# The Use of *Pythium oligandrum* for Biological Control
## of Preemergence Damping-Off Caused by *P. ultimum*

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## ABSTRACT

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Coating sugar beet seeds with oospores of *Pythium oligandrum* controlled preemergence damping-off caused by *P. ultimum* as effectively as treating seeds with fenaminosulf. Twenty-four hours after seeds were planted in field soil naturally infested with 13 propagules of *P. ultimum* per gram of soil, the pathogen had colonized the seed coats of 77% of the untreated seeds but only 10% of the seeds treated with *P. oligandrum*. Endosperm and radicle colonization by *P. ultimum* was also lower for treated seeds (3 and 7%, respectively) than for untreated seeds (63 and 71%, respectively). *P. oligandrum* actively colonized the endosperms and emerging radicles when seeds were treated with oospores (69 and 23% of radicle length, respectively). *P. oligandrum* was isolated from seedling roots predominantly at the junction between the primary root and the hypocotyl and rarely from secondary roots. Scanning electron microscopy confirmed the sites of colonization. Oospore seed treatment did not control postemergence damping-off as well as fenaminosulf seed treatment but did allow greater stands than untreated seeds. Seed treatment did not affect seedling growth or influence the frequency of root infection by *P. ultimum*. When seeds were planted in fumigated soil, however, the root length densities of seedlings from treated seeds were lower than those from untreated seeds. No tissue necrosis or reduction in shoot growth was observed. Amending soils with low propagule densities of *P. oligandrum* also reduced the incidence of preemergence damping-off caused by *P. ultimum*. When coated on the seed surface, *P. oligandrum* was nonpathogenic on 12 species of economic crop plants representing six families.

In the initial description of the species, Drechsler (12) observed that *Pythium oligandrum* Drechsler was commonly isolated from root lesions, but because it did not produce disease symptoms when inoculated singly, he considered it a secondary invader of diseased tissue that might be parasitic on other fungi. When *P. oligandrum* is grown in dual culture, the hyphae coil around and occasionally penetrate the mycelia of other *Pythium* spp., apparently parasitizing them (11). Tribe (33) reported that *P. oligandrum* grew in close association with cellulolytic fungi when grown on cellulose as a substrate but did not observe its mycoparasitic activity; because *P. oligandrum* cannot utilize cellulose as a carbon source, he postulated it was able to utilize nutrients released from the enzymatic degradation of the cellulose by the cellulolytic fungi. Deacon (8) subsequently demonstrated the ability of *P. oligandrum* to derive nutrients from a number of fungi in dual culture, including many phytopathogens such as *P. ultimum* Trow. The mycoparasitic nature of *P. oligandrum* in culture was confirmed by Vesely (34,35), Al-Hamdani (2), and Lutchmeah and Cooke (18).

In the soil, *P. oligandrum* occupies an ecological niche of an aggressive primary colonizing sugar fungus (sensu Garrett) (13) and is able to compete with *P. ultimum* for saprophytic colonization of substrate (22,23). Prior colonization of crop debris by *P. oligandrum* prevents subsequent colonization by *P. ultimum*. Because *P. ultimum* may depend on the saprophytic phase of its life cycle for the maintenance of soil inoculum (14), factors that reduce saprophytic activity prevent inoculum increases and lower inoculum potential in the soil (14). Saprophytic competition contributes to soil suppressiveness to *P. ultimum* in the San Joaquin Valley of California (22,23).

Deacon (8) initially investigated utilizing *P. oligandrum* as a biological agent for the control of damping-off of wheat and reported reduced incidences of disease with mycelial seed coatings. Vesely (36) observed that application of oospore suspensions to sugar beet (*Beta vulgaris* L.) seed reduced the incidence of damping-off compared with that of untreated seed, with the degree of control approaching that afforded by thiram treatment. Al-Hamdani et al (3) also observed reductions in the incidence of cress (*Lepidium sativum* L.) damping-off when seeds were pelleted with either culture homogenates or cultures of *P. oligandrum* grown on vermiculite. Lutchmeah and Cooke (19) reported a method for mass production of inoculum of *P. oligandrum*. When pelleted on the seed surface, it protected from damping-off of sugar beet and cress caused by *P. ultimum* and damping-off of carrot (*Daucus carota* L.) caused by *Mycocentrospora acerina* (Hartig) Deighton. None of these experiments investigated the colonization of seed or plant parts by *P. oligandrum* or its interactions with *P. ultimum*. All investigators found no evidence of higher plant parasitism by *P. oligandrum*.

In view of the involvement of *P. oligandrum* in soils naturally suppressive to *P. ultimum*, its ability to act as a mycoparasite in culture, and its previous success as a biological control agent, we investigated the effectiveness of *P. oligandrum* from suppressive soils in California for the biological control of damping-off. We also addressed the interactions between the biocontrol agent and the pathogen, *P. ultimum*. A preliminary report has been published (21).

## MATERIALS AND METHODS

**Soil preparation.** An Oceano loamy sand from Moss Landing, CA, naturally infested with *P. ultimum* was used as a growth medium in all greenhouse tests. After field collection from the top 12 cm of the furrow shoulder, the soil was air-dried, ground to pass a 1-mm-mesh sieve, and stored in polyethylene plastic bags at room temperature until used. Inoculum densities of *P. ultimum* were determined by the soil drop assay method (30) before use and expressed as germinable propagules per gram of air-dried soil.

When noninfested soil was needed, field soil was fumigated by mixing 15 kg of soil in a portable cement mixer and simultaneously spraying with 1 L of a metam-sodium solution (12 ml of Vapam [33% a.i.] per liter of $H_2O$). After the fumigant was applied, the soil was incubated in plastic bags for 5 days and subsequently aerated

on a greenhouse bench for at least 2 wk before use.

