

Rhizosphere Effects of Pea Seed Treatment With *Penicillium oxalicum*

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ABSTRACT

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When *Penicillium oxalicum*-treated seeds of five pea cultivars were planted in the field in 2 yr, a greater population density of *P. oxalicum* and other *Penicillium* spp. was isolated from the rhizosphere of plants grown from *P. oxalicum*-treated seeds than from the rhizospheres of plants grown from captan or untreated seeds of all cultivars; these fungi accounted for a significantly greater population of total fungi for all cultivars. Seed treatment had no effect on population densities of *Fusarium* spp. or bacteria (including actinomycetes). Numbers of rhizosphere

Additional key words: biological control.

colonies of *P. oxalicum* increased at 3, 7, and 10 days after the planting of treated seeds. *P. oxalicum* was most frequently recovered from the proximal 2.5 cm of taproot and from the secondary roots originating from this region and less frequently from distal portions of roots. Thus, application of *P. oxalicum* to seeds resulted in its recovery and in greater population densities of *Penicillium* spp., but this treatment did not affect other microorganisms in the rhizosphere.

An effective antagonist applied as a seed treatment should protect not only the seed but also the seedling roots by growing from seeds to roots or by changing the existing rhizosphere populations. Bacterial and fungal antagonists applied to seeds or tuber seed pieces were recovered from the rhizospheres of cotton (11), potato (1,6), soybean (8), sweet corn (8), and tomato (5,10). Kloepper et al (6) selected mutant strains of *Pseudomonas* spp. that colonized rhizospheres of roots emerging from treated potato seed pieces planted in the field. However, except for our brief report (19), there have been no reports on the effects of seed-applied organisms on populations of other organisms (saprophytes or pathogens) in the rhizosphere.

Penicillium oxalicum Currie and Thom was an effective seed treatment on peas (*Pisum sativum* L.) in the greenhouse and field (7,18). Also, it was recovered from the rhizosphere throughout the season (17).

Our objectives were to determine the effect of applying *P. oxalicum* to seeds on populations of fungi and bacteria in the pea rhizosphere, and the distribution of *P. oxalicum* on the pea root.

MATERIALS AND METHODS

P. oxalicum was grown on Difco Czapek Dox agar (CDA) for 4-5 wk at 23 ± 2 C, and conidia were harvested by gently scraping the colony surface with a bent glass rod or by brushing it with a

camel's hair brush. A 100-mg quantity of conidia and 200 seeds of pea were added to an Erlenmeyer flask and shaken by hand 150 times. Captan (80% WP) was applied as a dust (100 mg/200 seeds) in the same way. Untreated seeds were shaken 150 times in an autoclaved flask. The pea cultivars tested included Alaska, Green Giant 359, Little Marvel, New Era, and Perfection Dark Seeded. Harvesting of conidia and treating of seeds were done in a laminar-flow biological safety cabinet.

P. oxalicum-, captan-treated, and untreated seeds of the five pea cultivars were planted on 17 July 1978 and 6 June 1979 in a pea disease nursery. Peas had been planted in this disease nursery every year for several decades and soilborne pea pathogens there include: *Aphanomyces euteiches* Drechs., *Fusarium oxysporum* Schl. emend. Snyd. and Hans. f. sp. *pisi*, *F. solani* (Mart.) App. and Wr. emend. Snyd. and Hans. f. sp. *pisi*, *Pythium* spp., and *Rhizoctonia solani* Kühn. Fifty seeds were each planted 2.5 cm deep for each treatment of six replicates in a randomized block in both years.

Roots were collected at 2, 4, 6, and 8 wk after planting in 1978 and at 2, 4, and 6 wk after planting in 1979. Except for some variation among cultivars, these sampling times corresponded approximately to the seedling, blossom, harvest, and senescent stages, respectively. Five plants from *P. oxalicum*-, captan-treated, and untreated seeds of each cultivar were collected from each of three replicates. Plants were dug and roots were severed 1-2 mm below the point of seed attachment (to reduce exposure of roots to contamination by microorganisms colonizing the seed) and placed in a plastic bag. Nonrhizosphere soil samples were collected in 1979 with a soil sampling tube (2-cm diameter); 10 cores (6-8 cm depth) collected between pea rows were bulked for each of three replicates. Rhizosphere and soil samples were processed on the day of collection, or else stored overnight at 5 C in plastic bags or in the

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first dilution flask of each dilution series.

