# Control of Pythium Seed Rot and Preemergence Damping-off of Chickpea in the U.S. Pacific Northwest and Spain

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**ABSTRACT**

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Species of *Pythium* isolated from rotted chickpea seeds and damped-off seedlings and chickpea soils in the U.S. Pacific Northwest and southern Spain that caused seed rot and preemergence damping-off of chickpea were *Pythium ultimum* var. *sporangiiferum*, *P. ultimum* var. *ultimum*, and *P. irregulare*. *P. oligandrum* and unidentified *Pythium* spp. that were not pathogenic to chickpea were also isolated. *P. ultimum* var. *sporangiiferum* and *P. ultimum* var. *ultimum* were the most pathogenic to chickpea in field and growth chamber tests. In fumigated, reinfested soil in the field, these varieties caused 90 and 75% reduction in emergence and yield, respectively. In growth chamber studies, *P. irregulare* reduced emergence and caused stunting, but disease severity was highly influenced by the type and density of inoculum, temperature, soil type, and plant age. This species had no significant effect on emergence, plant growth, and yield in the field. Steamed soil infested with oospores or a sand-cornmeal mixture colonized with mycelium was used to reinfest fumigated soil with the test *Pythium* spp. in field trials at Central Ferry, Washington. Fumigation of field soil with methyl bromide reduced the population density of *P. ultimum* to nearly undetectable levels. Treating chickpea seed with the fluorescent pseudomonad *Pseudomonas fluorescens* strain Q29z-80 resulted in yield that was equivalent to that obtained with any of the fungicide seed treatments. Two other biological seed treatments, *Penicillium oxalicum* and *Pythium oligandrum*, were also effective against Pythium seed rot and preemergence damping-off in the field. Seed treatment chemicals metalaxyl, captan, benalaxyl and metalaxyl plus thiabendazole were effective in controlling Pythium seed rot of chickpea in both field and growth chamber tests. The four fungicide seed treatments were at least as effective as fumigating the soil with methyl bromide or treating seeds with various biological control agents.

Chickpea (*Cicer arietinum* L.), also called garbanzo or gram, is a traditional, food-legume crop of the dryland areas of southern Spain where approximately 90,000 ha of the large-seeded, cream-colored cultivars of chickpea, commonly referred to as kabuli types, are sown annually. Since 1982, chickpea has become a promising alternative crop for the dryland areas of the Palouse region of eastern Washington and northern Idaho in the U.S. Pacific Northwest. Chickpea is grown in rotation with wheat, barley, lentils, and peas. In 1987, about 4,500 ha of kabuli-type chickpeas were planted in the Palouse region.

Seed rot and preemergence damping-off caused by *Pythium ultimum* Trow is the most important soilborne disease of kabuli chickpeas in the Palouse (11). In Spain, the disease is not as prevalent in spring chickpea plantings (24) but can be severe in fall-winter plantings (24). *Pythium* spp. may be important in fall-winter commercial sowings of kabuli chickpea cultivars resistant to Ascochyta blight in southern Spain where increased yield and a reduction in severity of other important diseases, such as Fusarium wilt, results from fall seedings (23,24). Although several *Pythium* spp. are pathogens of chickpea (5,15,19), almost all the work on Pythium seed and root rot of chickpea has been conducted with *P. ultimum* (11,12,27). In the Pacific Northwest, *Pythium* spp. have also been implicated in diseases of peas, beans (16), and wheat (4,9,26).

Although metalaxyl and captan (11) control seed rot and preemergence damping-off of chickpea caused by *P. ultimum*, some farmers are interested in growing chickpeas organically (i.e., without pesticides) in response to consumer demand. Therefore, alternative means of disease control should be devised to meet the needs of both growers and consumers. Seed treatments with *Penicillium oxalicum* Currie & Thom (12) and fluorescent pseudomonads (14) have been tested with promising results; however, there is a need for more tests comparing the efficacy of biological and chemical methods in controlling Pythium disease.