**Inoculum preparation of *P. oligandrum* and seed treatment.** *P. oligandrum* was grown on oatmeal agar-water slants (14) at room temperature for 3–4 wk before the mycelia and oospores were harvested from the water surface. Cultures were homogenized in a blender for 1 min, and oospores were separated from mycelial debris by centrifugation at 5,000 *g* for 10 min. The pellet was resuspended in distilled water, mixed, and recentrifuged. This washing procedure was performed three times. After the final rinse, the oospores were suspended in a small quantity of water and densities were measured with a hemacytometer. Appropriate dilutions of oospores with distilled water were made to give $10^6$ thick-walled oospores per milliliter in a total volume of 5 ml. When methyl cellulose was part of the seed treatment, the oospore suspension was diluted with 1.5 ml of 2% methyl cellulose and additional distilled water to bring the volume to 5 ml. The oospore suspension was mixed with 4 g of nondecorticated sugar beet seeds (USDA breeding line V137 H8, Salinas, CA) in a plastic bag. (With this level of treatment, roughly 12,500 oospores are applied to each seed. Germination of oospores from 3- to 4-wk-old cultures of this particular isolate of *P. oligandrum* is about 5% on cornmeal agar [*unpublished*], so approximately 625 oospores are germinable per seed.) After treatment, the seeds were allowed to air-dry for 1 hr, then were sealed in plastic bags and stored at 4 C.

For control treatments, seeds were treated singly with either distilled water or the appropriate dilution of methyl cellulose. Sugar beet seeds were coated with fenaminosulf (Lesan 35WP) by placing 4 g of seeds in a plastic bag and adding 0.01 g of fenaminosulf with four drops of distilled water. The seeds and fungicide were mixed well before being stored at 4 C.

**Soil infestation with *P. oligandrum*.** Propagule densities of *P. oligandrum* were adjusted in field and fumigated soil by amendment with autoclaved soil that had been reinfested by adding culture-grown oospores of *P. oligandrum* isolated as previously described and moistening the soil to field capacity. After air-drying, the soil was ground to pass a 0.5-mm-mesh sieve and stored at 4 C until used. Propagule densities of *P. oligandrum* were determined by plating on a differential medium (23) before use and were expressed as propagules per gram of soil.

**Greenhouse trials.** Greenhouse trials were used to evaluate the effectiveness of seed treatments with *P. oligandrum* on controlling damping-off of sugar beet caused by *P. ultimum*. Plastic pots (8-cm diam.) containing 200 g of naturally infested field soil were each planted with 20 sugar beet seeds. Five replicate pots were planted per treatment, and all tests were conducted in a temperature-controlled greenhouse chamber at 21 C. Emergence and percentage of surviving plants were recorded on alternate days until harvest 18 days after planting. Shoots were then excised at the soil line and weighed immediately. Four random soil samples (exclusive of seedlings) per pot were taken with a No. 9 cork borer (16-mm diam.), and rootlets were collected by wet sieving, then plated on 2% water agar. On the basis of colony morphology, the number of colonies and length of roots colonized by *P. ultimum* and *P. oligandrum* were measured after 24 hr. Root density was determined by the Newman line intersect method (26). This experiment was repeated four times.

Pathogenicity tests on a variety of economic crops (Table 1) were conducted by coating seeds with oospores of *P. oligandrum* as previously described and planting the seeds in fumigated Oceano loamy sand soil. Trials were conducted as described above except that shoot weights were not measured. Seedlings were harvested after 17 days, rated for presence of root necrosis, and plated on 2% water agar to determine if *P. oligandrum* could be recovered from the roots. Treated seeds that were not used in this trial were plated on 2% water agar to ensure the presence of viable oospores on the seed surface. Seeds were allowed to germinate, and pathogenicity of *P. oligandrum* on seedlings in culture was rated. With the exception of sugar beet, both pathogenicity tests were conducted once with five replicates.

The influence of increased propagule densities of *P. oligandrum* in the soil on root infection by *P. ultimum* was investigated by amending field soil naturally infested with *P. ultimum* with

autoclaved soil reinfested with oospores of *P. oligandrum*. Twenty untreated sugar beet seeds were planted in 200 g of soil with five replicate pots per treatment as previously described. Pots were placed in plastic saucers to prevent the leaching of salts and amended with 0.85 meq of $Cl^-$ / 100 g of soil by a single irrigation with 1.3 ml of 1.4 M $CaCl_2$.

**Laboratory investigations.** To study the colonization of sugar beet seed coats and endosperm by *Pythium* spp., seeds coated with *P. oligandrum* and untreated seeds were planted in field soil naturally infested with 13 propagules of *P. ultimum* per gram of soil. Ten seeds were planted in 50 g of soil and placed in a 60-mm-diameter brass ring on a $-0.5$ bar ceramic tension plate. Soil matric potential was adjusted to $-0.1$ bar with a pressure plate extractor (23). After 12 hr of equilibration, the soil was removed and placed in a moist chamber (plastic bag with moist paper towels) and exposed to diurnal light fluctuations at room temperature (about 21 C). At 24-hr intervals, triplicate samples of each seed treatment were removed and the seeds recovered by wet sieving. Seed coats and aseptically isolated endosperms were plated on 2% water agar and subsequent frequencies of colonization recorded. Identification of isolated *Pythium* spp. was aided by plating on oatmeal agar-water slants and directly viewing reproductive structures in water with a compound microscope.

**Scanning electron microscopy.** Sugar beet seeds either untreated or treated with oospores of *P. oligandrum* were planted in field soil naturally infested with *P. ultimum* as outlined above. Seeds were collected daily from the soil by gentle wet sieving and either plated on water agar as previously outlined to identify sites of fungal colonization or fixed by a modified osmium-thiocarbohydrazide-osmium procedure (25). Seeds were fixed in 1% osmium textraoxide to which Kodak Photo-Flo 600 (1:600 dilution) had been added at a rate of 0.1 ml/5 ml of osmium tetraoxide. After 30 min, seeds were rinsed in three changes of distilled water for 15 min and treated with a saturated solution of thiocarbohydrazide for an additional 30 min. This was followed by another triple rinse in distilled water and a second fixation in osmium. After the final distilled water rinse, the seeds were frozen in liquid nitrogen and lyophilized. Samples were coated with platinum and stored in a desiccator under vacuum until viewed on an International Scientific Instruments Super II scanning electron microscope. This experiment was conducted at the same time as the experiment on seed colonization and was repeated twice.