Two to five roots, trimmed to 7–8 cm lengths (usually about 1–1.5 g of fresh roots and adhering soil), were added to a 250 ml Erlenmeyer flask containing 100 ml of 0.12% water agar and 15–20 g glass beads and either swirled manually for 1 min (in 1978) or placed on a rotary shaker for 3 min (in 1979). Tenfold serial dilutions in 0.12% water agar were prepared with 30 sec of manual swirling between each dilution. In 1978, population densities of total fungi and *P. oxalicum* were determined by pipetting and spreading 1 ml of a diluted suspension (10^{-3} , 10^{-4} , and 10^{-5}) on the surface of 20 ml of solidified peptone-dextrose rose bengal agar (PDRBA) in 9-cm-diameter petri dishes (4). In 1979, 15–18 ml of sterile molten agar (45–50 C) was added to a petri plate containing 1 ml of a diluted soil suspension (10^{-3} and 10^{-4}) and the plate was swirled.

Population densities of *Fusarium* spp., actinomycetes, and other bacteria in the rhizosphere of the cultivar Little Marvel, and in nonrhizosphere soil were determined in 1979. Rhizosphere samples were prepared as described in the preceding paragraph. Dilutions of 10^{-2} and 10^{-3} (at 2 wk) and 10^{-4} and 10^{-5} (at 6 wk) were used for *Fusarium* spp., 10^{-5} and 10^{-6} for actinomycetes, and 10^{-7} and 10^{-8} for other bacteria. For the nonrhizosphere samples, soil was mixed for 3 min in a 500-cc vessel, and then the equivalent of 1 g oven-dry soil was diluted as previously described. Dilutions of nonrhizosphere soil were 10-fold lower than those of rhizosphere bioassays. Pentachloronitrobenzene (PCNB) agar supplemented with chlorotetracycline HCl was used to determine population densities of *Fusarium* spp. (12), chitin agar (CA) for actinomycetes (3), and soil extract agar (SEA) supplemented with PCNB for other bacteria (2). In both years, five dishes were prepared for each of the two dilutions per sample.

Dishes of PDRBA were placed in an incubator at 21–23 C, or they were covered with brown paper (to avoid exposure to light so that the rose bengal would not decompose) and incubated at about 23 C for 5–7 days. They were then stored at 8 C until colonies were counted, usually within 2 wk. The total number of fungi, *P. oxalicum*, and other *Penicillium* spp. per dish were counted. Dishes of PDRBA inoculated with a pure culture of *P. oxalicum* were used as a reference to facilitate recognition of *P. oxalicum*.

Bioassay dishes for *Fusarium* spp., actinomycetes, and other bacteria were incubated at approximately 23 C. Colonies of *Fusarium* were incubated on PCNB agar for 7–14 days and then transferred to homemade potato-dextrose agar (PDA). Cultures were identified by using the system of Snyder and Hansen (15) after at least 10 days of incubation under fluorescent lamps (12 hr light:12 hr dark). Bacterial colonies were counted after plates had been incubated for 5–7 days and the number of actinomycete colonies were determined by counting clear areas in plates of CA after incubation for 14–21 days.

The term "colony" or "colonies" used throughout this paper refers to "colony-forming units" (cfu) since it was not determined if the colonies originated from one or more propagules.

The oven-dry weights of rhizosphere and nonrhizosphere soils were determined by pipetting 10 or 50 ml of suspension from the first flask of each dilution series into an aluminum or tin foil cup. The cups were heated in an oven at 105 C for 2–3 days and weighed.