The objectives of this study were to examine the etiology of Pythium seed rot and preemergence damping-off of kabuli chickpeas in the Palouse region and in southern Spain, to compare pathogenicity of the *Pythium* spp. isolated most frequently from rotted seeds and damped-off seedlings, and to compare the efficacy of various biological control agents against Pythium seed rot to chemical seed treatments in growth chamber and field studies.

## MATERIALS AND METHODS

**Isolation and identification of *Pythium* species.** Rotted chickpea seeds and diseased seedlings and soil samples were collected from commercial and experimental chickpea fields in the Palouse region and in Córdoba Province, Spain. Seeds and seedling roots were washed gently for 30 min in running tap water, surface-disinfested in 1% NaOCl for 3 min, rinsed twice in sterile, distilled water, and blotted dry on paper towels. Pieces of tissue were plated on 2% water agar (WA) or WA amended with rifampicin (50 mg/L) (10). After 48 hr at 23–26 C, hyphal tips of colonies of *Pythium* spp. growing from the plant tissues were transferred to potato-carrot agar (PCA) (25) and stored at 10 C. The soil dilution procedure and Mircetich's pimaricin-vancomycin agar medium (18) were used for direct isolation of *Pythium* spp. from soil and to estimate inoculum density in naturally and artificially infested soils.

Identification of *Pythium* to species or variety was based on the characteristics given in the monograph of van der Plaats-Niterink (25). Growth rates, colony morphology, and presence and dimensions of oogonia, antheridia, and oospores were determined from cultures growing on PCA. To induce formation of sporangia and zoospores, agar blocks of mycelium from PCA were transferred to petri plates containing a sterile solu-

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tion of dilute salts (6) plus several pieces of grass leaf (*Poa* sp.), boiled previously for 10 min in distilled water (25). To favor the discharge of zoospores, the dilute salts solution was changed frequently in combination with incubation temperatures at 10 and 20 C. Isolates of *P. ultimum* var. *ultimum* Trow, *P. ultimum* var. *sporangiiferum* Drechsler, *P. irregulare* Buisman, and *P. oligandrum* Drechsler from the American Type Culture Collection (ATCC) and from wheat (2) were also used to confirm the identification of the *Pythium* spp. used in this study.

*Pythium* spp. used in the pathogenicity studies were *P. irregulare* isolates Pi-1 (chickpea, Washington), Pi-2 (chickpea, Spain) and Pi-3 (chickpea, Washington); *P. u.* var. *sporangiiferum* isolates Pus-C1 (chickpea, Washington) and Pus-C2 (chickpea Spain); and *P. u.* var. *ultimum* isolates Puu-C (chickpea, Washington) and Puu-W (wheat, Washington). The *Pythium* spp. were isolated from rotted seeds or plant roots.

**Pathogenicity tests in the greenhouse and growth chamber.** All greenhouse, growth chamber, and field experiments were carried out with a large-seeded (100 seeds per 50 g), cream-colored chickpea cultivar (PI 458870) that is highly susceptible to Pythium seed rot and preemergence damping-off (11). Spofford silt loam soil from Central Ferry (11), Washington, was used in all experiments. Soil was passed through a 3-mm screen, air-dried, and stored in metal cans in the greenhouse. "*Pythium*-free" soil was obtained by treating the soil with live steam (100 C) for 60 min (10).

In a preliminary test, 27 *Pythium* spp. were grown in a sterilized mixture of sand-cornmeal (sand, water, and cornmeal in a 9:2:1 ratio (w/w), respectively) for 14 days. Inoculum of each isolate was incorporated into steamed field soil (10% w/w) that was then placed in plastic pots (15-cm diam.). Noninfested control pots received the sterile sand-cornmeal mixture without fungus. Ten untreated chickpea seeds were planted in each of five replicated pots.