TABLE 1. Economic crop plants on which *Pythium oligandrum* was nonpathogenic[a]

| Family<br>Species | Common name | Cultivar |
|---|---|---|
| Leguminosae | | |
| *Pisum sativum* L. | Pea | Little Marvel |
| *Phaseolus vulgaris* L. | Red kidney bean | ⋯ |
| *Glycine max* (L.) Merr. | Soybean | Flambeau |
| *Medicago sativa* L. | Alfalfa | Lahonton |
| Cucurbitaceae | | |
| *Citrullus vulgaris* Schrad. | Watermelon | Stone Mountain |
| *Cucumis sativus* L. | Cucumber | Poinsett 76 |
| *Cucumis melo*<br>var. *cantalupensis* L. | Cantaloupe | Hale's<br>Jumbo Best |
| *Cucurbita pepo* var. *medullosa* L. | Zucchini | Black Beauty |
| Cruciferae | | |
| *Raphanus sativus* L. | Radish | Comet |
| Solanaceae | | |
| *Lycopersicon esculentum* Mill. | Tomato | Marrieta |
| Compositae | | |
| *Lactuca sativa* L. | Lettuce | Great Lakes 188 |
| Chenopodiaceae | | |
| *Beta vulgaris* L. | Sugar beet | USDA breeding<br>line V137 H8 |

[a] Seed coats were treated with $10^6$ oospores of *P. oligandrum* per milliliter of $H_2O$, and data were recorded 17 days after seeds were planted in Oceano loamy sand fumigated with metam-sodium (12 ml of Vapam [33% a.i.] per liter of $H_2O$) and grown at 21 C.

## RESULTS

**Influence of seed treatment with *P. oligandrum* on damping-off and root infection by *P. ultimum*.** Coating sugar beet seeds with *P. oligandrum* significantly improved seedling emergence over untreated seeds and protected seedlings from preemergence damping-off as effectively as fenaminosulf seed treatment (Table 2). Survival of seedlings 18 days after planting, however, was significantly greater for seeds treated with fenaminosulf than for those treated with *P. oligandrum*. Seed treatment with *P. oligandrum* provided some protection from postemergence damping-off and allowed greater survival than that observed for untreated controls. No difference in disease control was apparent between oospores applied in water and those applied in methyl cellulose suspensions (Table 2). Seed treatments had no significant effect on the growth rate of sugar beet.

Seed treatment with *P. oligandrum* had no consistent effect on the root densities of 18-day-old seedlings of *B. vulgaris* grown in field soil compared with untreated seeds or any significant effect on root infection by *P. ultimum* (Table 2). *P. oligandrum* was rarely isolated from the roots or hypocotyls of 18-day-old seedlings.

**Effect of seed treatment on germination and colonization.** When planted in field soil naturally infested with 13 propagules of *P. ultimum* per gram of soil, 77 and 93% of the seed coats of untreated sugar beet seeds were colonized by *P. ultimum* 24 and 72 hr after planting, respectively (Table 3). For seeds treated with *P. oligandrum*, 10% of the seed coats were colonized by *P. ultimum* after 24 hr, with no increase in the degree of colonization thereafter. When viewed with the light microscope, treated seeds were covered with dense mycelium 24 hr after planting, whereas fungal growth on the surface of untreated seeds was minimal. The frequency of endosperm colonization by *P. ultimum* also was lower for treated seeds (3.4%) than for untreated seeds (63%). Endosperms colonized by *P. ultimum* often were necrotic, and the radicles were infected soon after emergence. Many of the seedlings from untreated seeds that germinated but did not emerge from the soil were infected by *P. ultimum* near the hypocotyl as the radicles emerged from the seed.

The length of 5-day-old sugar beet radicles colonized by *P. ultimum* was less for seedlings from seeds treated with *P. oligandrum* (7%) than for seedlings from untreated seeds (71%, Table 3). *P. oligandrum* was also an aggressive colonizer of endosperms and roots (69 and 23%, respectively) when coated on seed surfaces, colonizing the roots predominantly at the junction of the hypocotyl and taproot while in association with the seed coat but rarely in or acropetal to the zone of root hair formation. There was no evidence of infection or necrosis associated with seedling colonization by *P. oligandrum*. Germination of seeds planted in field soil was not significantly different for those treated with

oospores of *P. oligandrum* and those not treated (Fig. 1). Because of protection from preemergence damping-off, however, emergence was greater for treated seeds than for untreated seeds.

TABLE 3. Effect of treating sugar beet seeds with oospores of *Pythium oligandrum* ($10^6$/ml) on seed coat, endosperm, and root colonization by *P. oligandrum* and *P. ultimum*[a]

| Plant part colonized | Seed treatment | Colonization (%)[b] | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Seed coat | | | | | | |
| *P. ultimum* | None | 76.5 | 86.5 | 92.7 | ⋯[c] | ⋯ |
| | *P. oligandrum* | 10.0 | 10.0 | 6.7 | ⋯ | ⋯ |
| *P. oligandrum* | None | 0.0 | 0.0 | 0.0 | ⋯ | ⋯ |
| | *P. oligandrum* | 96.7 | 98.2 | 100.0 | ⋯ | ⋯ |
| Endosperm | | | | | | |
| *P. ultimum* | None | 11.7 | 62.8 | ⋯ | ⋯ | ⋯ |
| | *P. oligandrum* | 0.0 | 3.4 | ⋯ | ⋯ | ⋯ |
| *P. oligandrum* | None | 0.0 | 0.0 | ⋯ | ⋯ | ⋯ |
| | *P. oligandrum* | 18.4 | 69.2 | ⋯ | ⋯ | ⋯ |
| Root[d] | | | | | | |
| *P. ultimum* | None | ⋯ | 14.2 | 31.3 | 45.2 | 70.7 |
| | *P. oligandrum* | ⋯ | 3.4 | 0.3 | 1.3 | 6.8 |
| *P. oligandrum* | None | ⋯ | 0.0 | 0.0 | 0.0 | 0.0 |
| | *P. oligandrum* | ⋯ | 13.6 | 19.2 | 23.4 | 8.9 |

