Rhizosphere Competence of *Pythium oligandrum*

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ABSTRACT

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The associations of *Pythium oligandrum* with the root cortex, rhizoplane, and rhizosphere were measured with 11 crop species. This work was expedited by the use of a semiselective technique for isolation of *P. oligandrum* from soil and plant material. Cortical colonization of roots by *P. oligandrum* was not detected, and the rhizoplanes of the roots of most crops were free of the fungus. However, *P. oligandrum* was detected in large quantities with every crop tested when roots with adhering soil (rhizosphere soil) were assayed. Different crop species and cultivars of cantaloupe, cauliflower, and tomato varied in rhizosphere densities of *P.*

oligandrum, but rhizosphere population densities of the fungus were consistently higher than in nonrhizosphere soils with plants grown in *P. oligandrum*-infested sterilized potting mix or an unsterilized mineral soil. After transplanting tomatoes into potting mix infested with *P. oligandrum*, increases in CFU occurred over time in the rhizosphere but not in the nonrhizosphere soil. In trials on delivery methods of inoculum of *P. oligandrum*, the rhizosphere populations of tomato plants grown in potting mix were about sixfold higher compared to seed-coat treatments when ground, alginate pelleted oospores were applied to seedlings growing in plug containers prior to transplanting or to pots containing potting mix before direct seeding.

Pythium oligandrum Drechs. was isolated originally from discolored pea roots (*Pisum sativum* L.) (5). In a detailed description of the species, Drechsler (6) reported that he isolated *P. oligandrum* from the roots of several species of plants. Indeed, the majority of isolates of *P. oligandrum* that have been studied were obtained from a variety of plant roots (18,29). However, the nature of the relationship of *P. oligandrum* with plant roots has been unclear. *P. oligandrum* generally was nonpathogenic toward seeds and roots: Drechsler (6) failed to incite disease in pea in a pathogenicity test, and Martin and Hancock (14) confirmed its nonpathogenic nature in pathogenicity tests with 12 plant species in 6 agriculturally important families. Pieczarka and Abawi (22) found that *P. oligandrum* was associated commonly with roots of snap beans (*Phaseolus vulgaris* L.), but only mild root symptoms were found in their pathogenicity tests. *P. oligandrum* generally is not recognized as a root pathogen, but there are reports that some isolates of *P. oligandrum* may have small deleterious effects on certain crop plants (11,30). If it were a pathogen, reports of diseases caused by *P. oligandrum* would be anticipated because it is widespread and occurs in relatively high densities in many agricultural soils in the Central Valley of California (13,26).

The results of several studies suggest that *P. oligandrum* may be a successful biocontrol agent of damping-off caused by species of *Pythium* and other fungi (1,14,15,17,30). To protect roots from infection by soilborne pathogens, biocontrol agents must colonize the rhizosphere or rhizoplane, triggering active defense processes in root tissues or competing directly with pathogens for space and nutrients (27). In two investigations on the colonization of alfalfa roots by *Pythium* spp., with extensive sampling over a period of several years at two field sites in the Central Valley, *P. oligandrum* was isolated infrequently from roots (7,8). Although these studies

were with one crop, it raised the obvious question of whether *P. oligandrum* has an intimate enough association with plant roots to act as an effective biocontrol agent. In support of this doubt, research by Martin and Hancock (14) showed that *P. oligandrum* was a poor colonizer of radicles of sugar beet seedlings shortly after seed germination, even though it readily colonized sugar beet seeds, protecting them from preemergence damping-off by competition for space and nutrients.

This investigation was designed to consider the *P. oligandrum*-root association in greater detail. The primary objective was to establish the relationship of *P. oligandrum* to the root systems of several agricultural crops and their cultivars by measuring fungal colonization of the root cortex and rhizoplane and propagule densities in rhizosphere and surrounding nonrhizosphere soils. It was anticipated that determining the ability of *P. oligandrum* to colonize roots in a spatial-temporal context and determining its rhizosphere competence would serve as a basis for further evaluation of its potential as a biocontrol agent of root pathogens.

MATERIALS AND METHODS

Fungi and inoculum production. The sources of isolates of *P. oligandrum* and other species of *Pythium* and *Sclerotinia sclerotiorum* (Lib.) de Bary used in this study are listed in Table 1. Stock cultures of *Pythium* spp. were stored in hemp seed-water culture at room temperature (24). *S. sclerotiorum* was stored as dry sclerotia at 4°C. Transfers were made on 1.75% water agar (WA) or 1.7% cornmeal agar (CMA; Difco Laboratories, Detroit) for *P. oligandrum* or 5.2% potato dextrose agar (PDA; Difco) for *S. sclerotiorum*.

Large quantities of *P. oligandrum* oospores were obtained by growing each isolate on a synthetic minimal medium for *P. oligandrum* (MMPO) (2). The Fe and microelement solutions were prepared by a method described by Ribeiro et al. (25). Each isolate of *P. oligandrum* was grown at 24 ± 2°C for 6 weeks in at least 4 plastic petri plates (100 × 15 mm) or 20 glass test tubes (25 × 200 mm) sealed with autoclavable caps, each inoculated with a 10-mm-diameter disk from a 4-day-old WA culture. At harvest,

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mycelia growing on plates were transferred aseptically to sterile test tubes containing 10 ml of sterile distilled H₂O. The contents of tubes were sonicated for 80 s continuously in an ice bath at 50% duty cycle (Branson Sonifier, Cell Disruptor 185, Smith-Kline Corp., Danbury, CT). Such treatment insured the breakdown of about 99% of the mycelium without causing any obvious physical harm to oospores. Oospores were washed with sterile distilled H₂O by centrifugation at 3,000 × g for 10 min. The process was repeated at least three times until all mycelial debris was removed. Concentrated oospore suspensions of each isolate were stored separately at 4°C. Oospores (and the small fraction of surviving mycelia) of each isolate were formulated into calcium alginate pellets (2).

