots after 4 days. Without percolating water, dispersal of the bacterium to the apex of the elongating root did not occur. Parke et al (22) suggested that passive movement on the root apex as a sole means of transport probably could not account for the rapid, long-distance dispersal of the bacterium observed on roots in the greenhouse or in the field.

The aim of the present study was to quantify the effect of infiltrating water on the distribution of an introduced bacterium along a pea taproot and to assess the role of the root apex in transport. An understanding of the spatial and temporal characteristics of the early stages of root colonization and of the soil factors that affect this process should enable more accurate predictions of the fate of bacteria introduced to the rhizosphere. This, in turn, should lead to more effective management of bacterial populations to achieve biological control and enhance plant growth.

MATERIALS AND METHODS

Soil. The soil used was Plainfield sand (91.2% sand, 4.3% silt, 4.5% clay), pH 5.8, from the Hancock Agricultural Experiment Station near Hancock, WI. Soil was air dried, thoroughly mixed, and stored under cover at room temperature in unsealed plastic bags until used. It was sieved through a 4.75-mm screen before use, and no fertilizers or pesticides were applied. The moisture characteristic of sieved soil is shown in Figure 1. Although this soil was saturated at 30% moisture content, it drained freely at small matric potentials; by −10 kPa, the moisture content was approximately 13%. Most of the pore water drained between 0 and −10 kPa, and, therefore, this range was chosen for our experiments.

Bacterium. Strain PRA25 of *P. fluorescens* was isolated from the rhizosphere of pea grown in the Aphanomyces root rot nursery at the Arlington Agricultural Experiment Station near Arlington, WI. A spontaneous mutant strain, PRA25rif, resistant to 100 μg/ml of rifampicin, was used throughout this study. Strain PRA25 was effective in biological control of Aphanomyces root rot of peas in growth chamber and field studies (20); PRA25rif is as effective as the wild type in biological control in field experiments (Parke, *unpublished*). Stock cultures of the bacterium, derived from a single colony, were stored in 5% dimethyl sulfoxide at −80 C.

Seed treatment. PRA25rif was grown in nutrient broth yeast extract (NBY) (28) shake culture. After 48 hr, 2.5 ml of the turbid suspension was plated on NBY agar and incubated for 24 hr at room temperature. Bacterial growth was scraped from one

agar plate and thoroughly mixed with 20 captan-treated pea seeds (*Pisum sativum* L. 'Perfection 8221'). Control seeds were treated with sterile water. All seeds were dried for 24 hr in a sterile cabinet before sowing.

In each experiment, five pea seeds were assayed individually for populations of PRA25rif before sowing. Each seed was placed in 10 ml of sterile distilled water, sonicated for 20 sec, then dilution plated onto King's medium B (16) supplemented with 50 μg/ml of ampicillin, 75 μg/ml of cycloheximide, 12.5 μg/ml of chloramphenicol, and 100 μg/ml of rifampicin (KBACCR). Plates were incubated in the dark at room temperature for 48 hr when colonies were counted.

Control of soil-water matric potential. Plants were grown in soil columns, each consisting of a 25-cm-long polyvinylchloride (PVC) pressure pipe (inside diameter = 78 mm, wall thickness = 6 mm). Each column was placed inside a 600-ml Büchner funnel fitted with a fine-grade fritted glass plate (Kimax 28400-600F) (Fig. 2). Soil was packed to a bulk density of 1.5 g cm^{-3} to a depth of 24 cm and was in direct contact with the fritted plate. Before the soil column was packed, the funnel was connected to a length of plastic tubing and filled with degassed water under vacuum. The open end of the tube was placed in a water reservoir that was raised or lowered with respect to the fritted plate to establish the desired matric potential (12). After packing, soil columns were saturated to +1 kPa (1 kPa = 10 mbar) for 16–24 hr and covered to reduce evaporation. The desired matric potential was established in the soil column after time was allowed (4 hr) for the soil moisture to equilibrate. Soil-water matric potential varied by −2.4 kPa from the fritted plate to the top of the column.

Sowing and cultural conditions. Pea seeds were planted 3 cm deep in soil columns at the desired matric potential. A heavy (approximately 60 g, 65-mm-diameter) Syracuse watch glass was placed on the soil surface for 48 hr to promote uniform penetration of the radicles into soil of different shear strengths, which result from imposing different matric potentials. Soil columns were placed in a controlled environment chamber at 24–26 C, with a relative humidity between 60 and 90% and 325 μE m^{-2} sec^{-1} light on a 12-hr photoperiod from a bank of 16 cool-white fluorescent tubes.

Assessment of root colonization. All experiments were terminated 7 days after planting unless otherwise noted. The length of the taproot, depth of the lowest lateral root, frequency of recovery, and population density of PRA25rif along the taproot were determined. The seed and shoot were removed and the column was positioned horizontally. Soil gradually was pushed out of the column with a plunger, permitting 1-cm increments of the taproot and adhering soil to be removed. Thus, the root was sampled sequentially from the tip upwards with little risk of contamination from the treated seed and surrounding soil.