[a] Seeds were planted in Oceano loamy sand naturally infested with 13 propagules of *P. ultimum* per gram of soil and maintained at −0.1 bar at 21 C. Data reflect the average of two experiments and are significantly different in each column between seed treatments according to Duncan's multiple range test ($P = 0.05$).
[b] Percentage of seeds from which the respective plant part was colonized by either *P. ultimum* or *P. oligandrum*.
[c] Data not taken.
[d] Percentage of root length from which hyphae grew when roots were plated on water agar.
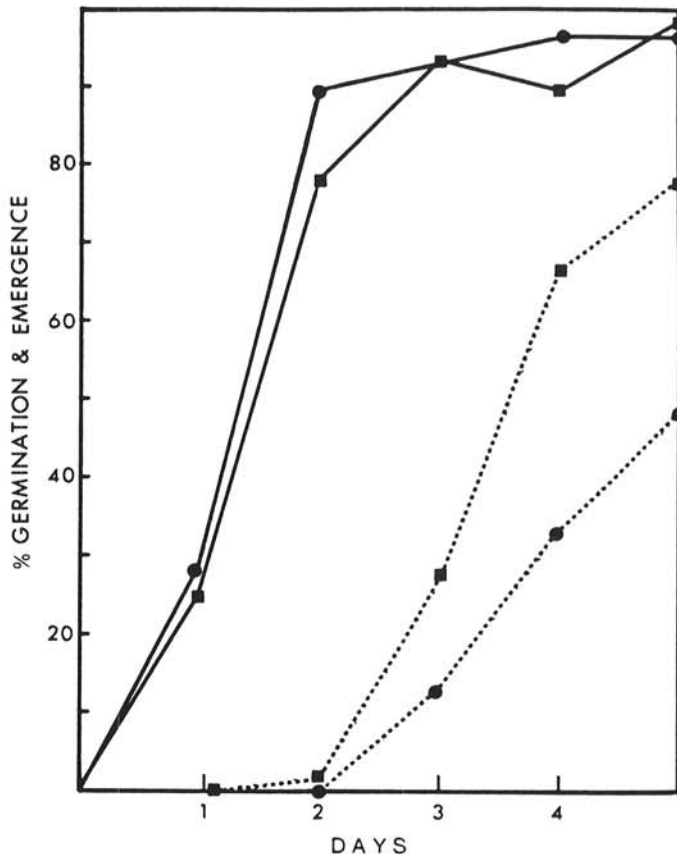


**Fig. 1.** Percent germination (—) and emergence (- - -) of seeds of *Beta vulgaris* either pelleted with oospores of *Pythium oligandrum* (■) or untreated (●) after incubation in field soil naturally infested with *P. ultimum* at 21 C and −0.1 bar.

TABLE 2. Emergence, survival, and plant growth of sugar beet seeds treated with oospores of *Pythium oligandrum* and planted in field soil[a]

| Seed treatment | Emergence (%) | Survival[b] (%) | Shoot weight (g) | Root length colonized by *P. ultimum* (%) |
|---|---|---|---|---|
| H₂O | | | | |
| 0 oospores/ml | 57.8 y | 64.0 y | 0.17 y | 6.2 y |
| $10^6$ oospores/ml | 87.0 z | 69.6 y | 0.16 z | 6.1 y |
| Methyl cellulose | | | | |
| 0 oospores/ml | 53.3 y | 47.1 x | 0.16 z | 4.2 y |
| $10^6$ oospores/ml | 81.0 z | 74.4 y | 0.18 z | 4.4 y |
| Fenaminosulf[c] | 92.0 z | 95.5 z | 0.18 z | 0.2 z |

[a] Data reflect the average of four experiments and were recorded 18 days after seeds were planted in Oceano loamy sand naturally infested with 13–58 propagules of *P. ultimum* per gram of soil. Data in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[b] Percentage of plants still standing after 18 days.
[c] Lesan 35WP, 0.01 g/4 g of seed.

**Scanning electron microscopy.** Twenty-four hours after being planted in field soil at −0.1 bar matric potential, sugar beet seeds coated with oospores of *P. oligandrum* were uniformly colonized by fungal mycelium. Oospores of *P. oligandrum* had germinated and the germ tubes were growing on the seed surface, often oriented toward the junction of the mesocarp and testa where the radicle was emerging. As radicles emerged from the seed, they were colonized by mycelia, predominantly basally and in the vicinity of developing hypocotyls (Fig. 2). Epiphytic fungal growth was not observed in or beyond the zone of root hair formation of radicles or on hypocotyl surfaces after seeds had completely germinated and seed coats were no longer in association with hypocotyls. These sites of colonization showed no signs of necrosis or discoloration of seedling tissue before fixation and corresponded to areas where *P. oligandrum*, but not *P. ultimum*, was recovered when tissue was transferred to water agar. Hyphal entwinings often associated with mycoparasitism by *P. oligandrum* were not observed.

