Biological Control of Seed Rot and Preemergence Damping-Off of Chickpea with *Penicillium oxalicum*

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ABSTRACT

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Seed treatment with conidia of *Penicillium oxalicum* significantly reduced seed rot and preemergence damping-off of chickpea (*Cicer arietinum*) caused by *Pythium ultimum* in two naturally infested soils from the Palouse region of eastern Washington. *P. oxalicum* was the only biocontrol organism of three tested that consistently enhanced emergence and increased seed yields over untreated seeds under field and greenhouse conditions. Seeds coated with conidia of *Trichoderma hamatum* or *T. harzianum* usually were ineffective in controlling the Pythium seed rot. In all tests, plant stands and yields from seeds treated with *P. oxalicum* as a dust (dry spores) or in a 1.6% methyl cellulose suspension were significantly better than those from untreated seeds and were statistically as effective as captan-treated seeds in field trials at Central Ferry, WA, but not Pullman, WA. Similar plant emergence and seed yields were obtained from *P. oxalicum*-coated seeds that had been stored for 8 mo or 5 days at 4 C and 35% RH, then planted in the field at Central Ferry, WA. Emergence and yields of metalaxyl-treated seeds were statistically equal or superior to all other treatments in greenhouse and field trials.

Chickpeas (*Cicer arietinum* L.) are a potential crop for the dryland areas of the Palouse region of eastern Washington and northern Idaho. Seed rot and preemergence damping-off of chickpea caused by *Pythium ultimum* Trow is a limiting factor in the cultivation of large-seeded (>25 g/100 seeds), cream-colored chickpea lines in this region of the Pacific Northwest (7). Fungicide seed treatment is necessary for good emergence and high yields of chickpea in many Palouse soils. Several fungicides have proven effective in controlling the Pythium disease of chickpea in the Palouse region (7), but only captan is currently registered for use on this crop. At the Regional Plant Introduction Station, Pullman, WA, where the USDA plant germ plasm collection for chickpea and other food legumes is maintained, we have been interested in developing alternative methods for controlling indigenous and/or introduced diseases of these crops. The objectives of this study were 1) to investigate biological agents for controlling Pythium seed rot and preemergence damping-off of chickpea by treating seeds with conidia of *Penicillium oxalicum* Currie & Thom, *Trichoderma hamatum* (Bon.) Bain, and *T. harzianum* Rifai; 2) to examine whether coating seeds with conidia of these fungi had a phytotoxic effect on emergence and growth of seedlings in sterile soil; and 3) to evaluate the efficacy of the biocontrol treatments in controlling the Pythium disease in naturally infested field soil when treated seed is stored for 8 mo at 4 C.

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MATERIALS AND METHODS

Fungus isolates. A culture of *Penicillium oxalicum* (ATCC 52658) was obtained from C. E. Windels, Department of Plant Pathology, University of Minnesota, St. Paul. Cultures of *T. hamatum* and *T. harzianum* were obtained from R. Baker, Department of Botany and Plant Pathology, Colorado State University, Ft. Collins. These three fungi have been effective biocontrol agents on other crops (1–4,9–11,14,15). Isolates were cultured on potato-dextrose agar (PDA) and stored on PDA slants at 4 C.

Isolation from chickpea seeds. Isolations were made from rotted seeds at weekly intervals up to 27 days after planting to identify the causal agent(s). Seeds were washed in running tap water for 15–30 min, surface-sterilized in 0.25% NaOCl for 5 min, and plated on 2% water agar.

Seed treatments. All greenhouse and field studies were carried out with a large-seeded (39 g/100 seeds), cream-colored chickpea line (PI 458870, USA) highly susceptible to Pythium seed rot and preemergence damping-off (7). Seed was selected for uniform size and tested for viability by standard germination techniques.