Taproot segments, 1 cm long, with adhering soil each were placed in 10 ml of sterile water, sonicated for 20 sec, and dilution plated on KBACCR. Lateral roots were trimmed off when present. Root apices (distal 1-cm segments of the taproot) were plated directly onto KBACCR and scored for presence or absence of the bacterium 48 hr later. In some experiments, the shoot was excised at the point of seed attachment, dried to constant weight at 70 C, and weighed.

Steady-state soil-moisture experiments. To assess the extent to which PRA25rif could colonize pea taproots under steady-state soil-moisture conditions, soil matric potential was maintained at a constant −1 or −6 kPa in two experiments and −1, −6, or −10 kPa in three experiments. There was no transient flow of water through the soil, and the evapotranspiration flux upward through the plant and soil was small and constant. All water required for plant growth was supplied by the reservoirs via the fritted plate. A minimum of five replicates was included for each treatment.

Infiltration experiments. To examine the effect of water infiltration into soil on the distribution of PRA25rif along pea taproots, water was gently added to the surface of the soil columns of −6 kPa matric potential 4 days after planting. A filter-paper disk was placed on the soil surface to reduce any disruption to the soil surface due to watering.

To examine the effect of adding different amounts of water on the distribution of PRA25rif, either 42 ml (= 8.8 mm), 130 ml (= 27.2 mm), or 260 ml (= 54.4 mm) of water was added 4 days after planting. The depth of the wetting front immediately before redistribution began was 3 cm for 8.8 mm, 10 cm for 27.2 mm, and 20 cm for 54.4 mm of added water. There were five replicates for each treatment, and the experiment was repeated three times.

Another experiment was conducted to determine whether the population distribution of PRA25rif along the taproot after 7 days (3 days after infiltration) was substantially different from that at 1 day after infiltration. The aim of this experiment was to evaluate the relative importance of transport or movement of the bacterium in the infiltrating water as opposed to growth of the bacterium after the infiltration event in determining the population distribution on the taproot after 7 days. This experiment was similar to the other infiltration experiment, except that only 27.2 mm of water was infiltrated on day 4. Plants were sampled at random on day 4 (before infiltration), day 5, and day 7. The population densities of PRA25rif along the taproot, taproot length, and shoot dry weight were determined. There were five replicates for each treatment, and the experiment was repeated once.

Experimental design and data analysis. All experiments were either a completely randomized design (CRD) or a randomized complete block design (RCB), where blocks consisted of groups of seeds treated on the same day. Each plant constituted an experimental unit, and each unit was replicated four to six times within each treatment. Analysis of continuous data was accomplished by one-way (CRD) or two-way (RCB) analysis of variance (ANOVA); the protected least significant difference (LSD) method was used for mean separation. Discrete data from several independent experiments first were tested for homogeneity among experiments by chi-square analysis, and contingency-table analysis was applied to the pooled data (26).

Regression analysis of PRA25rif population densities against distance below the seed as the independent variable was performed in some experiments to compare the distribution of PRA25rif along the roots among different watering treatments. A test of homogeneity of regression by Student's t distribution (26) for each plant showed that replicate plants behaved similarly, allowing comparison of treatments.

Data in Table 1 contain several zeros, making traditional ANOVA procedures invalid. Consequently, the data were analyzed in two ways. First, an ANOVA was performed on all of the nonzero values to obtain t-test limits for LSD comparisons. LSD values then were applied to all of the data to provide estimates of significant differences among means. A nonparametric analysis,

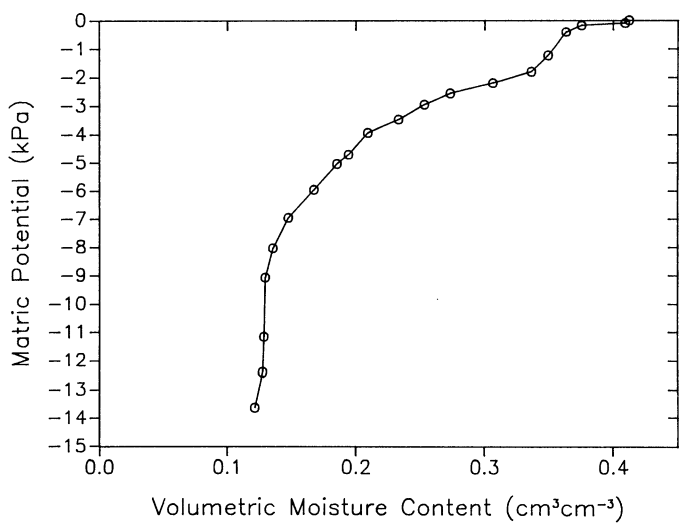

Fig. 1. Moisture characteristic of Plainfield sand.

including all the zero values, was performed to check this analysis. A Kruskal-Wallis test (9) gave substantially the same results as the modified ANOVA procedures shown in Table 1.