Untreated seeds planted in field soil showed little fungal colonization after 24 hr. Colonization was occasionally observed on the cortical coverings or stem pieces and was not uniform. Radicles emerging from the seed were often colonized at the base where they were in contact with the seed coat (Fig. 3); these areas of fungal colonization corresponded to necrotic and water-soaked lesions on seedlings observed before fixation and from which *P.*
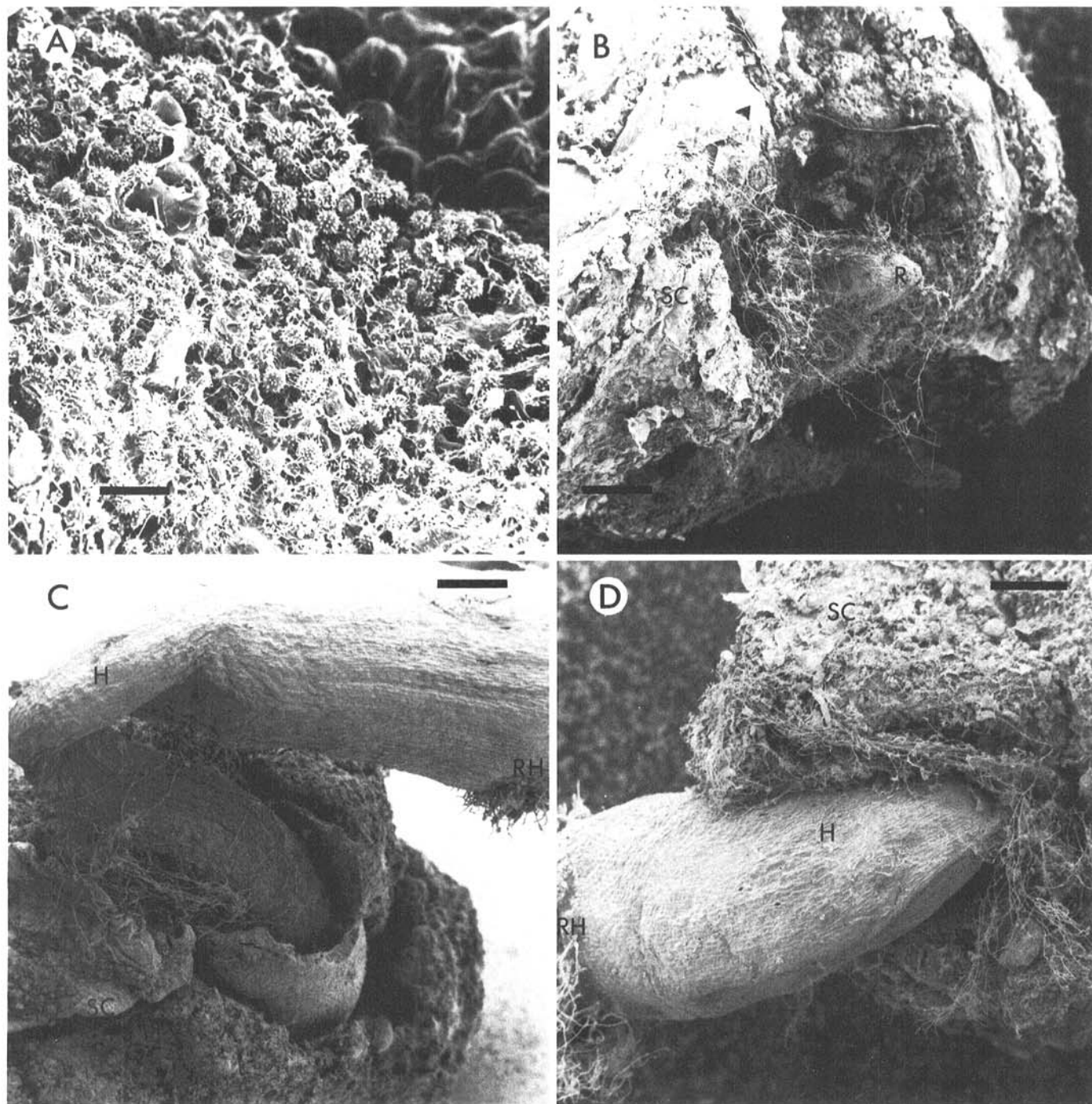
**Fig. 2.** Fungal colonization of sugar beet seeds pelleted with oospores of *Pythium oligandrum* and planted in field soil naturally infested with *P. ultimum* at 21 C and −0.1 bar. **A,** Oospores and mycelium of *P. oligandrum* on the seed surface (bar = 50 μm). **B,** Mycelium of *P. oligandrum* colonizing the seed coat (SC) and emerging radicle (R) 24 hr after planting (bar = 300 μm). **C and D,** Seed coat (SC) and hypocotyl (H) colonized by *P. oligandrum* 48 hr after planting (bars = 300 μm). The antagonist colonizes the hypocotyls only in association with the seed coat and does not grow in or beyond the zone of root hair formation (RH) but does grow down the hypocotyl into the interior of the seed. Fungal isolations correspond to these patterns of colonization.

*ultimum* was commonly isolated. The experiment was conducted twice, and observations were consistent both times.

**Pathogenicity test.** Coating seeds of 12 economic crop plants representing six families (Table 1) with oospores of *P. oligandrum* had no detrimental effects on seedlings planted in fumigated soil. As observed under the light microscope, none of the plants showed signs of root necrosis or root invasion by *P. oligandrum*. After 17 days, *P. oligandrum* was commonly isolated from the length of the taproot and from some lateral roots of radish, tomato, and soybean. With the other crops, *P. oligandrum* was isolated predominantly from the region of the hypocotyl-taproot junction and rarely from lateral roots. *P. oligandrum* was recovered from all treated seeds after transfer to water agar. After incubation for 7–10 days, seeds germinated and grew on the agar surface along with colonies of *P. oligandrum*. No necrosis of any part of the seedling was observed. For sugar beet, pelleting reduced the root length density of individual plants grown in fumigated soil but did not reduce shoot growth (Table 4). Data on root length density and shoot weight were not recorded for the other test crops.

**Soil amendment with *P. oligandrum*.** When field soil naturally infested with *P. ultimum* was amended with oospores of *P. oligandrum* to give 7–37 propagules per gram of soil, emergence of sugar beet seedlings was significantly greater than that of unamended controls (Table 5). Protection against preemergence damping-off was occasionally enhanced by soil amendment with 0.85 meq of Cl⁻ / 100 g of soil as $CaCl_2$. Chloride neither influenced the degree of root colonization by *P. ultimum* nor affected the incidence of disease. The frequency of isolation of *P. oligandrum* from 18-day-old roots grown in soil artificially infested with this fungus was low (1.1%).