The three fungi were cultured on PDA in petri dishes (100 × 15 mm) for 15–30 days at 22–25 C under fluorescent light (12-hr photoperiod at 4,300 lux). Conidia were collected as dry spores (*P. oxalicum*) or put into sterile distilled water (*P. oxalicum, T. hamatum,* and *T. harzianum*) after gently scraping the colony surface with a bent spatula. Dry conidia of *P. oxalicum* (50, 100, or 200 mg) were added to a 500-ml Erlenmeyer flask containing 100 seeds and agitated by hand for 2 min. No dry-spore treatment was included for the *Trichoderma* spp. because spores are more hydrophilic and cannot be collected in a dry state (dust). Conidial suspensions were prepared for each fungus in both sterile distilled water and 1.6% methyl cellulose (Methocel 4AC Premium) and adjusted to the desired conidial concentration with

a hemacytometer; 3 ml of each suspension was added to a 500-ml Erlenmeyer flask containing 100 seeds, agitated for 2 min, and air-dried for 3 hr at 25 C. Two seed-treatment fungicides were included in all trials as treated controls. These were metalaxyl (Ridomil 25W) and captan (Orthocide 50W) used at rates of 0.3 and 3.0 g a.i./kg of seed, respectively. Fungicides were applied to seeds as slurries by mixing each fungicide in 3 ml of water per 100 seeds in a 500-ml Erlenmeyer flask. Flasks were agitated for 2 min and seeds were air-dried for 3 hr at 25 C. Seeds treated with sterile distilled water or methyl cellulose or left untreated were included in all experiments. An untreated (dry) control treatment was not included in all field or greenhouse trials because emergence in this and the sterile distilled water (wet) control treatment was not significantly different in soils naturally or artificially infested with *P. ultimum* (7).

Greenhouse experiments. Two greenhouse experiments were conducted with naturally infested soil from Central Ferry, WA. Soil for each experiment was collected from different fields at Central Ferry. Each soil was amended with peat moss (20%, v/v) and placed in 15-cm-diameter plastic pots. Ten treated or untreated seeds were planted per pot to a depth of 1.5–2.0 cm. In one trial, untreated seeds were planted in amended soil that had been autoclaved for 3 hr on two consecutive days. Soil was watered to saturation whenever the soil moisture reached −0.3 bars as measured with a tensiometer (Irrometer Co., Inc., Riverside, CA). The experimental design was a randomized complete block with five replicates per treatment. Final emergence was recorded 27 days after planting. Soil samples for evaluating population density of *P. ultimum* in each amended soil were collected just before planting and were determined using Mircetich's selective medium (12), which was modified by reducing the agar by 50%.

Field experiments. Field trials were conducted at Central Ferry, WA, in 1981 and at Central Ferry and Pullman, WA, in 1982. The soil at Central Ferry is classified as a Spofford silt loam (pH 6.6, conductivity 0.6 mmhos/cm, 27% sand, 53.8% coarse silt, 2.8% fine silt, 16.4% clay, and 1.6% organic matter). The soil at Pullman is classified as a Palouse silt loam (pH 5.9, conductivity 2.5 mmhos/cm, 24% sand, 56% coarse and fine silt, 20% clay, and 3.2% organic matter). Seeds were planted 3.5–4.0 cm deep in mid- to late April with a tractor-driven, single-row cone planter in rows 5.3 m long with 1.5 m between rows. Soil temperatures at 4–7 cm deep ranged from 15 to 17 C. Each row constituted a replicate, and treatments were replicated four times with 50 seeds per replicate in a randomized complete block design. Final emergence was recorded 27 days after planting. Seed yields were determined for each single-row plot.

Storage experiment. Two lots of chickpea seeds were used in the storage experiment. Both were stored for 240 days at 4 C and 35% RH. One lot was treated with fungal spores or fungicides (as outlined before) and stored for 240 days before planting. The other was treated 5 days before planting. Appropriate untreated controls were included in this trial. Emergence was recorded at intervals up to 27 days and yields were determined for each plot. Seeds were planted in mid-April at Central Ferry, WA, in single rows 2.5 m long with 25 seeds per row. Plots were replicated four times. A *P. oxalicum* water treatment was not included because emergence and yields of seeds coated with *P. oxalicum* conidia in water were usually lower and more erratic than the *P. oxalicum* dry or methyl cellulose treatments.

Phytotoxicity experiment. Seeds were dusted with conidia of *P. oxalicum* at rates of 1, 2, and 4 mg/seed or coated with conidia in water or 1.6% methyl cellulose at 1 and 2×10^8 conidia per milliliter. Conidia of *T. hamatum* and *T. harzianum* suspended in water or 1.6% methyl cellulose were coated on seeds at rates of 1.6×10^9 and 8×10^8 conidia per milliliter, respectively. Ten seeds were planted in sterilized potting medium in 15-cm-diameter plastic pots. Each pot constituted a replicate and treatments were replicated five times in a randomized complete-block design. Greenhouse temperatures ranged from 15 to 25 C. Emergence counts were taken at weekly intervals up to 27 days after planting.