To determine the distribution of *P. oxalicum* in the rhizosphere, seeds of Little Marvel were either treated with *P. oxalicum* or not treated. Fifteen seeds were planted equidistant from each other in 13-cm-diameter clay pots containing 500 cc of soil collected from the pea disease nursery and mixed with vermiculite (9:1, v/v). Seeds were covered with 150 cc of soil, and the soil was watered; these pots (nine per treatment) were placed on a bench in a greenhouse at 21 ± 4 C. Population densities of *P. oxalicum* in the rhizosphere were determined 3, 7, and 10 days after the seeds were planted. At 3 days after planting, five seedlings from each of three replicates were removed from soil and the radicle was cut from the seed. At 7 and 10 days after planting, plants were removed from soil, the seeds were removed, and three roots of approximately equal length (10 cm at 7 days and 19 cm at 10 days) were selected from each of three replicates. The roots were divided into 2.5-cm segments from the proximal to distal end. Roots collected 10 days after planting had

secondary roots extending about 7.5 cm from the proximal end, and these were cut from each of the 2.5-cm-long root segments. Taproot segments and secondary roots removed from corresponding taproot segments were assayed for *P. oxalicum* following the same procedures outlined in the field rhizosphere studies conducted in 1978. Five dilution plates were processed from samples collected 3 and 7 days after planting and three dilution plates were processed for samples collected 10 days after planting.

To screen for pathogenicity of *F. solani* isolates collected from the rhizosphere and nonrhizosphere samples 6 wk after planting, two single-spore cultures were prepared from each of 88 isolates (two plates per isolate) and incubated on PDA for 7–10 wk at room temperature under diffuse light. Then, 20 ml of sterile distilled water was added to each of the two plates, the surface of the colony was gently scraped with a sterile bent glass rod to dislodge the spores, and 10 ml of spore suspension was pipetted from each culture and combined in a sterile glass test tube. Spore suspensions were stored at 5 C for 11–12 days. Meanwhile, seeds of pea cultivar Little Marvel were surface treated (5% NaOCl for 10 min, followed by three rinses in sterile distilled water) and planted in autoclaved sand. Seven days later, plants were removed from the sand, rinsed in sterile water, placed in sterile 100-cc screw cap jars (10 plants per jar) and 20 ml of a turbid spore suspension was added to each jar (one jar per *F. solani* isolate). Jars were covered with aluminum foil for 24 hr, the spore suspension was drained off, and 30 cc of autoclaved sand was added to cover seeds and roots. Jars were covered with glass to prevent moisture loss and set under fluorescent lamps (12 hr light:12 hr dark) for 7 days; the soil was moistened when necessary. Roots were washed and rated for root rot on a scale of 0–3; 0 = healthy roots, epicotyl and hypocotyl; 1 = slight to light brown discoloration of taproots and healthy secondary roots; 2 = taproot black but with secondary roots; and 3 = complete rooting of taproots with few or no secondary roots. Controls included seedlings treated with sterile distilled water, water washed from PDA plates, or a known isolate of *F. solani* f. sp. *pisi*.

Analyses of variance were calculated from data collected on the total number of fungi, *Penicillium* spp., and *P. oxalicum* in the rhizosphere of five pea cultivars, and for the number of *Fusarium* spp., actinomycetes, and other bacteria in the rhizospheres of cultivar Little Marvel ($P = 0.05$). Analysis of variance indicated differences in population densities of organisms for seed treatment means, but that there were usually no significant differences in population densities of organisms among cultivars for any seed treatment. Square root and log transformations did not correct for heterogeneity of error (F -tests that were not significant on nontransformed data were not significant on transformed data ($P = 0.05$)). Consequently, only seed treatment means were compared in all t -test comparisons, using nontransformed values ($P = 0.05$) (13,14). Unless indicated otherwise, populations for each sampling time were analyzed separately.

RESULTS

Rhizosphere populations from seed treatment. When *P. oxalicum* was used as a seed treatment it was always recovered from the rhizosphere of the five pea cultivars (Fig. 1). Analysis of variance showed that there were no statistical differences in *P. oxalicum* population densities among cultivars ($P = 0.05$). At each sampling date, some *P. oxalicum*-like colonies were isolated from the rhizospheres of captan-treated and untreated seed. The *P. oxalicum*-like colonies in the rhizosphere from captan-treated seeds made up 1% of the *P. oxalicum* population density from the rhizosphere of *P. oxalicum*-treated seeds in 1978 and 0.4% in 1979. In the rhizosphere of untreated seeds, *P. oxalicum*-like colonies made up 2.5% of the *P. oxalicum* population density from the rhizosphere of *P. oxalicum*-treated seeds in 1978 and 0.4% in 1979. The *P. oxalicum*-like colonies in nonrhizosphere soil made up 0.7% of the *P. oxalicum* population density of *P. oxalicum*-treated seeds in 1979. The presence of *P. oxalicum* in the rhizosphere of captan-treated and untreated seeds, and in nonrhizosphere soil, indicates that *P. oxalicum* was present in soil or that samples were

contaminated while they were being collected and processed.