Sclerotia were obtained by growing *S. sclerotiorum* as previously described, with a small modification (26). Three hundred grams of rolled oats was soaked in 450 ml of water overnight at 4°C and blended for 1 min at low speed in a Waring blender. Agar (30 g; Difco) was added, and the volume was brought to 2 liters with sterile distilled H₂O. After thoroughly mixing with a magnetic stirrer, the medium was dispensed into aluminum trays (30 × 50 × 5 cm) as 5-mm-deep layers (500 ml per tray). Aluminum covers over trays were sealed further with double layers of aluminum foil, and the trays were autoclaved for 30 min at 121°C and 0.1 MPa. Each tray was inoculated with at least 10 sclerotia of *S. sclerotiorum* grown on PDA, and trays were sealed in plastic bags and incubated in the dark at room temperature (24 ± 2°C) for 14 days. At harvest, the contents of trays were filtered aseptically through a layer of sterile cheesecloth, and the sclerotia retained on the cheesecloth were washed thoroughly with sterile water. Sclerotia were blotted dry on sterile filter papers and further dried in a laminar flow hood for 24 h at 25°C and 40 to 50% relative humidity (RH). Dried sclerotia were ground in a Wiley mill to pass through a 2-mm² mesh screen, and the ground material was stored at 4°C until use.

***P. oligandrum* semiselective medium (POSSM).** A CMA-based selective medium was developed for the enumeration of *P. oligandrum* in soil by modification of the 3P medium described by Ribeiro and Butler (26) and Tsao and Ocana (28). CMA was autoclaved and amended per liter with 400 mg of saponin, 25 mg of pimaricin, 50 mg of penicillin G, 50 mg of polymyxin B, 60 mg of streptomycin sulfate, and 20 mg of rose bengal. Stock cultures of saponin (20%, wt/vol) and antibiotics were sterilized by autoclaving and membrane filtering (0.2-mm² filter), respectively, and stored at 4°C until use. Antibiotics and saponin (from *Saponaria* sp.) were purchased from Sigma Chemical Co., St. Louis. Plates were stored in the dark at room temperature for no longer than 7

days before use. Immediately prior to use, ~5 to 10 mg of the ground, air-dried sclerotia of *S. sclerotiorum* was sprinkled over the surface of the solidified medium in each plate. Sclerotial lawns stimulate germination of pythiaceae mycoparasites, including *P. oligandrum* (26). POSSM amended with ground sclerotia is referred to as POSSM⁺. To count colonies of *P. oligandrum* after incubation for 24 or 48 h, soil and sclerotial particles were dislodged by rinsing under a gentle stream of tap water. Colonies of *P. oligandrum*, which normally grew within rather than on the surface of the medium, were easily observed by viewing washed plates with transmitted fluorescent light. The morphology of the colonies and the distinctive spiny oogonia were features that distinguished this mycoparasite from other species of *Pythium* that grew on this medium, which included other pythia with spiny oogonia, such as *P. periplocum* and *P. acanthicum* (29).

Effect of incubation temperature on recovery of *P. oligandrum* on POSSM. To evaluate the effect of incubation temperature on the recovery of *P. oligandrum* from soil on POSSM, a soil sample was taken from pots in which tomatoes (*Lycopersicon esculentum* Mill. 'UC 82L') were grown in UC mix infested with oospore pellets (20 pellets per pot). Three weeks after transplanting, five plants were uprooted, and samples of 100 cm³ of root-free soil per pot were pooled in a plastic bag and thoroughly mixed. Four dilutions of subsamples of this composite soil (0.25, 0.1, 0.02, and 0.01) were made in sterile distilled H₂O to a total volume of 100 ml. Soil subsamples were oven-dried at 110°C for 72 h for dry weight determinations. Soil suspensions were allowed to stand for 30 min, mixed thoroughly on a magnetic stirrer for 5 min, and aliquots of 1 ml were plated on POSSM⁺. For each soil dilution, 15 plates were used, with 5 plates incubated at 16, 25, and 33°C. After 24 h, the soil and sclerotial residues were rinsed from the surfaces of the media, and the colonies were counted and expressed as CFU per gram of oven-dried potting mix.