RESULTS

Isolation from seeds. Isolations from ungerminated chickpea seeds in greenhouse and field trials yielded primarily *Fusarium* spp. and *Pythium ultimum*. Isolates of *P. ultimum* were highly pathogenic to chickpea seeds, whereas the *Fusarium* spp. were not pathogenic in controlled inoculation tests (7). Other *Pythium*-like fungi were isolated occasionally from rotted chickpea seeds but these proved nonpathogenic to chickpea (7). In inoculated treatments, *P. oxalicum, T. hamatum,* and *T. harzianum* were not isolated from rotted seeds that remained in field soil longer than 27 days.

Greenhouse experiments. Several seed treatments significantly reduced seed rot and preemergence damping-off in the greenhouse trials (Table 1, Fig. 1), where the population of *P. ultimum* ranged from 517 to 689 colony-forming units per gram (cfu/g) of air-dried soil. Final emergence of seeds treated with metalaxyl was significantly better than all other treatments and ranged from 90 to 96%. Seeds failed to emerge in the control treatments. In all tests, emergence of seeds dusted with dry conidia of *P. oxalicum* was not significantly different from that of seeds treated with captan. Emergence from the *P. oxalicum*-dry and chemical treatments was significantly better than any of the treatments with the *Trichoderma* spp. and the controls (Table 1). The two *Trichoderma* spp. were not effective biocontrol agents in the greenhouse trials. Emergence of seeds in the *T. hamatum*-methyl cellulose treatment in trial A and the *T. harzianum*-

Table 1. Efficacy of *Penicillium oxalicum, Trichoderma hamatum*, and *T. harzianum* in controlling seed rot and preemergence damping-off of chickpea by *Pythium ultimum* in naturally infested field soil in the greenhouse[v,w]

Treatment[x]	Rate[y]	Emergence (%)[z]	
		Trial A	Trial B
Autoclaved soil	...	96 a	...
Metalaxyl	0.3 g a.i./kg	96 a	90 a
Captan	3.0 g a.i./kg	80 bc	38 b
P. oxalicum-dry	0.5 mg/sd	68 c	36 b
P. oxalicum-water	1×10^8 sp/ml	28 d	10 cd
P. oxalicum-mc	1×10^8 sp/ml	44 cd	20 c
T. hamatum-water	$4–8 \times 10^8$ sp/ml	8 e	0 d
T. hamatum-mc	$4–8 \times 10^8$ sp/ml	20 d	2 d
T. harzianum-water	$4–8 \times 10^8$ sp/ml	8 e	4 d
T. harzianum-mc	$4–8 \times 10^8$ sp/ml	14 e	16 c
Control-mc	...	0 e	0 d
Control-water	...	0 e	0 d
Control-untreated	...	0 e	...

[v] Seeds of a cream-colored chickpea PI 458870, USA, were planted in a Spofford silt loam soil from Central Ferry, WA, amended with peat moss (20%, v/v) where the population of *P. ultimum* was 689 (trial A) and 517 (trial B) colony-forming units per gram of air-dried soil.

[w] Seeds were planted (10 per pot) in 15-cm-diameter plastic pots, with five pots per treatment. Pots were incubated in a greenhouse at 20 ± 5 C and emergence counts were taken at intervals up to 27 days after planting. Each value represents mean percentage of emergence.

[x] Dry = loose conidia brushed off colony surface; water and mc = conidia suspended in water or a 1.6% methyl cellulose suspension, respectively; autoclaved soil = untreated seeds planted in autoclaved soil; and control-untreated = seed not treated with water or methyl cellulose before planting in naturally infested field soil.