To compare pathogenicity of *P. ultimum* and *P. irregulare*, isolates were grown in potato-carrot broth (PCB) amended with two drops of wheat germ oil (sterols) per liter to stimulate production of oospores. Mycelial mats were rinsed with water, comminuted with distilled water in a Waring blender at low speed for 2 min, and then added to small quantities of steamed, air-dried field soil. Infested soils were incubated in plastic bags for 3–5 days at 20 C. After being air-dried for 48 hr, infested soils were passed through a 3-mm screen, spread on clean paper, air-dried for an additional 48 hr, and stored in plastic bags at 4 C for up to 40 days before use. Appropriate dilutions of stock infested soils were made with steamed soil to provide desired inoculum densities of the *Pythium* spp. Inoculum densities of test soils were determined before use in each assay. Potting media were placed in plastic pots (15-cm diam.) and watered from the bottom until the soil was saturated. Chickpea seeds were placed on the soil surface and covered 2–2.5 cm deep with air-dried soil of the corresponding treatment. Steamed soil served as the control. Pots with seedlings were watered as needed thereafter to maintain soil moisture near "field capacity" (−30 K Pa) (10).

Comparative pathogenicity of isolates Puu-C and Pi-2 was tested by planting ungerminated chickpea seeds or established chickpea seedlings in two experimental soils infested with one isolate of the pathogen at 50 or 500 colony-forming units per gram (cfu/g) of air-dried soil, ranges commonly found in field soils in the Pacific Northwest and southern Spain (Table 1). Chickpea seedlings were germinated in vermiculite for 10 days and then the roots were carefully washed in tap water. Healthy seedlings were transplanted to infested soil at the same time as the ungerminated seeds. Seedlings and seeds planted in a steamed field soil from Central Ferry and in a nontreated potting medium (55% peat moss, 35% pumice, 10% sand) free of *Pythium* were used as control treatments. Ten seeds or five seedlings were planted in each of three replicate pots. The experiment was carried out in the greenhouse (18–24 C) in pots arranged in randomized complete block designs.

The influence of temperature on pathogenicity of *P. ultimum* and *P. irregulare* was tested in growth chambers at 10, 15, 20, and 25 C with a 14-hr photoperiod (324 $\mu$E $m^{-2}$ $sec^{-1}$) or in an outdoor lath house where temperatures ranged from 2 to 32.5 C (8.6, 23.4, and 15.9 C average minimum, maximum, and daily temperature, respectively) from 15 May to 9 June 1987. Soil used in these experiments was steam-sterilized at 100 C for 60 min. The growth chamber and lath house experiments were set up as a split-block design with three replications (pots) and five seeds per pot. Plants were grown at different temperatures for approximately 350 degree-days (base 0 C) until plants had five to seven leaves. Pathogenicity of fungal isolates was based on total emergence, plant height, and plant fresh weight (tops). The experiment was conducted twice.

**Pathogenicity tests in the field.** One isolate each of *P. u.* var. *ultimum*, *P. u.* var. *sporangiiferum*, and *P. irregulare* was tested for pathogenicity in field soil at Central Ferry. Plots were covered with 4-mil thick black polyethylene tarps and fumigated with a mixture of methyl bromide (98%) and chloropicrin (2%) at a rate of 70 g/$m^2$ of soil. Tarps were removed six days after treatment, and the soil was infested with each of the three *Pythium* isolates the following day.

Inoculum of *Pythium* spp. for the field experiment consisted of steamed soil reinfested with each isolate at 500 cfu/g or sand-cornmeal inoculum of each respective isolate mixed with steamed soil (38% w/w). The experiment was composed of single-row plots (5 m long, with 1.5 m between rows) in a split-plot design with four replications. Natural soil, fumigated soil, and fumigated soil that received either steamed soil or autoclaved *Pythium*-infested sand-cornmeal mixture served as controls. Inoculum was placed in manually opened furrows at a rate of 1 kg per 5-m row. The plots were seeded with 100 chickpea seeds 7 days after removal of the tarps (4 May 1987). The population density of *Pythium* spp. in natural and fumigated soil was determined in samples collected 2.0–2.5 cm deep on 24 April; 6, 13, 19 May; 2, 29 June; and 17 August.