When *P. oxalicum* was used as a seed treatment, the population density of other *Penicillium* spp. in the rhizosphere was the same from treated and untreated seeds at 2 wk in both years and at 4 wk in 1979 (Fig. 2). By 6 wk in both years, the population density of other *Penicillium* spp. in the rhizosphere of plants from *P. oxalicum*-treated seeds was greater than the population of *Penicillium* spp. in the rhizosphere of either captan-treated or untreated seeds.

In both seasons, 2, 4, and 6 wk after planting, the total population density of fungi per gram of oven-dry rhizosphere soil was significantly greater when *P. oxalicum* was used as a seed treatment than when seeds were treated with captan or not treated

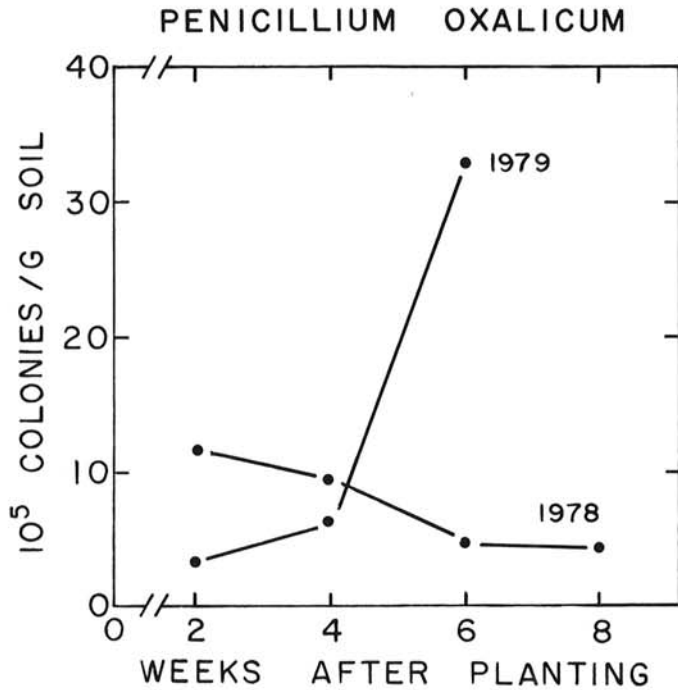


Fig. 1. Average number of colonies of *Penicillium oxalicum* per gram of oven-dry rhizosphere soil from plants grown from *P. oxalicum*-treated seeds of *Pisum sativum* planted in the field for two seasons. Each value is an average of 15 replicates (75 dilution plates).

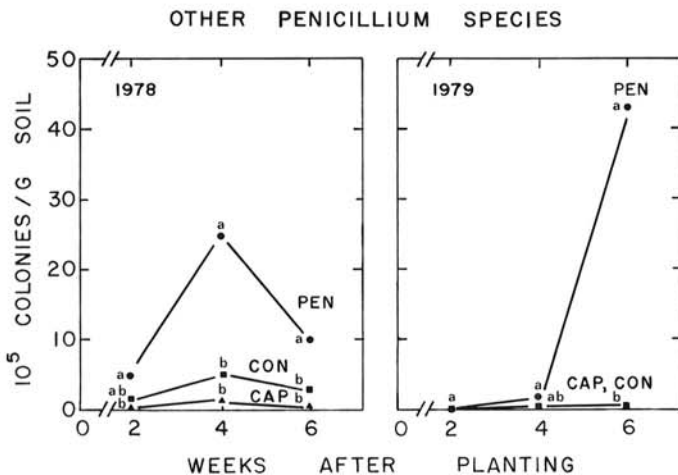


Fig. 2. Average number of colonies of *Penicillium* spp. per gram of oven-dry rhizosphere soil (excluding *P. oxalicum*) from plants grown from captan-treated, *P. oxalicum*-treated, and untreated seeds of *Pisum sativum* planted in the field for two seasons. Each value is an average of 15 replicates (75 dilution plates). Data from each sampling date were analyzed separately; means followed by the same letter are not significantly different (*t*-test, $P = 0.05$, 14 d.f.) (PEN = *P. oxalicum*, CAP = captan, CON = untreated control).