Selectivity of POSSM. *P. ultimum* (isolate 90-2), *P. sylvaticum* (isolate 90-7), *P. irregulare* (isolate 83-6), *P. aphanidermatum* (isolate 93-1), and *P. oligandrum* (isolate 76-13) were used in the evaluation of the selectivity of POSSM for *Pythium* spp. Inoculum of each isolate was prepared from isolate growth on autoclaved preparations of alfalfa leaves in water in test tubes. For each species, at least four tubes were inoculated with 10-mm-diameter WA disks of 4-day-old cultures. Tubes were incubated at room temperature (24 ± 2°C) for 2 weeks, the contents of the tubes were blended, and for each species, 10-ml aliquots were added to each of three pots containing UC mix. Twenty seeds of alfalfa (*Medicago sativa* L. 'Moapa 69') were sown in each pot. Pots were kept under greenhouse conditions for 1 week, after which seedlings were uprooted and the soil from the three pots for each species was pooled. Soil was mixed thoroughly in a plastic bag, and a sample was assayed. Soil suspensions were prepared by mixing 25 g wet weight of each sample in 175 ml of sterile distilled H₂O, and oven dry weights of equivalent samples were determined. The soil suspension was allowed to stand for 20 min, mixed well on a magnetic stirrer for 3 min, and 1-ml aliquots were plated either on a previously described *Pythium*-selective medium (23) or POSSM⁺, with five replicates per treatment. Soil plated on the *Pythium*-selective medium was incubated at 25°C, and soil plated on POSSM⁺ was incubated at 33°C for 48 and 24 h, respectively. Soil residues were rinsed from media surfaces, and colonies were counted and expressed as CFU per gram dry weight of potting medium.

To evaluate the selectivity of POSSM for isolation and quantification of *P. oligandrum* in mineral field soils, three loam soils were collected from agricultural fields in California and assayed. The soils were from the University of California's West Side Research and Extension Center (WSREC) at Five Points, in the San Joaquin Valley (Panoche loam); the Armstrong Research Facility at Davis, in the Sacramento Valley (Yolo loam); and a field near Soledad, in the Salinas Valley (Mocho loam). Representative col-

TABLE 1. Isolates of fungi and their sources

Species	Isolate no.	Source
<i>Pythium aphanidermatum</i>	75-6	Snap bean stem, West Side Research and Extension Center (WSREC), CA
	93-1	Tomato root, WSREC, CA
<i>P. irregulare</i>	83-6	Alfalfa root, Davis, CA
<i>P. oligandrum</i>	76-13	Snap bean root, Salinas Valley, CA
	86-21	ATCC 38472, sugar beet seedling, Berkeley, CA
	87-6	Collection of M. C. Middleton, University of California, Berkeley
	87-7	M. C. Middleton
<i>P. sylvaticum</i>	90-7	Tomato root, WSREC, CA
	91-4	Tomato root, WSREC, CA
<i>P. ultimum</i>	67-1	ATCC 32939, cotton seed, Porterville, CA
	90-2	Tomato root, WSREC, CA
<i>Sclerotinia sclerotiorum</i>	86-25	Potato stem, Tulalake, CA

onies were subcultured on WA amended with 100 mg of penicillin G per liter, subsequently cultured on autoclaved wheat leaf water, and examined microscopically. Monographs and keys by Middleton (18) and van der Plaats-Niterink (29) were used as aids in identification.

Potting mix and soil. The potting soil used in this study was UC mix (16). It was autoclaved at 121°C and 0.5 MPa for 10 h, cooled for 24 h, and reautoclaved. The field soil was Panoche loam from WSREC. Its composition was 38% sand, 39% silt, and 23% clay, with 0.85% organic matter. Its cation exchange capacity was 21 meq/100 g, and its nutrient composition (milligrams per kilogram of soil) was 8.4 NH₄-N, 55.3 NO₃-N, 55 P, and 309 K.

Plant growing conditions. Unless otherwise indicated, plastic pots (1.5 liter, 15 cm diameter, and 15 cm deep, containing 1.2 kg of air-dried potting mix or soil) were used throughout this study for growing plants. Transplants were grown in vermiculite in plastic trays (20 × 60 cm) divided into 96 cones (3 × 3 × 7 cm). Vermiculite was sterilized by autoclaving. Tomato and pepper seeds were treated with trisodium phosphate solution for 30 min, washed several times with water, and allowed to air-dry. The trisodium phosphate solution consisted of 10% Na₃PO₄·12-H₂O and 0.05% Triton X-100 and was used to inactivate seedborne tobacco mosaic virus. Plants were fertilized daily with half-strength Hoagland's solution (10) and watered twice a day. Greenhouse temperatures were 25 ± 2°C, with relative humidities ranging between 75 and 85%.

Comparison of delivery methods. To evaluate the efficiency of rhizosphere colonization of alginate formulated oospores and nonformulated oospores of *P. oligandrum*, three delivery methods were tested, including seed and transplant inoculations and direct infestations of potting mix prior to seeding.

Tomato seeds (cv. UC82L) were inoculated with nonformulated oospores by a procedure described previously, with small modifications (14). Three grams of seeds was placed in a plastic bag and shaken with 0.6 ml of 2% methyl cellulose, so each seed was coated with a film of methyl cellulose. Oospore suspensions (1 ml) of the five *P. oligandrum* isolates, containing 10⁶ oospores, were added to the seeds, and the mixture was reshaken. Treated seeds were allowed to air-dry in a laminar flow hood for 1 h at 26 ± 2°C and 40 to 50% RH. Air-dried seeds were stored until use at 4°C. For formulated oospores, oospore-alginate pellets were ground aseptically in a mortar with a pestle, and the fine powder was passed through a 0.2-mm² mesh sieve. The coating process consisted of mixing 3 g of seeds with 1 ml of 1.5% methyl cellulose in a plastic bag and adding 1 g (≈200 pellets, total of 10⁵ oospores) of the formulated oospore powder and 1 ml of sterile distilled H₂O and shaking the contents thoroughly. Treated seeds were air-dried and stored at 4°C. Treated seeds were sown in UC mix in plastic pots with eight replicates per treatment and maintained under greenhouse conditions. Autoclaved UC mix was used throughout all stages of these experiments. Several seeds were sown per pot, but seedlings were thinned to two per pot and grown for an additional 16 days. For the purpose of this experiment, day 0 was 7 days after germination, which also was the day on which seedlings of the same age were transplanted for the remaining treatments. For transplants, each cone was watered with an oospore suspension containing a mixture of the five isolates in equal proportions (10⁴ oospores) or was infested with five oospore pellets. In the potting mix test, untreated seeds were sown in transplanting trays. One week after germination, transplant seedlings were transplanted into eight pots, with two seedlings per pot, containing UC mix. For potting mix treatments, seedlings were transplanted into UC mix amended with 100 mg of oospore pellets per pot mixed into the upper 0.5 cm of soil or drenched with oospore suspensions containing 5 × 10⁴ oospores per pot.