Emergence counts of chickpea seedlings were made on 19 May and 2 June. (Only 2 June data is presented in Table 4.) Average yield per 5-m row in each plot was determined after the chickpeas were harvested (17 August). Ungerminated seeds were dug from the plots and attempts were made to isolate *Pythium* spp.

**Seed treatment for growth chamber and field experiments.** The six fungicide treatments tested in growth chamber and field experiments were fosetyl-Al (Aliette 80WP), thiabendazole (TBZ) (Mertect LSP 30%), captan (Orthocide 50WP), benalaxyl (MF-695, 25WP), metalaxyl (Apron 28% FL), and metalaxyl (45WP) plus TBZ (24WP) (Apron T69). The five biological seed treatments tested were *Penicillium oxalicum* (ATCC 52658) (12), *Pythium oligandrum* (D. M. Ingram, *unpublished*), *Rhizobium* sp. (*Cicer*), *Pseudomonas fluorescens* biovar I (Q29z-80) (14), and *P. fluorescens* biovar II (W4F1080) (D. Rhodes, *unpublished data*).

The effects of the fungicide and biological seed treatments were tested in naturally infested soil both in the field and in the growth chamber at 15 C. Fungicide slurries were prepared by mixing each fungicide with 40 ml of water in 2-L widemouth Erlenmeyer flasks. The seeds (300 g) and fungicide slurry were agitated for about 5 min until all the liquid was absorbed by the seeds. The seeds were immediately air-dried for 2–3 hr in a vented hood. Seeds agitated with and without water alone constituted the "wet" and "dry" controls, respectively.

The dry method of Kaiser and Hannan (12) was used to treat chickpea seeds with *P. oxalicum* (4 mg conidia per gram of seed). Two days after treatment, the conidial concentration per gram of seed was checked by placing five treated seeds in 100 ml of distilled water in each of two 250-ml flasks. Flasks with seeds were immersed in a Bronson ultrasonic cleaner

(Bronson Cleaning Equipment Co., Shelton, CT) for 30 sec to remove all conidia adhering to the seeds. A hemacytometer was used to count the concentration of conidia in the wash water of the two flasks, which was determined to be $6 \times 10^7$ conidia per gram of seed.

Chickpea seeds were coated with oospores of *P. oligandrum* produced on PCB (10). The final concentration of oospores was $7.2 \times 10^4$ per gram of seed. The two strains of *P. fluorescens*, Q29z-80 and W4F1080, were applied to chickpea seeds by the method of Weller and Cook (26) at $10^8$ cfu/g of seed. Bacterial seed treatments and oospores of *P. oligandrum* were applied to seed in 1% methylcellulose and a methylcellulose control was included. A commercial peat formulation of *Rhizobium* sp. (*Cicer*) ($10^9$ cfu/g of peat) was applied to chickpea seeds with gum arabic serving as the sticker.

**Field experiments with seed treatments.** A seed treatment experiment was conducted at Central Ferry in soil naturally infested with *P. ultimum* (90 cfu/g). Eleven treatments were tested in single-row plots (5 m long, 1.5 m row spacing) arranged in a randomized complete block design with five replications. Seeds were planted 3.5–4.0 cm deep on 4 May 1987 with a tractor-driven, single-row cone planter. Emergence counts were made on 19 May and 2 June. (Only 2 June data is presented in Table 5.) Average yield was determined for each plot after harvest (17 August). Populations of *Pythium* were monitored throughout the growing season in both the fumigated and natural soils. Soil samples were taken from the fumigated plots and the nonfumigated checks. Soil dilutions (1:100) were made of the samples to determine populations of *Pythium* spp.