(Fig. 3A). At 8 wk after planting in 1978, there were no significant differences in the number of fungi in the rhizosphere, regardless of seed treatment. Captan- and untreated seeds yielded approximately the same number of fungi in the rhizosphere at each sampling time, except at 6 wk in 1979, when the rhizosphere population of fungi from captan-treated seeds was significantly greater than that from untreated seeds. Analyses of variance showed that for each seed treatment, there were no statistical

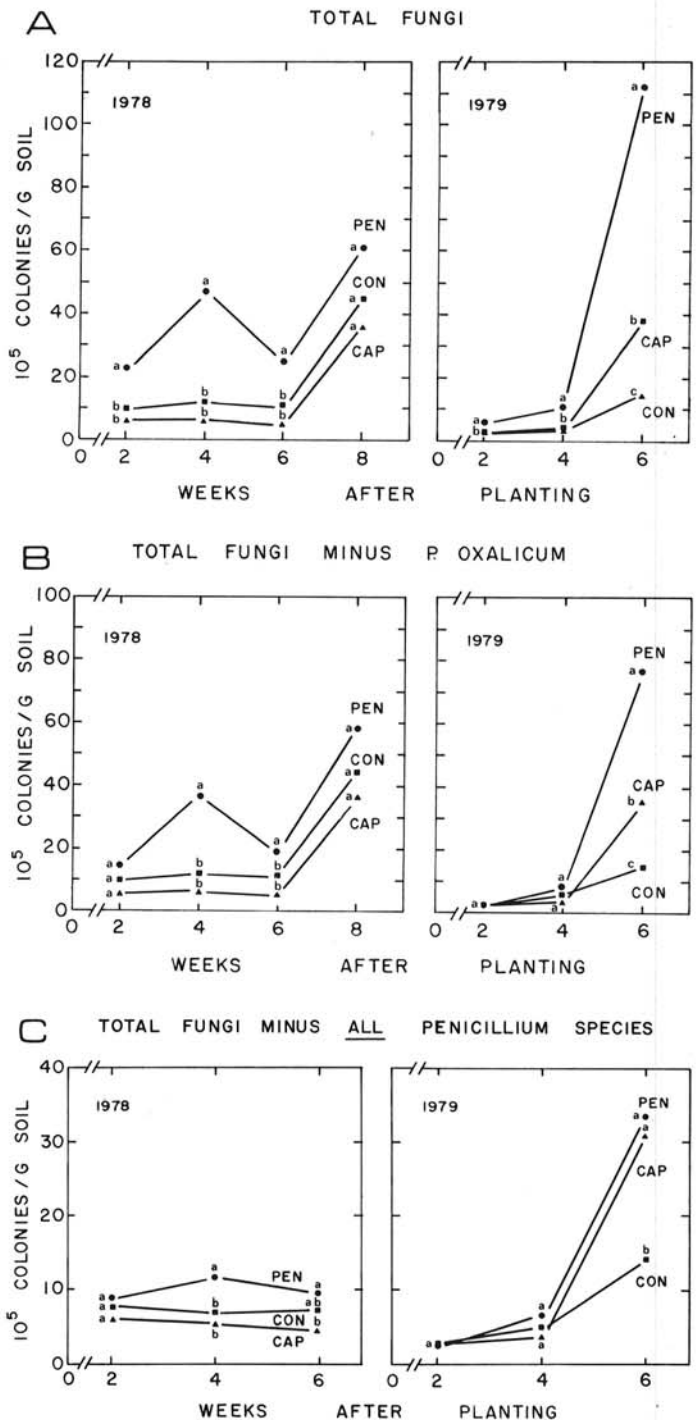


Fig. 3. Average number of colonies of A, total fungi (including *Penicillium oxalicum*), B, total fungi minus *P. oxalicum*, and C, total fungi minus *P. oxalicum* and other *Penicillium* spp. per gram oven-dry rhizosphere soil from plants grown from captan-treated, *P. oxalicum*-treated, and untreated seeds of *Pisum sativum* planted in the field for two seasons. Each value is an average of 15 replicates (75 dilution plates). Data from each sampling date were analyzed separately; means followed by the same letter are not statistically different (*t*-test, $P = 0.05$, 14 d.f.) (PEN = *P. oxalicum*, CAP = captan, CON = untreated control).

differences among cultivars at any of the sampling times ($P=0.05$).