At harvest, plants were gently uprooted from pots to preserve the small feeder roots. Soil adhering to these roots was shaken off,

and the roots from the plants from each pot were cut into 2- to 3-cm segments. Root segments from each pot were weighed and suspended in sterile distilled H₂O to a 1:8 dilution (wt/vol) in test tubes. Tubes were allowed to stand for 30 min, vortexed at high speed for 1 min, and 1-ml aliquots were pipetted onto each of five POSSM⁺ plates. Additionally, two 1-ml aliquots were pipetted onto two preweighed filter papers placed individually on aluminum trays. Filter papers were oven-dried at 110°C for 48 h, the dry weights of soil residues were determined, and these data were used to calculate the CFU per gram (inoculum density) of rhizosphere soil (RS). Root-free soil was assayed in a similar way for nonrhizosphere (NRS) populations. The experiment was performed during January through February 1994 and repeated during March through April 1994.

Distribution of *P. oligandrum* in relation to soil and root systems. Seeds of 11 plant species representing 7 plant families were sown in autoclaved UC mix and allowed to stand for 2 days (Table 2). Three pots were used per species. Oospore pellets containing a mixture of the five isolates of *P. oligandrum* in equal proportions were mixed into the surface 0.5 cm of soil at a rate of 200 mg of pellets per pot. After germination, seedlings were thinned to three plants per pot. Pots were arranged in a completely randomized design, and seedlings were grown for 20 days after germination.

At harvest, plants were gently uprooted, and roots were shaken carefully to remove adhering clumps of potting mix. Root systems for plants in a pot were treated as a unit. For each crop species, all roots were cut into 2- to 3-cm segments and mixed well to insure random, representative samples. Mixed root segments were divided into four batches of equal size. The batches of pooled root segments were labeled for further assay as follows: root-rhizosphere (R-Rs), root-rhizoplane (R-Rp), and root-cortex (R-C). The R-Rs root segments included adhering soil particles, whereas the R-Rp root segments were washed several times with tap water, soaked for 20 s in 1% sodium hexametaphosphate to dislodge soil particles that might be adhering to the root surface, rinsed several times with tap water, blotted dry on tissue paper and plated on POSSM. The R-C root segments were treated the same as those of R-Rp, but prior to blotting they were additionally disinfected for 2 min in 1% sodium hypochlorite, washed three times in sterile water, and blotted dry on sterile filter papers. The R-C segments were plated aseptically on POSSM. Root segments were aligned in a parallel pattern in five rows on the surface of plates. Nor-

TABLE 2. Crop species and cultivars used to investigate the associations between *Pythium oligandrum* and their roots and rhizospheres

Family Species	Common name	Cultivar
Leguminosae		
<i>Phaseolus vulgaris</i> L.	Common bean	Bush Plus
<i>Medicago sativa</i> L.	Alfalfa	Moapa 69
Cucurbitaceae		
<i>Cucumis melo</i> var. <i>cantalupensis</i> L.	Cantaloupe	Primo
<i>Cucumis sativus</i> L.	Cucumber	Straight Eight
Cruciferae		
<i>Rhaphanus sativus</i> L.	Radish	Scarlet Gobe
<i>Brassica oleracea</i> L.	Cauliflower	Early White
Solanaceae		
<i>Lycopersicon esculentum</i> Mill.	Tomato	Early Pack 7
<i>Capsicum annuum</i> L.	Pepper	California Wonder
Compositae		
<i>Lactuca sativa</i> L.	Lettuce	Crisp Head
Chenopodiaceae		
<i>Beta vulgaris</i> L.	Sugar beet	USH 11
Graminaceae		
<i>Triticum aestivum</i> L.	Wheat	Modoc

mally, a meter of root segments was plated on the surface of three plates. Four replicates per treatment were used. All plates were incubated at 33°C, and the colonies were counted at 24, 36, and 48 h. Colonization was expressed as the number of colonies per meter of root (CFU per meter). Assay techniques for *P. oligandrum* in RS and NRS were identical to those described in experiments on delivery methods with POSSM⁺. The experiment was carried out during March through April 1994 and repeated during June through July 1994. Identical procedures were used in the repeated experiment, except the roots were harvested 29 rather than 20 days after transplanting.