## RESULTS

**Isolation and identification of *Pythium* species.** *P. ultimum* was the most prevalent species isolated from rotted chickpea seeds, roots, and field soils in the Palouse region, whereas *P. irregulare* and *P. oligandrum* were isolated most frequently from plant tissues and soils in southern Spain (Table 1). *P. u.* var. *ultimum* and *P. u.* var. *sporangiiferum* differed only by the ability of *P. u.* var. *sporangiiferum* isolates to produce zoospores in water culture. The inoculum level of *P. irregulare* and *P. oligandrum* in chickpea soils of southern Spain was over 550 cfu/g, compared to less than 150 cfu/g in Palouse soils (Table 1). The characteristics of these fungi matched the descriptions given by van der Plaats-Niterink (25), but chickpea isolates of *P. irregulare* had very few projections and did not produce zoospores in water culture. Unknown *Pythium* spp. were isolated occasionally from both Spanish and Palouse soils, but because they were not pathogenic to chickpea, no additional work was done on identification.

**Pathogenicity tests in the greenhouse and growth chamber.** Using soil infested with sand-cornmeal inoculum in the greenhouse, three isolates of each variety of *P. ultimum* and 10 isolates of *P. irregulare* were pathogenic to chickpea, causing 100%, 100%, and 82% seed rot and preemergence damping-off, respectively. Six isolates of *P. oligandrum* and the five isolates of the unknown *Pythium* spp. were not pathogenic to chickpea.

A complete lack of emergence occurred in the greenhouse potting mixture and field soil infested with *P. u.* var. *ultimum* either at 50 or 500 cfu/g. *P. irregulare* caused a significant reduction and delay in chickpea emergence in field soil but not in the greenhouse potting mixture (Table 2). Although *P. irregulare* did not cause seed rot and preemergence damping-off in the greenhouse potting mix, chickpea seedlings were slightly stunted and their fresh weight was reduced about 28% (Table 2). Ten-day-old chickpea seedlings planted in both types of soil infested with *P. ultimum* or *P. irregulare* at 50 and 500 cfu/g were asymptomatic and grew similarly to those of the control. No damage to the feeder roots was observed in these plants.

Both *P. u.* var. *ultimum* and *P. u.* var. *sporangiiferum* were highly pathogenic to chickpeas grown in artificially infested (500 cfu/g) soil in the growth chamber at 10, 15, 20, and 25 C and in an outdoor lath house. There was no emergence at any temperature when untreated seeds were sown in soil infested with either *P. u.* var. *sporangiiferum* isolate C1, or *P. u.* var. *ultimum* isolates C or W (Table 3). Isolate Pi-1 was pathogenic to chickpeas grown between 10 and 20 C and in the outdoor lath house but did not result in any damage at 25 C (Table 3). This isolate also reduced both plant height and fresh weight of seedlings at all temperatures tested. Isolates Pi-2 and Pi-3 caused 30 and 58%, respectively, fewer emerged seedlings in the outdoor lath house trial.

**Pathogenicity tests in the field.** The type of inoculum used in field trials at Central Ferry did not influence the effect of the introduced *Pythium* spp. on plant growth, thus, results were averaged for presentation. Both varieties of *P. ulti-*

**Table 1.** Frequency of isolation and soil population of *Pythium* spp. associated with seed rot and preemergence damping-off of chickpea in southern Spain and the U.S. Pacific Northwest

| | Fields infested[a] (no.) | | *Pythium* cfu/g of soil[b] (mean no.) | |
|---|---|---|---|---|
| *Pythium* spp. | Spain | USA | Spain | USA |
| *P. irregulare* | 5 | 2 | 567 | 145 |
| *P. oligandrum* | 4 | 4 | 561 | 120 |
| *P. ultimum* | 1 | 6 | 92 | 194 |
| *Pythium* spp. | 3 | 2 | 130 | 31 |

[a] For each country, six fields with a history of seed rot and preemergence damping-off of chickpea were sampled. Isolations were made from rotted chickpea seeds, diseased seedlings, and soils.
[b] Soil dilutions were plated on Mircetich's pimaricin-vancomycin agar medium (18).