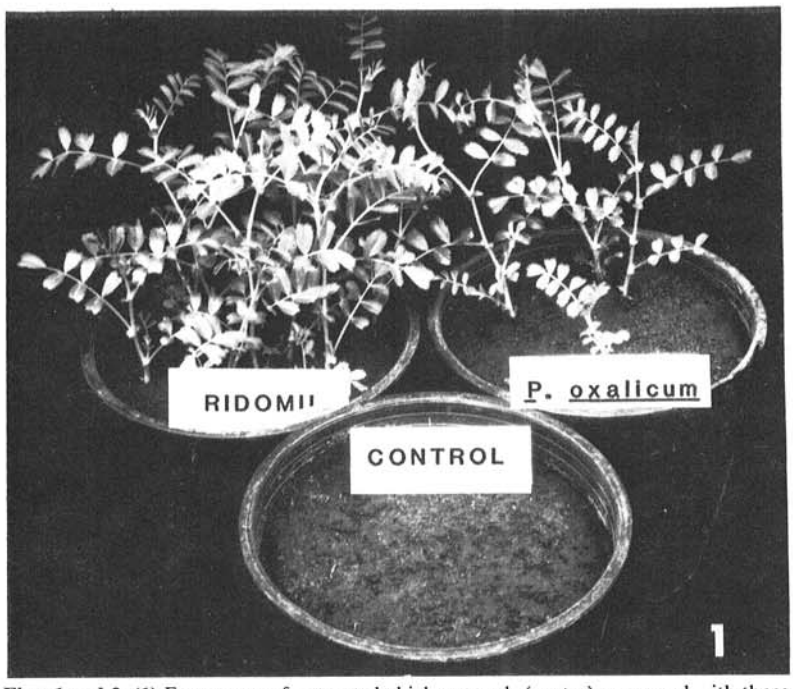
[y] g a.i./kg = Grams of active ingredient per kilogram of seed; mg/sd = milligrams of dry spores per seed; and sp/ml = spores per milliliter.

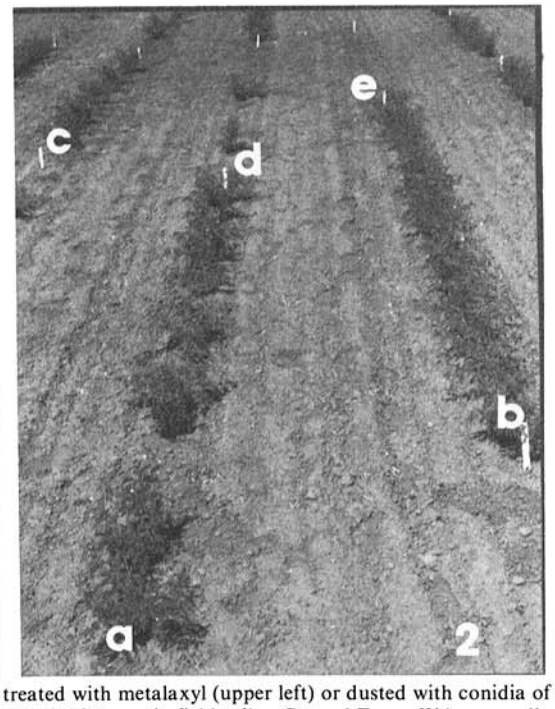
[z] Numbers in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

methyl cellulose treatment in trial B was significantly better than that of the controls and other *Trichoderma* treatments.

Efficacy of the *P. oxalicum* treatments varied by method of application of conidia to seeds and from trial to trial. Application of *P. oxalicum* to seed as dry conidia (dust) increased emergence 36 and 68% over control treatments in trials B and A, respectively. In both trials, there was no significant difference between the *P. oxalicum*-dry and captan treatments. The *P. oxalicum* treatment using methyl cellulose resulted in a significant increase in emergence (20–44%) over the controls, but in trial B, it was not as effective as the fungicide or *P. oxalicum*-dry treatments.

Field experiments. In field trials at Central Ferry, emergence data generally supported results of the greenhouse trials. In all treatments except metalaxyl, seedling emergence at Central Ferry was considerably higher in 1981 than in 1982 (Table 2), but yields varied according to treatment and were greater in 1982 for the fungicide and *P. oxalicum*-dry and methyl cellulose treatments. One factor that could have contributed to reduced yields in these four treatments in 1981 was virus infection. The incidence of virus diseases in the chickpea biocontrol trials was considerably higher in 1981 than 1982. In 1981, there was at least a three-fold increase in yield in the control and *Trichoderma* treatments at Central Ferry compared with 1982 at the same location but in a different field with a different crop history. Emergence and yields of the





Figs. 1 and 2. (1) Emergence of untreated chickpea seeds (center) compared with those treated with metalaxyl (upper left) or dusted with conidia of *Penicillium oxalicum* in field soil naturally infested with *Pythium ultimum*. **(2)** Emergence of chickpeas in field soil at Central Ferry, WA, naturally infested with *Pythium ultimum* when seeds were (a) dusted with conidia of *Penicillium oxalicum*, (b) treated with metalaxyl, (c) coated with spores of *P. oxalicum* in a water suspension, (d) coated with conidia of *Trichoderma hamatum* in a water suspension, and (e) not treated (water control).