Densities of *P. oligandrum* in RS and NRS with plants grown in field soil and autoclaved UC mix. This experiment was carried out in a manner similar to the above experiment, except the seeds of each crop were sown in Panoche loam or autoclaved UC mix. Seedling weeds were removed manually from pots containing WSREC field soil. Roots of a given crop in a pot were considered to be a unit and were assayed as described above for RS. NRS samples were processed as described above. Three plates of POSSM⁺ per replicate were used, and the average number of col-

onies was used to calculate the CFU per gram of dry RS or NRS. For field soil, *P. oligandrum* colonies were counted, and confirmation of fungal identity was carried out with wheat leaf-water cultures.

Effects of cultivars on rhizosphere densities of *P. oligandrum*. We observed in the initial experiment with crop species that *P. oligandrum* rhizosphere (R-Rs or RS) populations varied markedly between species. To further assess the significance of these findings, differences in rhizosphere densities of *P. oligandrum* between cultivars of several individual crop species were investigated. The crop species selected on the basis of their support in initial experiments of high, moderate, or low R-Rs populations of *P. oligandrum* were cantaloupe, tomato, and cauliflower, respectively. Five cultivars of each crop were selected (Table 3). Seeds of each cultivar were sown in autoclaved UC mix, with five plastic pots per cultivar, and each pot was infested with 200 mg of oospore pellets as described in the experiment on crop species. Seedlings were thinned to three plants per pot, and all of the procedures described earlier were maintained. Seedlings were allowed to grow for 4 weeks postgermination. At harvest, plants were uprooted and processed as in the earlier experiment. However, only R-Rs and RS were assayed. For R-Rs, 4 m of root segments was examined per cultivar, and 10 g of root segments (including adhering soil) for RS was randomly selected and used to prepare the soil suspension. Other details of the assays were identical to those in the crop species experiment.

Dynamics of *P. oligandrum* in the rhizosphere of tomato. Three trials with tomato (cv. UC82L) were performed to assess changes in densities of CFU of *P. oligandrum* in RS and NRS over time. Seedlings were prepared by sowing seeds into a transplanting tray and thinning to one per cone. There were five rep-

TABLE 3. Cultivars of cantaloupe, tomato, and cauliflower used for studies on rhizosphere development of *Pythium oligandrum*

Plant	Cultivar
Cantaloupe	Primo
	Eastern Star
	Argonaut
	Challenger
	Columbia
Tomato	Milano
	Nahomi
	Reliant
	King's Choice
	GS20
Cauliflower	Montano
	Blizzard
	NVH1511
	Early White
	Snow Ball Y

Fig. 1. Influence of temperature on enumeration of inoculum densities of *Pythium oligandrum* with a semiselective medium. Vertical bars show the standard error of the mean.

TABLE 4. Measurement of inoculum densities of *Pythium* species in reinfested soil with selective media

<i>Pythium</i> sp.	CFU g ⁻¹ of soil ^x	
	PSM ^y	POSSM ^z
<i>P. oligandrum</i>	540 a	650 a
<i>P. irregulare</i>	270 a	0 b
<i>P. sylvaticum</i>	300 a	0 b
<i>P. ultimum</i>	420 a	0 b
<i>P. aphanidermatum</i>	360 a	340 a

^x CFU per gram of oven-dried soil. Mean values followed by the same letter in rows were not significant at $P < 0.01$ according to the Student-Newman-Keuls test.

^y *Pythium*-selective medium at 25°C.

^z *P. oligandrum* semiselective medium amended with ground sclerotia of *Sclerotinia sclerotiorum*.

TABLE 5. Quantitative measurement of *Pythium* species in raw field soils with a semiselective medium for *P. oligandrum* (POSSM)

Soil source ^w	CFU g ⁻¹ of soil ^x				
	<i>P. oli.</i>	<i>P. aph.</i>	<i>P. par.</i>	<i>P. aca.</i>	<i>P. per.</i>
West Side Research and Extension Center	104 ± 2 ^y	10 ± 4	146 ± 13	67 ± 8	75 ± 9
Davis	89 ± 5	ND ^z	13 ± 4	ND	61 ± 6
Salinas	151 ± 8	ND	ND	28 ± 3	55 ± 12

^w Soil was collected from field sites at the University of California's West Side Research and Extension Center at Five Points; the Armstrong Research Facility at Davis; and the Salinas Valley.

^x CFU per gram of oven-dried soil of each of the following fungi: *Pythium oligandrum* (*P. oli.*), *P. aphanidermatum* (*P. aph.*), *P. paroeandrum* (*P. par.*), *P. acanthicum* (*P. aca.*), and *P. periplocum* (*P. per.*).

^y Values are the means and their standard errors of results obtained from three subsamples assayed on POSSM at 33°C, based on five plates per subsample.

^z ND = not detected.

licates per sample period, and pots were arranged in a completely randomized design. Assays were performed at 3, 10, 16, and 42 days after transplanting. In the first trial, oospore suspensions (produced on MMPO and concentrated as described above) were poured at a rate of 2.5×10^5 per pot (1.2 kg of soil) into the top layer of potting mix in which a single tomato seedling (12 days old) was transplanted. For the second trial, oospore pellets were added directly to the germination tray at a rate of five pellets (i.e., total of 5 CFU) per cone ($\sim 2.5 \times 10^3$ oospores per cone, in which soil dry weight ≈ 10 g). In the third trial, the inoculum was mixed into the surface 0.5 cm of UC mix during transplanting at a rate of 200 mg of oospore pellets per pot (2.0×10^4 oospores). In all trials, inoculum was a mixture of the five isolates in equal proportions. At each time interval, plants were harvested on the basis of a pretrial, assigned random number. Procedures for harvesting and measuring inoculum densities of RS and NRS were similar to those described in previous experiments.