To determine if the presence of *P. oxalicum* in the rhizosphere of plants from *P. oxalicum*-treated seeds resulted in a greater than normal rhizosphere population of fungi, the number of *P. oxalicum* colonies per gram of oven-dry rhizosphere soil was subtracted from the total number of fungi in the rhizosphere. When this was done, the population density of fungi in the rhizosphere was still significantly greater when *P. oxalicum* was used as a seed treatment at 4 and 6 wk in 1978, and at 6 wk in 1979, but seed treatment had no significant effect at other sampling times (Fig. 3B). Analyses of variance showed that there were no significant differences among cultivars for any seed treatment ($P=0.05$).

When the population density of all *Penicillium* spp. (including *P. oxalicum*) was subtracted from the total number of fungi in the rhizosphere, the number of fungi in the rhizosphere of *P. oxalicum*-treated seeds did not differ significantly from the number of fungi in the rhizosphere of at least one of the controls (captan-treated or untreated seeds) for all but one sampling time (Fig. 3C). Analyses of variance showed that there were no statistical differences among cultivars for any seed treatment ($P=0.05$).

The population density of *Fusarium* spp. in the rhizosphere 2 wk after planting was the same for all seed treatments and did not differ from the population of *Fusarium* in nonrhizosphere soil (Table 1). The *Fusarium* population comprised *F. oxysporum* (42%), *F. solani* (37%), *F. roseum* (Lk.) emend. Snyder and Hans. 'Equiseti' (16%) and *F. episphaeria* (Tode) Snyder and Hans. (5%). At 6 wk after planting, seed treatment had no effect on rhizosphere

TABLE 1. Colonies of *Fusarium* spp. in rhizosphere soil 2 wk after planting and colonies of *Fusarium* spp., *F. solani*, and *F. oxysporum* in rhizosphere soil of plants 6 wk after planting captan-treated, *Penicillium oxalicum*-treated, and untreated seeds of *Pisum sativum* 'Little Marvel' in field plots

Treatment	10^4 colonies <i>Fusarium</i> per gram oven-dry soil			
	<i>Fusarium</i> spp. ²		<i>F. solani</i> ²	<i>F. oxysporum</i> ²
	2 wk	6 wk	6 wk	6 wk
Captan	1.9 a	198.9 b	177.9 c	19.7 d
<i>P. oxalicum</i>	5.7 a	166.1 b	142.9 c	22.9 d
None	6.5 a	154.7 b	113.9 c	40.5 d
Nonrhizosphere soil	1.9 a	0.8	0.3	0.3

²Each value is the average of three replicates, five plates per replicate. Means in columns followed by the same letter are not statistically different (t -test, $P=0.05$). Nonrhizosphere soil samples at 6 wk were excluded from the statistical analysis.

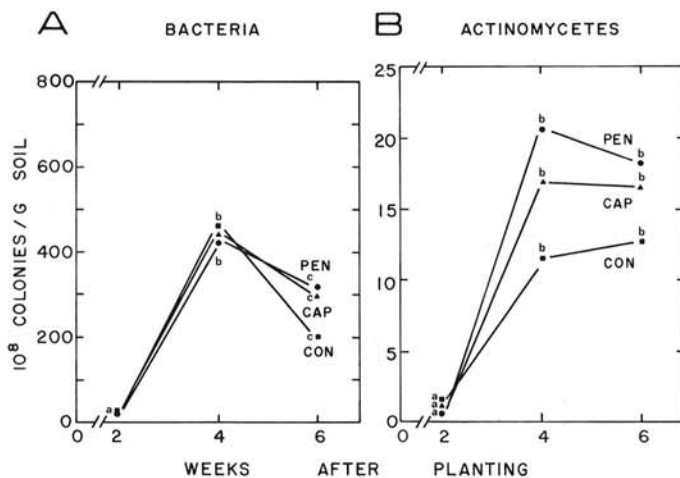


Fig. 4. Average number of colonies of A, bacteria, and B, actinomycetes per gram oven-dry rhizosphere soil from plants grown from captan-treated, *P. oxalicum*-treated, and untreated seeds of *Pisum sativum* 'Little Marvel' planted in the field. Each value is an average of three replicates (15 dilution plates); means followed by the same letter are not statistically different (t -test, $P=0.05$) (PEN = *P. oxalicum*, CAP = captan, CON = untreated control).