Table 2. Effect of coating seeds with conidia of *Penicillium oxalicum, Trichoderma hamatum*, and *T. harzianum* on emergence and yield of chickpea in field trials at Central Ferry and Pullman, WA[v]

		Location[y,z]					
		Central Ferry				Pullman	
		1981		1982		1982	
Treatment[w]	Rate[x]	Emergence (%)	Yield (g)	Emergence (%)	Yield (g)	Emergence (%)	Yield (g)
Metalaxyl	0.3 g a.i./kg	91.6 a	504 a	94.0 a	1,738 a	95.6 a	1,617 a
Captan	3.0 g a.i./kg	84.6 ab	495 ab	78.0 ab	1,485 ab	62.0 b	1,227 b
P. oxalicum-dry	0.5 mg/sd	88.6 ab	444 ab	24.0 c	832 c	26.6 c	682 cd
P. oxalicum-water	1×10^8 sp/ml	50.0 de	167 d	6.6 d	100 d	26.6 c	869 c
P. oxalicum-mc	1×10^8 sp/ml	73.0 bc	295 cd	49.0 b	1,271 b	10.6 d	286 e
T. hamatum-water	$4–8 \times 10^8$ sp/ml	69.0 c	341 c	6.0 d	119 d	5.6 d	144 e
T. hamatum-mc	$4–8 \times 10^8$ sp/ml	61.6 cd	348 c	1.6 d	10 e	6.6 d	150 e
T. harzianum-water	$4–8 \times 10^8$ sp/ml	63.0 cd	367 c	4.0 d	110 d	12.6 cd	309 de
T. harzianum-mc	$4–8 \times 10^8$ sp/ml	63.6 cd	345 c	0.6 d	26 e	7.6 d	215 e
Control-mc	···	35.6 ef	159 d	0.0 d	0 e	7.0 d	106 e
Control-water	···	32.0 f	166 d	0.6 d	5 e	6.6 d	87 e

[v] A total of 200 seeds of chickpea PI 458870, USA, were planted per treatment (four replicates of 50 seeds each). The natural population of *Pythium ultimum* was 1,011 and 956 colony-forming units per gram of air-dried soil (cfu/g) at Central Ferry in 1981 and 1982, respectively, and 678 cfu/g at Pullman.

[w] Dry = loose conidia brushed off colony surface; water and mc = conidia suspended in water or a 1.6% methyl cellulose suspension, respectively.

[x] g a.i./kg = Grams of active ingredient per kilogram of seed, md/sd = milligrams of dry spores per seed, and sp/ml = spores per milliliter.

[y] Emergence counts were taken at intervals up to 27 days after planting; numbers represent mean percentage of emergence and mean yield per row from four single-row plots (each row 5.3 m long).

[z] Numbers within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

fungicide treatments in 1982 were similar at Pullman and Central Ferry and in all but one instance (*P. oxalicum*-methyl cellulose treatment at Central Ferry); both were significantly higher than the biocontrol agents and control treatments. In 1981 at Central Ferry, the *P. oxalicum*-dry treatment was the best of the biocontrol agents (Table 2, Fig. 2), and emergence and yield were not significantly different from either of the fungicide treatments. Emergence of the *P. oxalicum* treatment with methyl cellulose in 1981 was significantly different from the metalaxyl treatment but not significantly different from the captan or *P. oxalicum*-dry treatments. In 1982, however, emergence and yield of the *P. oxalicum*-methyl cellulose treatment was significantly higher than the *P. oxalicum*-dry treatment, statistically equivalent to the captan treatment, but significantly lower than the metalaxyl treatment. In 1982 at Pullman, the *P. oxalicum*-dry and *P. oxalicum*-water treatments significantly increased emergence and yields over the controls and all but the *T. harzianum*-water treatment. At Central Ferry in 1981, emergence and yields of seeds coated with conidia of the two *Trichoderma* species were statistically similar. They were significantly higher than the water or methyl cellulose controls, but significantly less than the *P. oxalicum*-dry or fungicide treatments. In the 1982 trials at Central Ferry and Pullman, there were no significant differences in emergence and yields between the *Trichoderma* treatments and the controls, except for yield in the *T. harzianum*-water treatment at Central Ferry.