RESULTS

Steady-state soil-moisture experiments. PRA25rif generally was recovered from a greater depth and at higher population densities at −1 kPa than at −6 or −10 kPa, although the influence of matric potential on mean population density at different depths was significant only at 1–2 and 2–3 cm below the seed (Table 2). The frequency with which PRA25rif was recovered from the root segments declined with increasing depth, which reflected the similar effect of soil matric potential on population density of the bacterium. PRA25rif was recovered from 5% of taproots 4–5 cm below the seed at −1 kPa but was not recovered from any roots more than 3 cm from the seed in the treatments of −6 and −10 kPa (Table 3).

The length of the taproot after 7 days was strongly influenced by matric potential, but the depth of lateral branching and shoot dry weight were similar at matric potentials of −1 and −6 kPa (Table 4). Seed treatment with PRA25rif had no effect on taproot length, depth of lateral branching, or shoot dry weight after 7 days (Table 4).

Infiltration experiments. Water infiltration into soil columns 4 days after planting seeds treated with PRA25rif resulted in significantly increased recovery of the bacterium from more distant locations on the taproot than in treatments in which no water was added (Table 1). PRA25rif was not recovered from any of the root sections sampled, including the section 3–4 cm below the seed, in the absence of added water. With the addition of 27.2 or 54.4 mm of water, there was a significant increase ($P < 0.05$) in populations recovered 3–4, 5–6, and 7–8 cm below the seed after 7 days. With 54.4 mm of water, recovery at 9–10 cm below the seed also increased significantly ($P < 0.05$) (Table 1). The bacterium was not detected at or near the root tip ($\bar{x}$ = 19.5 cm below the seed).

In a similar experiment, PRA25rif was recovered 4–5, 9–10, or 13–14 cm below the seed with 8.8, 27.2, or 54.4 mm of water

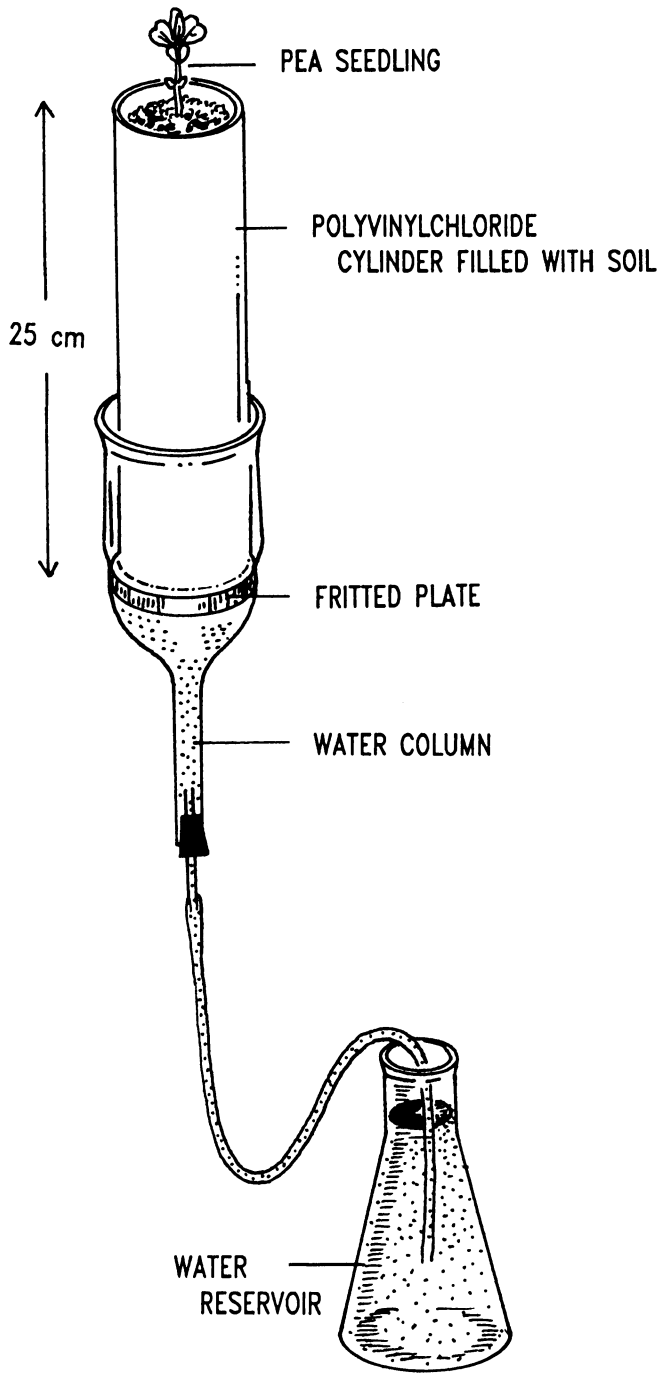

Fig. 2. Soil column and funnel apparatus for control of soil-water matric potential.