## DISCUSSION

Coating sugar beet seeds with oospores of *P. oligandrum* controlled preemergence damping-off caused by *P. ultimum* equivalent to treating the seeds with fenaminosulf (Table 2). This protection was manifested within 24 hr of planting and coincided with seed colonization by *P. oligandrum* (Table 3). Those sites of colonization by *P. oligandrum* were similar to those colonized by *P. ultimum* when untreated seeds were planted in naturally infested

soil. Because the parts of the seed and seedlings colonized by *P. oligandrum* had a lower frequency of colonization by *P. ultimum* than untreated seeds, early colonization by *P. oligandrum* apparently protects against subsequent infection by *P. ultimum*. A similar antagonism was observed in soils suppressive to *P. ultimum* in California (22,23) where prior colonization of crop debris by *P. oligandrum* prevented subsequent colonization by *P. ultimum*.

The control of preemergence damping-off caused by *P. ultimum* after seed treatment with oospores of *P. oligandrum* reported in this investigation agrees with other reports (3,19,36–38). Vesely and Hejdanek (36,37) observed similar levels of disease control for sugar beets with oospore and thiram seed treatments. Likewise, Al-Hamdani et al (3) and Lutchmeah and Cooke (19) reported

TABLE 4. Emergence, fresh shoot biomass, and root length density of sugar beet seedlings when seeds were treated with oospores of *Pythium oligandrum* and planted in fumigated soil[a]

| | Percent of fungicide-treated control | | |
|---|---|---|---|
| Seed treatment[b] | Emergence | Fresh shoot biomass | Root length density[c] |
| $H_2O$ | | | |
| 0 oospores/ml | 87.9 z | 110.0 z | 91.6 z |
| 10⁶ oospores/ml | 93.3 z | 105.0 z | 67.9 x |
| Methyl cellulose | | | |
| 0 oospores/ml | 92.3 z | 115.0 z | 77.5 y |
| 10⁶ oospores/ml | 86.0 z | 100.0 z | 69.5 x |

[a] Data reflect the average of three experiments and were recorded 18 days after seeds were planted in Oceano loamy sand fumigated with metam-sodium (12 ml of Vapam [33% a.i.] per liter of $H_2O$) and grown at 21 C. Results were compared with those for seeds treated with fenaminosulf (Lesan 35WP, 0.01 g/4 g of seed), and values shown are percentages of these fungicide-treated seeds. Data in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[b] Seeds (4 g) were placed in a small plastic bag and mixed well with 5 ml of solution, then air-dried.
[c] Calculated by multiplying root length density obtained by the Newman line intersect method by soil weight per pot and dividing by the mean number of plants per pot.
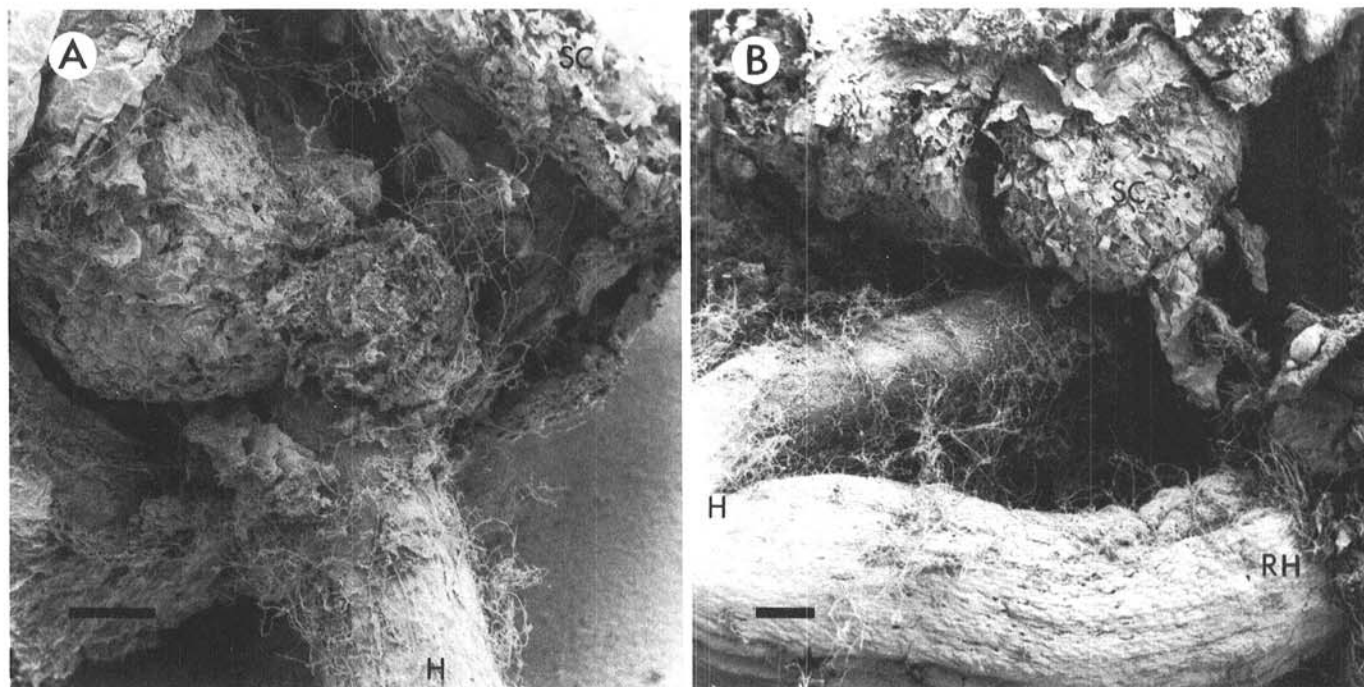


**Fig. 3.** Fungal colonization of untreated sugar beet seeds planted in field soil naturally infested with *Pythium ultimum* at 21 C and −0.1 bar. *P. ultimum* colonizing the seed coat (SC) and hypocotyl (H) after **A,** 48 hr and **B,** 72 hr (bars = 300 μm). Sites of colonization correspond to location of necrotic tissue before fixation and areas of isolation of *P. ultimum* when plated on water agar.

disease control after oospore treatment of cress seeds and cress and sugar beet seeds, respectively. Because fungicide controls were not included in these two studies, however, we do not know how the levels of biocontrol compared with commercial seed treatments. Walther and Gindrat (38) also obtained equivalent control of damping-off of sugar beet caused by *P. ultimum* and *Phoma betae* Frank with oospores of *P. oligandrum* and captan seed treatments.