Statistical analysis. Results were analyzed by CoStat, version 3.0 (CoHort Software, Berkeley, CA). When suitable, linear regression or analysis of variance were performed with the least significant difference of the Student-Newman-Keuls test. Unless otherwise indicated, data from repeated experiments were pooled for statistical analyses and graphic presentations.

Fig. 2. Rhizosphere and nonrhizosphere populations of *Pythium oligandrum* after infestation of potting mix or treatment of tomato seeds and transplants with formulated oospores (FO) or nonformulated oospores (NFO). Untreated transplants were planted in the infested potting mix. Readings were made 23 days after seeding and 16 days after transplanting. $\text{LSD}_{0.01} = 212 \text{ CFU g}^{-1}$ of soil for rhizosphere soil and 35 CFU g^{-1} of soil for nonrhizosphere soil. Vertical bars show the standard error of the mean.

RESULTS

Effect of incubation temperature on isolation. The frequency of isolation of *P. oligandrum* was higher when isolation plates were incubated at 33 than at 25°C ($P < 0.001$) at the soil dilutions tested (Fig. 1). *P. oligandrum* was not detected when soil was incubated on POSSM⁺ at 16°C for 24 h.

Selectivity of POSSM⁺. *P. oligandrum* and *P. aphanidermatum* were detected with POSSM⁺ when sterile soil infested with a variety of *Pythium* species was assayed (Table 4). Other species (*P. irregulare*, *P. sylvaticum*, and *P. ultimum*) were detected with a *Pythium*-selective medium (23) but not with POSSM⁺ at 33°C. However, when natural field soils were assayed with POSSM⁺, other *Pythium* spp. were detected (Table 5). Species detected on POSSM⁺ from natural field soils included *P. oligandrum*, *P. aphanidermatum*, *P. paroecandrum*, *P. periplocum*, and *P. acanthicum*. Colonies of *P. oligandrum* were easily differentiated from other species based on their growth patterns and colony and oogonial morphology. Identities were confirmed after growth on wheat leaf-water culture according to the taxonomic criteria of van der Plaats-Niterink (29).

Comparison of delivery methods. Regardless of the delivery method, RS inoculum densities of *P. oligandrum* were significantly higher ($P < 0.001$) when formulated oospores were used than when nonformulated oospores were used (Fig. 2). RS populations were significantly higher ($P < 0.001$) when nonformulated oospore inocula were delivered directly into the potting mix than when inocula were delivered with transplants or seeds, but transplants supported equivalent increases in inoculum densities in the rhizosphere when formulated oospores were applied. Seed coatings were the least effective delivery method, with population growth 12% of that achieved by either transplants or potting mixtures with formulated oospore inoculum (Fig. 2). Formulated oospore delivery with either transplants or potting mixtures allowed the highest densities of CFU in RS and NRS.

Spatial distribution of *P. oligandrum* in relation to root systems. The highest amounts of *P. oligandrum* were found in the R-Rs of the crops tested ($P < 0.001$) (Fig. 3). Values obtained on the basis of CFU per meter of root and CFU per gram of RS for all crops varied similarly (Fig. 3 and Table 6). R-C colonization by *P. oligandrum* was not detected with the crops tested in this investigation (Fig. 3). R-Rp colonization by *P. oligandrum* also was not detected with most crops. However, low degrees of R-Rp colonization with alfalfa, cucumber, radish, and sugar beet were found, but the CFU per meter of root did not exceed 5 to 10% of the

Fig. 3. *Pythium oligandrum* inoculum densities (CFU 100 cm $^{-1}$ of root) associated with the root-cortex, root-rhizoplane, and root-rhizosphere of 11 plant species grown in potting mix (UC mix) under greenhouse conditions for 21 days.

rhizosphere populations of these crops (Fig. 3). There were no significant differences between *P. oligandrum* densities in the rhizoplane and the root cortex in the crops examined ($P > 0.05$).

The rhizosphere densities of *P. oligandrum* varied greatly between crops, such as alfalfa, cantaloupe, beans, and wheat, with high densities of CFU in R-Rs or RS, and cauliflower, with low rhizosphere densities (Fig. 3 and Table 6). The inoculum densities of *P. oligandrum* in the NRS were always less than its RS population with all crops. The ratio of RS/NRS varied between crops and between experiments but was always higher than 1.0 (Table 6). In many cases, the ratio was ~2.5, but ratios of up to 40 were measured (Table 6).

Inoculum densities of *P. oligandrum* in the rhizosphere and nonrhizosphere in UC mix and soil. There were highly significant differences between *P. oligandrum* inoculum densities in the rhizosphere of UC mix and soil, with a mean density in the autoclaved UC mix more than three times that measured in soil (Fig. 4 and Table 7). The RS inoculum densities of *P. oligandrum* were significantly higher ($P < 0.001$) than the NRS inoculum densities regardless of crop or soil. Variation of *P. oligandrum* RS inoculum densities between crops was observed. With most crops, the RS/NRS ratios were similar in UC mix and field soil (Table 7). Ratios of RS/NRS were between 2.5 and 22 for crops grown in autoclaved UC mix and 1.1 to 8.3 for crops grown in field soil. However, when the inoculum densities in RS for UC mix were divided by densities in RS for WSREC soil, the value was usually between 4 and 20 (Table 7).