TABLE 1. Mean population (log colony-forming units [cfu]) of strain PRA25rif of *Pseudomonas fluorescens* recovered from 1-cm segments of the taproot of 7-day-old pea seedlings grown in soil at −6 kPa and watered with three amounts of distilled water at day 4

Taproot segment (distance below seed)	Mean population of PRA25rif (log cfu) with amount of water added on day 4			
	0 mm	8.8 mm	27.2 mm	54.4 mm
3–4 cm	0 a[y]	1.78 ab	4.78 b	3.82 b
5–6 cm	0 a	0.95 ab	4.05 b	3.49 b
7–8 cm	0 a	0.75 ab	2.66 b	3.03 b
9–10 cm	0 a	0 a	1.41 ab	3.06 b
Root apex[z]	–	–	–	–

[y]Values within a row followed by the same letter are not significantly different ($P = 0.05$) according to a least significant difference test. All values are the means of five replicates.
[z]Distal 1-cm taproot segments were directly plated and scored for presence (+) or absence (−) of PRA25rif. Taproot length ranged from 8 to 23.5 cm ($\bar{x}$ = 19.5 cm).

TABLE 2. Mean population densities of strain PRA25rif of *Pseudomonas fluorescens* recovered from seeds and 1-cm segments of the taproot of 7-day-old pea seedlings grown in soil at three matric potentials

	Population density means (log cfu[w]) at:		
	−1 kPa	−6 kPa	−10 kPa
Seeds at harvest[x]	7.4 a[y]	7.5 a	7.4 a
Taproot segment (distance below seed)			
0–1 cm[x]	6.2 a[y]	5.7 a	5.6 a
1–2 cm	5.6 a	3.5 b	2.4 b
2–3 cm	2.7 a	1.1 ab	0 b[z]
3–4 cm	0.8	0	0
4–5 cm	0	0	0
5–6 cm	0	0	0
Total population on roots 0–6 cm from seed	6.3	5.7	5.6
Length of taproot at 7 days (cm)	13.4 a	19.2 b	19.6 b

[w]cfu = colony-forming units.
[x]Initial seed population density of *P. fluorescens* was 8.1 log cfu per seed, as determined from five seeds selected from those sown in the experiment. Determined from five single seeds or root segments per matric potential treatment.
[y]Values within a row followed by the same letter are not significantly different ($P = 0.05$) according to least significant difference tests. All values are the means of five replicates.
[z]Limit of detection = 100 cfu/cm root.

added 3 days previously (Fig. 3). Comparison of the slopes of regression lines generated from the data indicated that, with no added water, the distribution of the bacterium along the root was significantly different ($P < 0.05$) from any treatment in which water was added. The distribution of the bacterium 3 days after addition of 54.4 mm of water was significantly different ($P < 0.05$) from that 3 days after 8.8 or 27.2 mm of water was added. The addition of different amounts of water had no effect on either root length ($\bar{x}$ = 21.3 ± standard error 3.3 cm) or shoot dry weight ($\bar{x}$ = 44.0 ± standard error 6.9 mg) at 7 days as compared with the control.

In the third experiment, the distribution of PRA25rif, without the addition of 27.2 mm of water, did not change significantly from day 4 to day 7 (Fig. 4). In the watered treatment, the distributions of PRA25rif on day 5 and day 7 were significantly different than on day 4. However, there was no significant difference between the distribution of the bacterium on day 5 and day 7, nor was the increase in population density 9–10 cm below the seed significant as assessed by paired *t*-tests.

DISCUSSION

It is well known that water percolation through soil can transport bacteria long distances in the absence of plant roots (3,10,18,24,31,32). There remains, however, some doubt about the importance of water flow in the colonization of roots by introduced rhizobacteria. Some researchers believe that passive movement with the elongating root tip is most important (15,30). Others have emphasized movement with soil water as the overriding determinant (1,8,18,22). We suggest from our results that both phenomena are involved but that the overall pattern of root colonization in our system was determined primarily by the addition of water to the soil.

We have demonstrated that passive movement with infiltrating water can be a significant determinant of the distribution of strain PRA25rif of *P. fluorescens* in the rhizosphere. In the absence of infiltrating water, the recoverable population of PRA25rif after 7 days generally was restricted to 0–4 cm below the seeds. This occurred even though steady-state soil matric potentials of −1 to −10 kPa were optimal for motility of *Pseudomonas* (33) and within the range favorable for colonization by this strain (Table 2). In contrast, PRA25rif was recovered from root segments up to 13–14 cm below the seeds if 27.2 mm of water was added to the top of the columns and allowed to infiltrate soil (Fig. 4). Further, the depth at which the bacterium was recovered was related to the amount of water added because the bacterium was recovered from greater depths when larger volumes of water were applied (Fig. 3).