The absence of colonization by *P. oligandrum* from the primary root system of sugar beet may indicate why seed treatment did not reduce the frequency of root colonization by *P. ultimum* or protect from postemergence damping-off as well as fenaminosulf seed treatment did. However, seed treatment occasionally reduced the incidence of postemergence damping-off compared with untreated seeds, perhaps because of reduced seed and hypocotyl infection by *P. ultimum*. The reduced effectiveness of seed treatment with *P. oligandrum* for controlling postemergence damping-off concurs with the observations of Al-Hamdani et al (3) and Vesely (36) on damping-off of cress and sugar beet, respectively. Lutchmeah and Cooke (19), however, reported otherwise; seed treatment with a vermiculite culture in a clay carrier protected from postemergence damping-off of cress and sugar beet caused by *P. ultimum* and of carrot caused by *M. acerina*. The pelleting treatment with *P. oligandrum* delayed seed germinaton by 3–5 days, perhaps allowing *P. oligandrum* to become better established in the spermosphere and protect from infection.

When coated on the seed surface, *P. oligandrum* may protect from damping-off by several different mechanisms. Competition for space and nutrients is one possibility. Because of germination and growth rates similar to *P. ultimum* (albeit slightly slower, *unpublished*), *P. oligandrum* germinates and starts to grow on the seed surface at much the same time as *P. ultimum*. Because of its presence on the seed coat, however, *P. oligandrum* has a competitive advantage over *P. ultimum* and should be able to colonize and become established in the spermosphere. Prior colonization by *P. oligandrum* may result in formation of a physical barrier to *P. ultimum* (7), reduction in the quantity of nutrients exuding into the soil (15,40), or alterations in the composition of the exudates diffusing through the soil. Alterations in the composition of seed exudates by the presence of metabolically active *P. oligandrum* may prevent colonization by *P. ultimum* in much the same manner as the presence of other primary colonizers inhibited subsequent colonization of substrates by *P. mamillatum* Meurs (4).

Protection from seed colonization by *P. ultimum* in the presence of *P. oligandrum* may also be due to direct hyphal interactions between the fungi. Lutchmeah and Cooke (18) observed that when the fungi were grown in dual culture on cellophane, hyphae of *P. ultimum* lost opacity as they came in contact with hyphae of *P. oligandrum*. Because hyphae of *P. ultimum* were not subsequently penetrated, Lutchmeah and Cooke (18) proposed hyphal interference as the mechanism of antagonism. In the original description of mycoparasitism by *P. oligandrum*, Drechsler (11) reported a similar loss of opacity as *P. oligandrum* came in contact with hyphae of *P. myriotylum* Drechsler. Unlike Lutchmeah and Cooke (18), Drechsler (11) observed penetration of hyphae of *P. myriotylum*, *P. ultimum*, and several other *Pythium* spp. by *P. oligandrum* after degradation of host protoplasm. These differences in ability to penetrate host mycelium may be due to isolate variability; variations among isolates in ability to penetrate *Rhizoctonia solani* Kühn have been reported (18). Other factors may be involved, however. Al-Hamdani (2) observed penetration of hyphae of *P. ultimum* by the same isolate of *P. oligandrum* (IMI 133857) used by Lutchmeah and Cooke (18).

Although the mycoparasitic capabilities of *P. oligandrum* in dual culture are well documented (2,8,9,11,18,35), the evidence implicating it as a mechanism of disease control is not conclusive. Several limitations of this phenomenon may preclude it from being a primary mechanism of seed protection in field soil, most significant of which is the vegetative growth rates of *P. ultimum*. In field soil, sporangia of *P. ultimum* may germinate in 1.5 hr (31), grow at a rate of 300 $\mu$m/hr (32), and colonize seed coats within 24 hr. Therefore, parasitism by *P. oligandrum* (e.g., hyphal entwining and penetration of the host) would have to occur soon after germination of propagules of *P. ultimum* and render the hyphae nonviable before seed infection. Compared with seed infection by *P. ultimum*, however, mycoparasitism is not an immediate and rapid process; there is a lag time between recognition of host mycelium and subsequent initiation of parasitism and resultant hyphal death. The length of this lag time in the spermosphere is not known, but it may be difficult for mycoparasitism to keep pace with the high growth rate of *P. ultimum* and prevent subsequent seed colonization. Therefore, it appears more likely that the primary mechanism by which *P. oligandrum* protects seed from infection is alteration of the quality and quantity of seed exudates in the spermosphere combined with potential hyphal interference at the infection court. Similar mechanisms are believed to be involved in the prevention of substrate colonization by *P. ultimum* when organic debris is previously colonized by *P. oligandrum* (23).

In addition to protecting from damping-off when applied to the surface of sugar beet seeds, *P. oligandrum* also reduces the incidence of disease when applied as a soil amendment. This protection was greater for preemergence than for postemergence damping-off and was occasionally enhanced by the addition of calcium chloride. Other investigations (22,23) indicate that *P. ultimum* is relatively intolerant and *P. oligandrum* is relatively tolerant to chloride in the soil. At the concentration used in this experiment, calcium chloride had no effect on the incidence of damping-off or the growth rate of sugar beet (20) (Table 5). The levels of control were not as effective as fungicide seed treatment, but this may be a reflection of the initial propagule densities of *P. oligandrum* with which the soil was infested and not the relative efficacy of the organism in biocontrol. These results contrast with the conclusions of Vesely and Hejdanek (37) on the ability of *P. oligandrum* to contribute to reductions in disease when present in the soil.