Cultivar impact on *P. oligandrum* RS densities. RS inoculum densities of *P. oligandrum* were significantly different among cantaloupe, tomato, and cauliflower (Fig. 5). No significant differences were detected between experiments ($P > 0.05$). *P. oligandrum* RS inoculum densities varied significantly among cultivars of the same crop. The majority of the cantaloupe cultivars had the highest *P. oligandrum* RS inoculum densities, whereas those of tomato usually had the lowest RS inoculum densities (except for Nahomi), whereas cauliflower had intermediate RS

inoculum densities. The two methods (CFU per gram of soil and CFU per meter of root) of evaluating rhizosphere populations of *P. oligandrum* gave similar results with different cultivars (Fig. 5).

***P. oligandrum* population dynamics.** The inoculum densities of *P. oligandrum* in the rhizosphere of tomato increased sharply during the initial 2 weeks after transplanting, followed by a stationary phase for the following 2.5-week period (Fig. 6). A slight decline was noted in the third trial between day 16 and 42. Second-degree polynomial curve fits of rhizosphere data from the three trials yielded R^2 values of 0.991, 0.987, and 0.825 for trials 1 through 3, respectively, and the rates of increase were 313, 311, and 295 CFU per day for the three trials, respectively. However, the rates of change during the first 3 days were 11, 85, and 347 CFU per day for the three trials, perhaps reflecting variation between the types of inoculum used and the methods of delivery (nonformulated oospores delivered through UC mix, formulated oospores delivered through transplants, and formulated oospores delivered through UC mix, respectively). Yet, except for differences in their initial rate, the RS population growth curves were the same for the three trials, with no significant differences between trials. The NRS population curves indicate that an initial decline occurred to a minimum level by day 10, after which a steady population increase occurred to a stationary phase in all

TABLE 6. Populations of *Pythium oligandrum* in the rhizospheres and nonrhizospheres of agricultural crops

Crop ^v	CFU g ⁻¹ of soil ^w					
	RS		NRS		RS/NRS ^x	
	Exp. 1 ^y	Exp. 2 ^y	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Bean	8,930	1,510	3,440	210	2.6	7.3
Alfalfa	7,950	2,380	1,230	260	6.5	9.0
Cantaloupe	8,350	2,090	2,910	360	2.9	5.8
Cucumber	5,690	545	1,990	190	2.9	2.8
Radish	3,200	1,020	2,240	400	1.4	2.5
Cauliflower	700	220	560	54	1.3	4.0
Tomato	1,030	1,890	630	370	1.6	5.2
Pepper	5,840	960	740	44	7.9	22
Lettuce	1,680	1,055	42	196	40	5.4
Sugar beet	490	2,440	240	336	2.0	7.2
Wheat	NT ^z	1,730	NT	163	NT	10.6

^v Both experiment 1 (Exp. 1) and experiment 2 (Exp. 2) were carried out in autoclaved UC mix under greenhouse conditions. Exp. 1 was harvested 20 days after germination, and Exp. 2 was harvested 29 days after germination.

^w CFU per gram of dry rhizosphere soil (RS) or nonrhizosphere soil (NRS) incubated on a semiselective medium for *P. oligandrum* at 33°C for 24 h.

^x RS/NRS is the ratio of inoculum densities of *P. oligandrum* between rhizospheres and nonrhizospheres for each crop.

^y The *F* value in experiment 1 was 27.6 between crops (columns) and 36.5 between the RS and NRS (rows) of a given crop. The least significant difference (LSD) values were 1,470 and 770 CFU g⁻¹, respectively, for these two comparisons. The *F* value in experiment 2 was 23.2 between crops (columns) and 62.3 between the RS and NRS (rows) of a given crop. The LSD values were 248 and 104 CFU g⁻¹, respectively. *F* values were calculated according to two-way analysis of variance at $P < 0.001$, and the LSD was calculated according to the Student-Newman-Keuls test at the same *P* value.

^z NT = not tested.

Fig. 4. Rhizosphere (Rs) and nonrhizosphere (NR) soil populations of *Pythium oligandrum* of 11 crop plants grown in sterilized potting mix (UC mix) and field soil (Panoche loam) for 21 days. Vertical bars show the standard error of the mean.

Fig. 5. Rhizosphere inoculum densities of *Pythium oligandrum* with five cultivars each of cauliflower, tomato, and cantaloupe. The least significant difference was 600 CFU g⁻¹ of rhizosphere soil and 8.5 CFU m⁻¹ of root rhizosphere at $P < 0.05$ according to the Student-Newman-Keuls test. Vertical bars show the standard error of the mean.

trials. Parallel declines were observed between RS and NRS populations in the third trial (Fig. 6).

DISCUSSION

The results of this study established that *P. oligandrum* colonized the rhizosphere of 11 crop species in unsterilized field soil and

Fig. 6. Population dynamics of *Pythium oligandrum* in rhizosphere and nonrhizosphere soil of tomato (cv. UC82L) grown in sterilized UC mix under greenhouse conditions.