Howie et al (15) postulated two stages in root colonization by introduced organisms: phase 1, in which bacterial cells are moved by passive carriage on the root apex; and phase 2, in which the bacterial cells deposited along the root multiply. In our experiments, the introduced strain was not recovered from the root apex if water was not added, even 4 days after planting. At this time, the taproots had extended more than 7 cm beyond the recoverable population of the bacterium. By 7 days, this distance had increased to 17 cm. This suggests that populations of the bacterium remain associated with the root apex for only a short period. Once the root apex has elongated beyond the location of the introduced bacterium, the root itself does not

TABLE 3. Percent of taproot segments from which strain PRA25rif of *Pseudomonas fluorescens* was recovered from 7-day-old pea seedlings grown at three matric potentials

	Percent of taproot segments colonized at:		
	−1 kPa	−6 kPa	−10 kPa
Root segment (distance below seed)			
0–1 cm	100[x,y]	100	100
1–2 cm	100 a	91 a	64 b
2–3 cm	62 a	23 b	7 b
3–4 cm	29 a	0 b[z]	0 b
4–5 cm	5 a	0 a	0 a
5–6 cm	0	0	0
Root length of taproot at 7 days (cm)	13.4 a	20.0 b	20.0 b

[x]Data are pooled from five experiments (−1 and −6 kPa: n = 22; −10 kPa: n = 14).
[y]Values within a row followed by the same letter are not significantly different ($P > 0.05$) according to an r × c contingency table, chi-square analysis, followed by pair-wise comparisons among the treatments.
[z]Limit of detection = 100 colony-forming units/cm root.

TABLE 4. Mean length of taproot, depth to lowest lateral root, and shoot dry weight of 7-day-old pea seedlings grown from seeds treated or not treated with strain PRA25rif of *Pseudomonas fluorescens* in soil at two matric potentials

	Characteristics of pea seedlings grown at different soil matric potentials			
	−1 kPa		−6 kPa	
	Treated seeds	Untreated seeds	Treated seeds	Untreated seeds
Taproot (cm)	14.1 a[z]	14.5 a	21.8 b	20.6 b
Deepest lateral (cm)	6.3 a	8.2 a	8.7 a	8.8 a
Shoot dry weight (mg)	33.4 a	38.0 a	28.5 a	30.1 a

[z]Values within a row followed by the same letter are not significantly different (P = 0.05) according to protected least significant difference tests. All values are means of at least five replicates. There was no significant seed treatment × matric potential interaction.

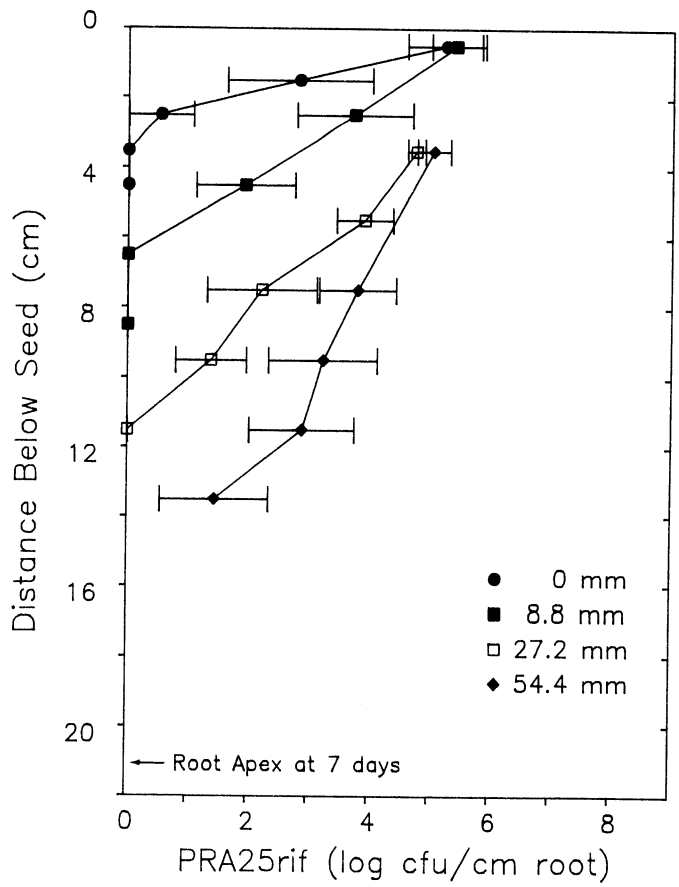

Fig. 3. Population density ($\bar{x}$ ± standard error) of strain PRA25rif of *Pseudomonas fluorescens* recovered from various locations on 7-day-old pea taproots maintained at −6 kPa, or watered on day 4 with 8.8, 27.2, or 54.4 mm and drained to −6 kPa. Average root length at 7 days, indicated by the arrow, was not significantly different among the treatments.

appear to contribute directly to further downward movement of the introduced bacterium, at least, in the absence of infiltrating water. This is clearly shown in Figure 4, where the depth of recovery of the marked strain remained unchanged from day 4 to day 7 when water was not added.