Storage experiment. Seedling emergence from *P. oxalicum*-treated seeds stored 240 days at 4 C and 35% RH was not impaired compared with those planted 5 days after treatment (Table 3). Emergence and yields from chickpea seed treated with fungicides or conidia of *P. oxalicum* were significantly higher than from seed treated with *Trichoderma* and the control treatments. Emergence and yields tended to increase in the *P. oxalicum*-treated series as dosage rates increased. Only the highest rate of the *P. oxalicum* conidia in methyl cellulose gave results similar to captan seed treatment, however, but it was significantly lower than results from the metalaxyl treatment. Although the *P. oxalicum*-methyl cellulose treatment at 4×10^8 spores per milliliter after storage for 240 days gave control similar to captan, a rate of 2×10^8 spores per milliliter caused phytotoxicity in pathogen-free soil in a greenhouse trial. Seed treatments with the *Trichoderma* spp. at both storage times were not significantly different from the control treatments.

Phytotoxicity experiment. In one greenhouse trial with sterile potting soil, emergence from seeds coated with conidia of *T. hamatum* and *T. harzianum* in water or methyl cellulose at 1.6×10^9 and 8×10^8 spores per milliliter, respectively, dusted with conidia of *P. oxalicum* at 1 or 2 mg/seed or coated with conidia of *P. oxalicum* in water at 1 or 2 $\times 10^8$ spores per milliliter was not significantly different from that of the fungicide or control treatments, which was >95%. However, emergence of seeds treated with conidia of *P. oxalicum* in methyl cellulose at 1 or 2×10^8 spores per milliliter or as a dust at 4 mg/seed was reduced significantly by 36, 38, and 14%, respectively. *P. oxalicum* sporulated profusely on some ungerminated seeds and a few seedlings in the *P. oxalicum*-methyl cellulose series were stunted.

DISCUSSION

P. oxalicum was the only biocontrol agent tested in our field and greenhouse trials that consistently enhanced seedling emergence and increased yields of chickpea comparable to the fungicide treatments in two Palouse soils naturally infested with *P. ultimum*. Kommedahl and Windels (9) first identified *P. oxalicum* as an antagonist and demonstrated its effectiveness as a seed protectant in controlling seedling blight and seed rot of peas caused by a complex of soilborne pathogens, including *Pythium* spp. (13–15). They (15) also observed that *P. oxalicum* was more effective in preventing preemergence than postemergence damping-off of peas. In our studies, seed treatment with *P. oxalicum* was effective as a seed protectant against seed rot and preemergence damping-off of chickpea. Under field and greenhouse conditions, emergence and yields in *P. oxalicum* treatments were frequently increased by >50% over control treatments. When seeds were not treated with a fungicide or spores of *P. oxalicum* before planting, emergence in field soil was often reduced by >90%. In most greenhouse and field tests, little or no control of the Pythium seed rot and preemergence damping-off disease of chickpea resulted when seeds were coated with conidia of *T. hamatum* or *T. harzianum*. Seed treatment with *P. oxalicum* applied as a dust or in a methyl cellulose suspension resulted in final stands and yields that were significantly better than the controls and usually statistically equivalent to those treated with captan, except for the 1982 Pullman field trial. In one instance, emergence and yield of *P. oxalicum*-treated seeds was statistically similar to those treated with metalaxyl, which was shown in an earlier study (7) to be one of the most effective seed treatment fungicides to control the Pythium disease of chickpea.