TABLE 7. Rhizosphere and nonrhizosphere populations of *Pythium oligandrum* on agriculturally important crops grown either in field soil (from the University of California's West Side Research and Extension Center [WSREC]) or autoclaved UC mix under greenhouse conditions^w

Crop	CFU g ⁻¹ of soil ^x				RS/NRS ^y		UC mix/ RS WSREC/RS ^y
	RS		NRS		UC mix	WSREC	
	UC mix ^z	WSREC ^z	UC mix	WSREC			
Bean	1,510	145	210	24	7.3	6.2	10.4
Alfalfa	2,380	145	260	18	9.0	8.3	16.5
Cucumber	545	131	190	37	2.8	3.6	4.16
Radish	1,020	131	400	46	2.5	2.9	7.78
Cauliflower	220	35	54	31	4.0	1.1	6.33
Tomato	1,890	69	370	29	5.2	2.4	27.4
Pepper	960	48	44	25	22	1.9	20
Lettuce	1,055	55	196	28	5.4	2.0	19.1
Sugar beet	2,440	35	336	21	7.2	1.6	69.6
Wheat	1,730	222	163	36	10.6	6.2	7.78

^w Inoculum was 200 mg of oospore pellets per pot (40 pellets per 1.2 kg of soil, i.e., 0.03 CFU g⁻¹ of soil) in sterilized soil and 9.03 CFU g⁻¹ in field soil, which included the indigenous inoculum of *P. oligandrum*.

^x CFU per gram of dry rhizosphere soil (RS) or nonrhizosphere soil (NRS) incubated on a semiselective medium for *P. oligandrum* at 33°C for 24 h.

^y RS/NRS is the ratio of inoculum densities of *P. oligandrum* between RS and NRS for different crops and soils. UC mix/RS WSREC/RS is the ratio of inoculum densities of *P. oligandrum* in the rhizosphere between UC mix and WSREC soil.

^z The least significant difference according to the Student-Newman-Keuls test at $P < 0.01$ for comparisons between crops was 62.4, between RS and NRS was 26.6, and between UC mix and WSREC was 26.6.

sterilized potting mix and that crop species and their cultivars varied widely in their rhizosphere populations of *P. oligandrum*. We found also that the method of delivery of oospores affected the success of the fungus in colonizing the rhizosphere. The ability of the fungus to actively colonize the rhizosphere was demonstrated by the progressive increase of inoculum densities in the rhizosphere of tomato over a period of 6 weeks in infested soil, which did not occur in NRS.

The variation between cultivars of the same crop in rhizosphere populations of *P. oligandrum* suggests that underlying genetic differences in plants affect rhizosphere population sizes. Studies by Neal et al. (19) attributed variation in rhizosphere microbial populations of two wheat cultivars to genetic differences. Rhizosphere competence is a trait of the microbe, but the extent of rhizosphere colonization by a given microbe also depends on host traits (20). The extent of development of beneficial microbes in the rhizosphere of different cultivars should be assessed and carefully considered in evaluating their effects on disease control and other beneficial activities. In the future, selection of genetic traits of plants that favor rhizosphere colonization by selected antagonistic biologicals will be viewed as an important component of biological control (9,20).

The qualitative and quantitative nature of microbial populations in the rhizosphere are related directly or indirectly to the rhizodepositions that include root exudates (4,12,32,33). The variation between agricultural crops in their *P. oligandrum* rhizosphere populations observed in this study may be related to the rhizodepositions, especially the water-soluble exudates. Root exudate quality and quantity are affected by many factors, including both crop species and cultivars, plant growth stage, and physical and chemical environments under which plants are grown (21,33). In a preliminary trial, when roots of tomato transplants were either wounded by pruning or not wounded before being transplanted into sterile UC mix infested with *P. oligandrum*, rhizosphere and nonrhizosphere population densities were significantly higher with wounded roots than with nonwounded roots (A. K. Rawahi, unpublished data). Although this suggests that a positive correlation exists between the quantity of exudates and *P. oligandrum* populations, these observations are limited, and the mechanisms underlying species and cultivar differences found in rhizosphere populations of *P. oligandrum* await further experimentation.

Because *P. oligandrum* is a mycoparasite, it may act as a parasite of other fungi in the rhizosphere (30). However, we found that this fungus colonized the rhizosphere in a sterilized potting mix, which indicates it is capable of growth in the rhizosphere in the absence of other fungi. Although its degree of rhizosphere development was less in raw soil than in UC mix, the ability of *P. oligandrum* to flourish in this habitat shows that it also can compete successfully for nutrients with native soil microflora.

P. oligandrum rarely was isolated from feeder roots of alfalfa in previous field studies (7,8); therefore, it was not surprising that it was a poor colonizer (R-C and R-Rp) of alfalfa roots, as well as roots of other crops, in this investigation. Nevertheless, these findings conflict with reports that imply that this fungus is a root parasite. Drechsler (6) concluded that the isolation of *P. oligandrum* from root lesions represented "an association with congeneric species familiar as causal agents of these diseases," indicating he regarded *P. oligandrum* as a secondary root colonizer. In earlier work, Hancock found this fungus occasionally associated with *P. ultimum* in stem lesions in snap bean but was unable to demonstrate its pathogenicity in greenhouse trials (J. G. Hancock, unpublished data). Based on our knowledge of the life history of *P. oligandrum* learned over the last two decades, it could be anticipated that this fungus would be present in lesions as a mycoparasite of plant-pathogenic fungi rather than as a primary pathogen (3,13,30,31). Indeed, because of its success as a rhizosphere inhabitant and its unusual biology as both a nonspecialized myco-

parasite and a microbial competitor, *P. oligandrum* must be regarded as a strong candidate as a biocontrol agent for root as well as seedling diseases.

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