These results may be partly due to the rapid rate of root growth in our experiments. Root growth was 3.5 cm day^{-1} between 4–7 days at matric potentials of −6 and −10 kPa and averaged 2.9 cm day^{-1} over the 7-day period after planting. However, other investigators also have shown that populations of introduced bacteria remain restricted to a short distance from the source of inoculum in the absence of infiltrating water (8,18,22). Contrary to these conclusions, Weller (29) indicated that strain 2-79 of *P. fluorescens* could be detected from the entire length (7 cm) of wheat roots during the first month after planting in the field. It is not surprising that results of field experiments would be different, since soil water was not controlled. However, Bahme and Schroth (1) reported that strain A1-B of *P. fluorescens* could be recovered in small numbers from 50% of the apices of 16-cm-long potato roots in unwatered field plots. Similarly, Howie et al (15) reported that strain 2-79 of *P. fluorescens* could be recovered from wheat roots 7–9 cm from the seed in soil columns without added water. The reasons for differences in the apparent efficiency of root apices to transport rhizobacteria introduced on seeds in these different systems are not understood, but these studies involved different bacterial strains, plant hosts, soils, and temperatures.

Infiltrating water appears to redistribute bacteria from the seed or seed piece to lower parts of the root system and to renew the population on the root tip, as suggested by Bahme and Schroth (1). The population density of PRA25rif appeared to increase between days 5 and 7 on root segments 9–14 cm below the seed after 27.2 mm of water was added (Fig. 4). This may be attributed to multiplication of the bacterium, downward redistribution of the bacterium as the soil continued to slowly drain (5), or transportion of the bacterium by the reinoculated root tip. It was not possible to distinguish among these possibilities in our experiment.

It is important to note that we did not detect an overall increase in the total population density of the introduced bacterium associated with the seed and root, possibly because the population density at locations near the seed already exceeded the "carrying capacity" of the root (2). As discussed above, population density increases may occur only if the bacterial cells are transported, by percolating water or some other means, to the root tip or ahead of the root tip to colonize newly produced root tissue. Consequently, phase 1 colonization sensu Howie et al (15) appears to be restricted to a brief period immediately after seed germination. Perhaps, the concept of phase 1 colonization should be expanded to include passive transport of the introduced bacterium by any means, including roots, percolating water, and other dispersal agents.

Phenotypic characteristics of *P. fluorescens* are likely to influence its movement through soil. Although the roles of reversible sorption and permanent adhesion (19) in root colonization have not been resolved, surface charge properties of the bacterium and the plant root, and the type and quantity of soil colloids, could greatly influence dispersal with infiltrating water. Only those bacterial cells reversibly sorbed, and not those attached to roots or soil colloids by permanent adhesion, are