Both *T. hamatum* and *T. harzianum* have been reported to be effective biocontrol agents of *Pythium* and *Rhizoctonia* diseases of peas, beans, and radishes (1–5,11). In most field and greenhouse trials, however, both fungi failed to protect chickpeas against *P. ultimum*. Several factors may have contributed to the poor performance of the *Trichoderma* spp. in the two Palouse soils. Temperature of Palouse soils at the time of seeding may have been too cold (<17C) for the *Trichoderma* spp. to effectively protect treated seeds from attack by *P. ultimum* (5). At soil temperatures of <17 C, *P. ultimum* may have been able to infect and damp-off *Trichoderma*-treated chickpea seeds in

Table 3. Effect of storage of chickpea seeds treated with conidia of *Penicillium oxalicum*, *Trichoderma hamatum*, and *T. harzianum* on control of seed rot and preemergence damping-off caused by *Pythium ultimum* in field trials at Central Ferry, WA[u]

		Time seed stored[x]			
		5 Days		240 Days	
Treatment[v]	Rate[w]	Emergence[y] (%)	Yield (g)	Emergence (%)	Yield (g)
Metalaxyl	0.3 g a.i./kg	94.0 a[z]	869 a	97.2 a	996 a
Captan	3.0 g a.i./kg	78.0 ab	743 ab	81.2 ab	864 ab
P. oxalicum-dry	0.5 mg/sd	24.0 c	416 b	33.2 de	554 c
	1.0 mg/sd	···	···	34.8 de	530 c
	2.0 mg/sd	···	···	45.2 c	750 bc
P. oxalicum-mc	1×10^8 sp/ml	49.2 b	636 b	···	···
	2×10^8 sp/ml	···	···	29.2 e	440 cd
	4×10^8 sp/ml	···	···	68.0 b	768 b
T. hamatum-mc	4×10^8 sp/ml	1.6 d	5 c	0.0 f	0 e
T. harzianum-mc	4×10^8 sp/ml	0.8 d	13 c	4.0 f	11 e
Control-mc	···	0.0 d	0 c	0.0 f	0 e
Control-untreated	···	0.8 d	3 c	0.0 f	0 e

[u] A total of 100 seeds of chickpea PI 458870, USA, were planted per treatment (four replicates of 25 seeds each). The natural population of *P. ultimum* was 956 colony forming units per gram of air-dried soil.

[v] Dry = loose conidia brushed off colony surface, mc = conidia suspended in a 1.6% methyl cellulose suspension, and untreated = seeds not treated with methyl cellulose or water before planting.

[w] g a.i./kg = Grams of active ingredient per kilogram of seed, mg/sd = milligrams of dry spores per seed, and sp/ml = spores per milliliter.

[x] Seed stored at 4 C and 35% RH until planted.

[y] Emergence counts were taken at intervals up to 27 days after planting; numbers represent mean percentage of emergence and mean yield per row from four single-row plots (each row 2.5m long).

[z] Numbers within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

naturally infested Palouse soils before the *Trichoderma* conidia had time to germinate and initiate sufficient mycelial growth to protect the seed. The pH of the Spofford and Palouse silt loam soils used in this study ranged from 5.9 to 6.9 and may not have been acidic enough for the *Trichoderma* spp. to be effective seed protectants. Marshall (11) showed that *T. harzianum* was more effective as a seed treatment in reducing the incidence of damping-off of snap beans by *R. solani* in greenhouse soil of pH 3.5 than of pH 5.6. The natural population of *P. ultimum* in the Central Ferry and Pullman soils ranged from 678 to 1,011 cfu/g. The effectiveness of both *Trichoderma* spp. in preventing seed decay and preemergence damping-off of chickpea may have been reduced because of the high inoculum levels of *P. ultimum* in these soils. Marshall (11) showed that when *T. harzianum*-treated bean seeds were planted in soil artificially infested with increasing inoculum levels of *R. solani*, there was a corresponding increase in the incidence of damping-off of bean seedlings. If our field soils were low in available iron, siderophore-producing bacteria may have inhibited growth of *Trichoderma* spp. and interfered with their effectiveness as biocontrol agents (2,6). Competition and/or antagonism from soilborne fluorescent pseudomonads may have contributed to the ineffectiveness of the *Trichoderma* spp. as seed-treatment biological control agents. This was shown to occur in some soils with *T. hamatum* used as a seed treatment of peas to prevent seed rot (6).

At Central Ferry in 1981, increased yields in all *Trichoderma* spp., *P. oxalicum*-water, and control treatments were probably due to the higher emergence and survival of plants in these treatments even though virus incidence was greater. Seed yields of virus-infected chickpea plants can be reduced significantly, particularly if infection occurs before pod set (8).