Increasing the propagule density of *P. oligandrum* in the soil may protect from disease by increasing the competition for nutrients in the rhizosphere, thereby decreasing the activity of the pathogen. Bouhot and Joannes (5,6) observed this kind of interaction between a phytopathogenic *Pythium* spp. and saprophytic fungi in the Mucoraceae, e.g., increased propagule densities of the saprophytes in the soil decrease the levels of disease. Likewise, Watson (39) concluded this form of nutrient competition

TABLE 5. Influence of increased propagule densities of *Pythium oligandrum* and calcium chloride concentration in field soils naturally infested with *Pythium ultimum* on the incidence of preemergence and postemergence damping-off of sugar beet seeds[a]

| Soil treatment | Experiment 1 | | Experiment 2 | |
| --- | --- | --- | --- | --- |
| | Emergence (%) | Survival[b] (%) | Emergence (%) | Survival (%) |
| None | 40.8 x | 36.4 v | 42.0 w | 55.4 y |
| CaCl$_2$[c] | 39.0 x | 48.7 vw | 46.5 wx | 64.9 y |
| *P. oligandrum*[d] | | | | |
| 6.7 propagules/gram of soil | 63.2 y | 61.3 wx | ⋯ | ⋯ |
| 15 propagules/gram of soil | ⋯ | ⋯ | 52.0 wx | 47.3 y |
| 20 propagules/gram of soil | 68.0 y | 86.1 y | ⋯ | ⋯ |
| 37 propagules/gram of soil | ⋯ | ⋯ | 62.0 xy | 46.8 y |
| CaCl$_2$ + *P. oligandrum* | | | | |
| 6.7 propagules/gram of soil | 73.6 y | 84.8 y | ⋯ | ⋯ |
| 15 propagules/gram of soil | ⋯ | ⋯ | 71.0 y | 63.8 y |
| 20 propagules/gram of soil | 69.6 y | 79.6 xy | ⋯ | ⋯ |
| 37 propagules/gram of soil | ⋯ | ⋯ | 89.0 z | 47.1 y |
| Fenaminosulf[e] | 92.0 z | 95.5 z | 92.0 z | 95.5 z |

[a] Data were recorded 18 days after seeds were planted in Oceano loamy sand naturally infested with 11 and 15 propagules of *P. ultimum* per gram of soil, respectively, and incubated at 21 C. Data in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

[b] Percentage of plants still standing after 18 days.

[c] Added at 0.85 meq of Cl$^-$/100 g of soil.

[d] Propagules in the soil after amendment.

[e] Lesan 35WP, 0.01 g/4 g of seed.

was responsible for reductions in damping-off caused by *P. ultimum*, and Alabouvette et al (1) observed it was involved in *Fusarium*-suppressive soils in France.

*P. oligandrum* also may compete with *P. ultimum* for colonization of infection sites on the root, thereby reducing the number of infection courts accessible to the pathogen. In preliminary investigations (*unpublished*), *P. ultimum* was isolated at a lower frequency from roots of sugar beet plants grown in soil amended with *P. oligandrum* than from those of plants grown in unamended soil. Because the frequency of root colonization by *P. oligandrum* is low, this is contrary to what would be expected if site competition was responsible for root protection. Dolan et al (10) reported similar results when roots of *Persea* spp. were inoculated with *Phytophthora parasitica* Dastur (which is nonpathogenic on this crop) and challenged with pathogenic *Phytophthora* spp. The frequency of recovery of both species was low. Similar interactions between pathogenic and nonpathogenic *Fusarium* spp. have been reported (27,29).

When coated on seed surfaces, *P. oligandrum* was found to be nonpathogenic in vitro and in vivo on 12 crop species representing six families (Table 1). On sugar beet, *P. oligandrum* slightly reduced the root length densities of seedlings planted in fumigated soil, with no detrimental effects on shoot weight or stand counts (Table 4). There was no visible sign of root necrosis, and root length density was not reduced when pelleted seeds were planted in field soil. Vesely (36) reported reductions (3%) in sugar beet stand counts owing to seed pelleting, but in view of the size of the experiment it is doubtful this reduction is beyond the limits of experimental error. Other investigators found *P. oligandrum* to be slightly pathogenic on wheat (16) and on snap beans when grown at 30 C but not at 21 C (28) and to be capable of damping-off *Pisum sativum* L. (24) (which was not observed in this investigation). However, after looking at a number of different isolates from various diseased hosts, Drechsler (12) was able to find few isolates of *P. oligandrum* capable of causing disease singly. Therefore, it appears that pathogenicity may be an isolate-specific phenomenon and not widespread in the species. Other investigators have reported *P. oligandrum* to be nonpathogenic on barley and oats (16), pineapple (17), cress (2,3,19), and carrot (19).

The use of *P. oligandrum* for the biological control of *P. ultimum* has several advantages. Ecologically, the fungus occupies a similar niche as *P. ultimum* and is vegetatively active under similar moisture and temperature regimes (22,23). The antagonistic influences toward *P. ultimum* are twofold, i.e., increases of pathogen inoculum density are prevented by reductions in pathogen saprophytic activity (22,23) and seeds and seedlings are protected from damping-off. This protection can be provided from either soil treatment or seed treatment with oospores, the latter controlling preemergence damping-off equivalent to fenaminosulf seed treatments. Control of postemergence damping-off may be increased by manipulation of procedures for applying the antagonist to the seed (19) and by isolate selection. Its ecological niche and dual antagonism toward *P. ultimum* make *P. oligandrum* ideal for use as a biological control agent for container-grown crops, for reinfestation of fumigated or pasteurized soils, and for seed treatments.

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