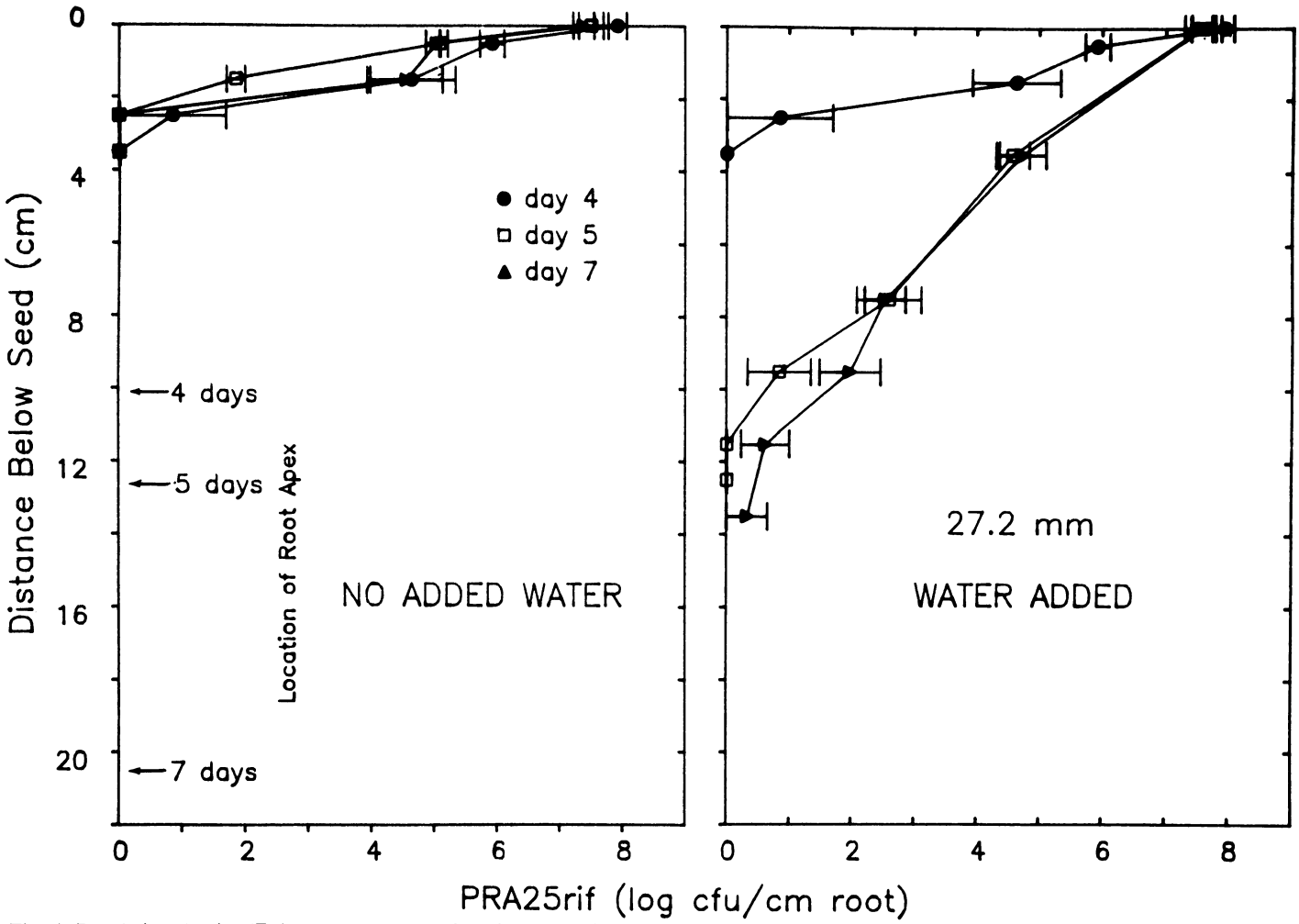

Fig. 4. Population density ($\bar{x}$ ± standard error) of strain PRA25rif of *Pseudomonas fluorescens* recovered from pea taproots grown at −6 kPa with or without the addition of 27.2 mm of water at day 4 and harvested on day 4, day 5, or day 7. Average root length for each day is indicated by the arrows. There were no significant differences in root length among the treatments with and without added water.

likely to be removed by infiltrating water (14). The ability of strain PRA25 of *P. fluorescens* to move readily with water may permit the bacterium to rapidly disperse to areas of the roots not already occupied by large numbers of bacteria. This may be an important attribute for disease control or plant growth promotion. Conceivably, however, the bacterium also could be flushed out of the root zone by heavy rainfall or irrigation.

The role of bacterial motility in root colonization by *P. fluorescens* under steady-state soil-moisture conditions has not been resolved (11,15,25). The importance of motility in combination with percolating water, similar to what would occur during rain events or irrigation, has not been evaluated. Lawrence et al (17) speculate that motility of *P. fluorescens* is important in overcoming attractions between the bacterium and solid surfaces and may be necessary for the bacterium to detach from roots or soil particles and to enter into the lanes of fast-moving water beyond the quiescent boundary layer, similar to the phenomenon described by Duniway and McKeen (13) for zoospores of *Phytophthora*. Motility under these circumstances could permit the bacterium to move into the "jet stream" and enable them to travel rapidly and over large vertical distances in biopores. To confirm this hypothesis, the rhizosphere distribution of nonmotile mutants should be compared with their motile wild-type parents in the presence of flowing water.

Within the range of steady-state matric potentials tested, −1 kPa was most favorable for colonization by strain PRA25rif of *P. fluorescens* (Table 2). This was substantially wetter than optimal conditions found for strain 2-79 of *P. fluorescens* (15,23). Strain PRA25 originally was isolated from soil from an experimental site infested with *Aphanomyces euteiches* Drechs. f. sp. *pisi* Pfender & Hagedorn Aphanomyces root rot is favored by near-saturated soil conditions (6), and a matric potential of −1 kPa in Plainfield sand is conducive to severe disease (Parke, *unpublished*). Thus, strain PRA25rif, an effective biological control agent of Aphanomyces root rot, appears to be well suited for root colonization under the same environmental conditions favorable to disease.

We have found that vertical dispersal of an introduced bacterium with percolating soil water is likely to be an important determinant of its distribution in the rhizosphere. Soil water appears to affect passive carriage as well as multiplication of the bacterium in the rhizosphere. Investigators, therefore, should take this important variable into consideration in carrying out experiments and in interpreting results on root colonization by bacteria. Timely application of irrigation water could significantly affect rhizosphere colonization by introduced bacteria and determine the success or failure of experiments on biological control or plant growth promotion.