The effectiveness of *P. oxalicum* in preventing seed rot and preemergence damping-off of chickpea by *P. ultimum* was affected by the method used to apply spores to seeds. In both greenhouse and most field trials at Central Ferry and Pullman, dusting seeds with conidia of *P. oxalicum* was significantly as good or better than coating seeds with conidia in a methyl cellulose or water suspension. Because of the hydrophobic nature of the conidia of *P. oxalicum*, coverage of the seed surface by conidia suspended in methyl cellulose or water at lower application rates (1×10^8 conidia per milliliter) may have been less efficient than by dusting seeds with conidia. However, improved coverage of the seed might result when higher concentrations of conidia are suspended in methyl cellulose, as was observed in the storage experiment.

Although *P. oxalicum* was the most efficient biocontrol agent tested in our studies, performance between locations and years was generally more erratic than that of the fungicide seed treatments. This variability in performance of *P. oxalicum* could be attributed to a number of factors, including differences in physical and chemical properties of the soils used, time of planting, inoculum concentration of *P. ultimum*, crop history, and environmental conditions before and during emergence. The erratic performance of *P. oxalicum* that we observed in our trials tends to support the conclusions of Kommedahl et al (10) that variability in performance of biological control organisms is more pronounced for micoorganisms than for chemical seed treatments. Chickpeas have the ability to compensate for reduced stands with an increase in plant growth and seed yields (W. J. Kaiser and R. M. Hannan, *unpublished*). This phenomenon could be observed at both field locations with captan and the different biocontrol agents, eg, *P. oxalicum*-methyl cellulose treatment at Central Ferry in 1982.

In greenhouse trial B, there was a significant reduction in emergence in the captan and *P. oxalicum*-dry treatments over that observed in trial A. Soils used in both greenhouse tests were collected from different fields at Central Ferry. The population of *P. ultimum* was similar in both soils. Differences in the physical, chemical, and/or biological properties of these two soils, which were both classified as a Spofford silt loam, could have contributed in part to the reduced emergence observed in the captan and *P. oxalicum*-dry treatments in trial B. Another possibility is that incomplete coverage of the chickpea seed in trial B with captan and *P. oxalicum* conidia failed to protect seeds from *P. ultimum* and resulted in a higher incidence of disease. With metalaxyl, a systemic fungicide, complete coverage of the seed is not as critical.

In a sterile potting medium, chickpea emergence was significantly reduced when spores of *P. oxalicum* were applied to seeds as a dust or in a methyl cellulose suspension at higher inoculum rates than used in our field and greenhouse studies. It is possible that at high inoculum levels and higher temperatures (>20 C), *P. oxalicum* becomes pathogenic to germinating chickpea seeds in a sterile medium where competition from other soil microorganisms is greatly reduced or eliminated. Under field conditions where soil temperatures during emergence are cold (<20 C) and abundant soil microflora exist, phytotoxicity of *P. oxalicum* at high inoculum levels may be reduced, which may in fact enhance germination and emergence. In sterile soil, Windels (13) showed that small black lesions and some discoloration occurred on cotyledons of pea seeds treated with spores of *P. oxalicum*. She observed that in autoclaved soil, emergence of *P. oxalicum*-treated seed was statistically lower than that of captan-treated or untreated seed (C. E. Windels, *personal communication*).

This appears to be one of the first reports of the biological control of a disease of chickpea. Use of *P. oxalicum* as a biocontrol agent to prevent seed decay and preemergence damping-off of chickpea appears to offer certain advantages over use of chemical seed treatments like captan and metalaxyl. The fungus is effective as a protectant of chickpea seed in Palouse soils that are heavily infested with *P. ultimum*. Spore viability of *P. oxalicum* on treated seeds is long lasting in storage. Emergence and yields of *P. oxalicum*-treated seeds were nearly as effective as fungicide seed treatments, even after storage of treated seeds for 8 mo at 4 C. Problems associated with handling hazardous chemicals and pollution of the environment are eliminated when *P. oxalicum*-treated seeds rather than chemically treated ones are planted in *Pythium*-infested soils. If inoculum of *P. oxalicum* could be produced economically and in sufficient quantities, it might be feasible for some farmers to treat seeds with spores of *P. oxalicum* rather than fungicides and raise chickpeas for specialty markets at home and abroad where premium prices are frequently paid for nonchemically treated food products.

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