population densities of the total number of colonies of *Fusarium* spp., *F. solani*, or *F. oxysporum* (Table 1). At this time, *F. solani* made up 83.7% and *F. oxysporum* 16% of the total population of *Fusarium*.

When 88 of these cultures of *F. solani* were tested for pathogenicity on pea seedlings, all isolates caused some root rot and symptoms ranged from distinct lesions and light root discoloration to severe rot of the taproots and secondary roots. The water controls had no symptoms of root rot (index value of 0) and the known isolate of *F. solani* f. sp. *pisi* caused severe root rot (index value of 3). Cultures of *F. solani* from the rhizosphere of plants from captan-treated (23 isolates), *P. oxalicum*-treated (23 isolates), and untreated (21 isolates) seeds, and from nonrhizosphere soil (21 isolates) gave an average root rot index of 2.4, 2.5, 2.3, and 2, respectively.

Seed treatment had no effect on the number of bacteria in the rhizosphere at any sampling date, and population densities of bacteria in the rhizosphere were statistically greater at 4 wk than at 2 or 6 wk after planting (Fig. 4A). Nonrhizosphere population densities were always lower than rhizosphere populations of bacteria and averaged 0.5×10^8 , 1.4×10^8 , and 0.2×10^8 colonies per gram of soil at 2, 4, and 6 wk after planting, respectively.

Seed treatment had no effect on the population densities of actinomycetes in the rhizosphere at any sampling time and were significantly greater at 4 and 6 wk than at 2 wk after planting (Fig. 4B). Nonrhizosphere populations were lower than rhizosphere populations and averaged 0.5×10^7 , 1.6×10^7 , and 0.4×10^7

TABLE 2. Taproot length, *Penicillium oxalicum* colonies per gram of rhizosphere soil, and *P. oxalicum* colonies per root of plants grown from *P. oxalicum*-treated and untreated seeds of *Pisum sativum* 'Little Marvel' in the greenhouse in soil collected from a pea disease nursery

Seed treatment	Days after planting	Taproot length (cm) ^a	<i>P. oxalicum</i> ^a	
			Colonies per g soil	Colonies per root
<i>P. oxalicum</i>	3	1.5	770,000	6,300
	7	9.7	328,500	36,700
	10	18.7	978,400	291,400
None	3	1.4	400	3
	7	10	7,500	800
	10	20	6,100	1,400

^aEach value is the average of three replicates (15 dilution plates at 3 and 7 days, and nine dilution plates at 10 days); there were five plants per replicate at 3 days, and three plants per replicate at 7 and 10 days after planting.

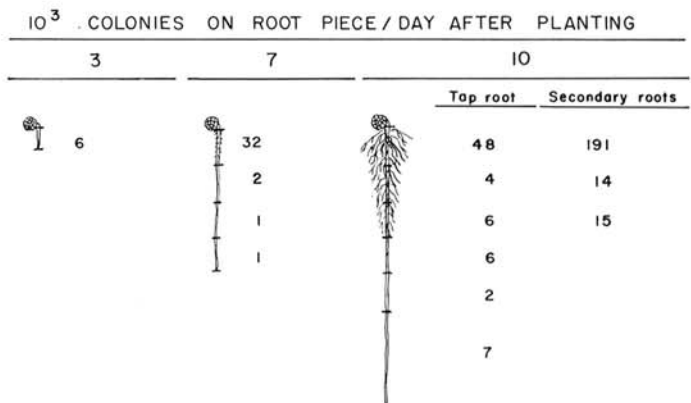


Fig. 5. Average number of colonies of *Penicillium oxalicum* per 2.5-cm root segment from plants grown from *P. oxalicum*-treated seeds of *Pisum sativum* 'Little Marvel' at 3, 7, and 10 days after planting in a greenhouse (21 ± 4 C) in soil collected from a pea disease nursery. Each value is an average of three replicates (15 dilution plates at 3 and 7 days, and nine dilution plates at 10 days); there were five plants per replicate at 3 days, and three plants per replicate at 7 and 10 days after planting.

colonies per gram of soil at 2, 4, and 6 wk after planting, respectively.