LITERATURE CITED

1. Bahme, J. B., and Schroth, M. N. 1987. Spatial-temporal colonization patterns of a rhizobacterium on underground organs of potato. Phytopathology 77:1093-1100.
2. Bennett, R. A., and Lynch, J. M. 1981. Colonization potential of bacteria in the rhizosphere. Curr. Microbiol. 6:137-138.
3. Bitton, G., Lahav, N., and Henis, Y. 1974. Movement and retention of *Klebsiella aerogenes* in soil columns. Plant Soil 40:373-380.
4. Bowen, G. D., and Rovira, A. D. 1976. Microbial colonization of plant roots. Annu. Rev. Phytopathol. 14:121-144.
5. Bresler, E., Kemper, W. D., and Hanks, R. J. 1969. Infiltration, redistribution and subsequent evaporation of water from soil as affected by wetting rate and hysteresis. Soil Sci. Soc. Am. Proc. 33:832-847.
6. Burke, D. W., Mitchell, J. E., and Hagedorn, D. J. 1969. Selective conditions for the infection of pea seedlings by *Aphanomyces euteiches* in soil. Phytopathology 59:1670-1674.
7. Burr, T. J., and Caesar, A. 1984. Beneficial plant bacteria. CRC Crit. Rev. Plant Sci. 2:1-20.
8. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
9. Conover, W. J. 1980. Practical Nonparametric Statistics. 2nd ed. John Wiley & Sons, New York. 493 pp.
10. Dazzo, F., Smith, P., and Hubbell, D. 1973. Vertical dispersal of fecal coliforms in Scranton fine sand. Proc. Soil Crop Sci. Soc. Fla. 32:99-102.
11. De Weger, L. A., van der Vlugt, C. I. M., Wijfjes, A. H. M., Bakker, P. A. H. M., Schippers, B., and Lugtenberg, B. 1987. Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. 169:2769-2773.
12. Duniway, J. M. 1976. Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. Phytopathology 66:877-882.
13. Duniway, J. M., and McKeen, C. D. 1987. Dispersal of *Phytophthora cryptogea* zoospores in soils and glass microbeads by water flow. (Abstr.) Phytopathology 77:1744.
14. Fletcher, M., Latham, M. J., Lynch, J. M., and Rutter, P. R. 1980. The characteristics of interfaces and their role in microbial attachment. Pages 67-78 in: Microbial Adhesion to Surfaces. R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds. Ellis Horwood Ltd., Chichester, W. Sussex, UK.
15. Howie, W. J., Cook, R. J., and Weller, D. M. 1987. Effects of soil matric potential and cell motility on wheat root colonization by fluorescent pseudomonads suppressive to take-all. Phytopathology 77:286-292.
16. King, E. O, Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
17. Lawrence, J. R., Delaquis, P. J., Korber, D. R., and Caldwell, D. E. 1987. Behavior of bacterial stream populations within the hydrodynamic boundary layers of surface microenvironments. Microb. Ecol. 14:15-27.
18. Madsen, E. L., and Alexander, M. 1982. Transport of *Rhizobium* and *Pseudomonas* through soil. Soil Sci. Soc. Am. J. 46:557-560.
19. Marshall, K. C. 1980. Adsorption of microorganisms to soils and sediments. Pages 317-329 in: Adsorption of Microorganisms to Surfaces. G. Bitton and K. C. Marshall, eds. John Wiley & Sons, New York.
20. Parke, J. L. 1987. Biological control of *Aphanomyces euteiches* f. sp. *pisi* by bacteria applied to pea seeds. (Abstr.) Phytopathology 77:1688.
21. Parke, J. L. 1990. Root colonization by indigenous and introduced microorganisms. Plant Soil (In press).
22. Parke, J. L., Moen, R., Rovira, A. D., and Bowen, G. D. 1986. Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent, *Pseudomonas fluorescens*. Soil Biol. Biochem. 18:583-588.
23. Parke, J. L., Rovira, A. D., and Bowen, G. D. 1984. Soil matric potential affects colonization of wheat roots by a pseudomonad suppressive to take-all. (Abstr.) Phytopathology 74:806.
24. Roughley, R. J., and Worrall, V. S. 1984. Vertical movement of *Rhizobium trifolii* in soil. Pages 39-40 in: Proc. Aust. Conf. *Rhizobium*, 7th, Sydney.
25. Scher, F. M., Kloepper, J. W., Singleton, C., Zaleska, I., and Laliberte, M. 1988. Colonization of soybean roots by *Pseudomonas* and *Serratia* species: Relationship to bacterial motility, chemotaxis, and generation time. Phytopathology 78:1055-1059.
26. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.
27. Suslow, T. V. 1982. Role of root-colonizing bacteria in plant growth. Pages 187-223 in: Phytopathogenic Prokaryotes. Vol. 1. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
28. Vidaver, A. K. 1967. Synthetic and complex media for rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of carbon source. Appl. Microbiol. 15:1523-1524.
29. Weller, D. M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology 73:1548-1553.
30. Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
31. Wilkinson, H. T., Miller, R. D., and Millar, R. L. 1981. Infiltration of fungal and bacterial propagules in soil. Soil Sci. Soc. Am. J. 45:1034-1039.
32. Wollum, A. G., and Cassel, D. K. 1978. Transport of microorganisms in sand columns. Soil Sci. Soc. Am. J. 42:72-76.
33. Wong, P. T. W., and Griffin, D. M. 1976. Bacterial movement at high matric potentials. 1. In artificial and natural soils. Soil Biol. Biochem. 8:215-218.