Rhizosphere distribution from *P. oxalicum* seed treatment. *P. oxalicum* was isolated from all portions of the root (primary and secondary) at 3, 7, and 10 days after seeds were planted (Fig. 5). Greatest number of colonies were isolated from the 2.5 cm of primary (tap) root proximal to the seed and on the secondary roots in that region. Also, more propagules of *P. oxalicum* were isolated in this region at each subsequent sampling time.

Calculations of number of *P. oxalicum* colonies per root showed a lower population density than the calculations of number of *P. oxalicum* colonies per gram oven-dry rhizosphere soil (Table 2). The number of colonies per gram of oven-dry soil were comparable to field-experiment estimates of *P. oxalicum* recovered from the rhizosphere of *P. oxalicum*-treated seed.

DISCUSSION

P. oxalicum was recovered from the rhizosphere of plants grown from *P. oxalicum*-treated seeds of all cultivars tested in two field seasons. In an earlier report (16), *P. oxalicum* conidia were observed microscopically on roots of plants from *P. oxalicum*-treated seeds, but no germinated conidia were observed on roots within 2 wk after seeds were planted in field soil. Recovery of *P. oxalicum* from the rhizosphere and its distribution on all portions of roots, especially nearest the seed, indicates that the seed, not the soil, is the source of inoculum, and that at least some of the observed conidia were viable. Others report that: organisms applied to seed were recovered from the rhizosphere for only a short time (5,10); in low numbers (9); were not recovered, but affected other organisms in the rhizosphere (8); or were effective colonizers of the rhizosphere (6). Whether *P. oxalicum* multiplied in the rhizosphere during the two field seasons cannot be validly interpreted from Fig. 1, because the determination of population densities in the rhizosphere is dependent upon soil moisture. Soil moisture content of nonrhizosphere samples collected in this study varied from 13 to 28% during a season, and would skew estimates of populations of *P. oxalicum* in the rhizosphere. The number of colonies per root system (Table 2) is a more realistic approach to determining populations of *P. oxalicum* than are calculations based on rhizosphere soil.

The presence of *P. oxalicum* in the rhizosphere was not associated with effectiveness of the antagonist as a root protectant. Although seed treatment with *P. oxalicum* increased seedling stand in 1 of 2 yr, at harvest in both years there were no significant differences in plant weight or yield between organism-treated or untreated seeds (*unpublished*). The ability of conidia of *P. oxalicum* to germinate, and for the fungus to sporulate on the seed coat within 3 days after the seed is planted in soil (16), suggests that the antagonist must be active at the infection court in order to act as a protectant.

Greater population densities of fungi in the rhizosphere of plants from seeds treated with *P. oxalicum* compared to population densities from rhizospheres of plants from captan-treated and untreated seeds, were accounted for by the presence of *P. oxalicum* and an increase of other *Penicillium* spp. None of the seed treatments apparently affected population densities of other groups of microorganisms in the rhizosphere. No significant differences in numbers of colonies of *Penicillium* spp. were found at 2 wk after planting regardless of seed treatment, but significantly greater population densities of *Penicillium* spp. in the rhizosphere of seeds treated with *P. oxalicum* were apparent at 4 wk and clearly at 6 wk after planting. Since populations of *Penicillium* spp. increased more following seed treatment with *P. oxalicum* than from captan-treatment, or no seed treatment,

presumably there was a delayed ecological effect of the *P. oxalicum* propagules introduced into the rhizosphere via seed treatment. Sporulating fungi such as *Penicillium* spp. are favored in soil dilution plate analyses, but this is not likely to explain the results obtained because of the relatively lower frequency of *Penicillium* spp. in the controls for each sampling date. The increased populations of *Penicillium* spp. could affect pathogens, although populations of pathogenic isolates of *F. solani* were not affected. Despite the relative imprecision of rhizosphere analyses, we conclude that *P. oxalicum* applied to seeds can be introduced into the rhizosphere where it can increase population densities of other *Penicillium* spp.

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