**Table 2.** Emergence and fresh weight of chickpea grown in two soils infested with *Pythium ultimum* var. *ultimum* isolate Puu-C and *P. irregulare isolate* Pi-2

| *Pythium* isolate | Soil[w] | Inoculum density[x] (cfu/g) | Emergence (%)[y] 1 wk | Emergence (%)[y] 3 wk | Fresh weight (g/plant) |
|---|---|---|---|---|---|
| Puu-C | G | 50 | 0 | 0 | 0 |
| | | 500 | 0 | 0 | 0 |
| | CF | 50 | 0 | 0 | 0 |
| | | 500 | 0 | 0 | 0 |
| Pi-2 | G | 50 | 96.7 a[y] | 96.7 a | 1.8 b |
| | | 500 | 93.3 a | 93.3 a | 2.1 b |
| | CF | 50 | 76.7 b | 80.0 b | ND[z] |
| | | 500 | 30.0 c | 56.7 c | ND |
| Control | G | 0 | 100.0 c | 100.0 a | 2.7 a |
| | CF | 0 | 93.3 a | 93.3 a | ND |
| LSD (0.05) | | | 8.19 | 6.19 | 0.39 |

[y] Untreated seeds planted 10 per pot in 15-cm-diameter plastic pots, three pots per treatment. Emergence counts were taken 1 and 3 wk after planting.
[w] G = greenhouse potting medium (55% peat moss, 35% pumice, 10% sand); CF = Spofford silt loam soil collected from Central Ferry, Washington.
[x] Inoculum (colony-forming units per gram (cfu/g) of air-dried soil) of each isolate was adjusted by diluting stock infested soil with steamed (100 C, 60 min) CF soil to achieve a desired initial inoculum density.
[y] Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).
[z] ND = no data collected.

*mum* reduced emergence of chickpeas in fumigated, reinfested field soil (Table 4), but *P. irregulare* did not. Both varieties of *P. ultimum* caused a 90% failure of seedlings to emerge as compared to the fumigated control. *P. irregulare* was not pathogenic as measured by the number of emerged seedlings. Only 40% of the seedlings emerged in natural soil.

Yield was reduced by more than 75% by both varieties of *P. ultimum* as compared to yield from either fumigated soil or treaments receiving steamed soil or autoclaved *Pythium* (Table 4). *P. irregulare* did not reduce yield in the field test. Yield was reduced by 40% in natural soil when compared with that obtained from plants grown in fumigated soil.

Soil dilutions of naturally infested Spofford silt loam from Central Ferry indicated that the *P. ultimum* population density was about 90 cfu/g of air-dried soil at the time of seeding (4 May). Two wk after planting, population counts peaked at 200 cfu/g and slowly declined over the next 2–3 wk to about 90 cfu/g. Population density of *P. ultimum* in fumigated soil was nearly undetectable, with anywhere from 0 to 13 cfu/g over the growing season.

**Seed treatment experiments in the field.** Treating chickpea seeds with either metalaxyl, captan, benalaxyl, or metalaxyl plus TBZ resulted in greater than 89% emergence in naturally infested field soil (Table 5). Seedling emergence in fumigated field soil was equivalent to that from these seed treatments. Significantly fewer (43%) seedlings emerged when seeds were treated with fosetyl-Al as compared to the most effective fungicide seed treatments (metalaxyl or metalaxyl plus TBZ) but resulted in 25 times more emerged seedlings when compared to the wet control, in which only 2% of the seedlings emerged. Seed treatment with TBZ alone was ineffective.

Seed treated with *P. oligandrum*, *P. oxalicum*, and *P. fluorescens* strain Q29z-80 resulted in seedling emergence ranging from 42 to 52% (Table 5). Treating seeds with *Rhizobium* sp. (*Cicer*) or *P. fluorescens* strain W4F1080 did not significantly improve the emergence in naturally infested soil when compared to the wet control.

The greatest yields resulted from treatments that gave the best emergence. Metalaxyl plus TBZ, metalaxyl, captan, benalaxyl, and fumigation with methyl bromide each resulted in yields greater than 1,000 g per plot (Table 5), with metalaxyl plus TBZ yielding the best (>1,200 g of seed per 5-m row). The yield of the fosetyl-Al treatment was only 43% of the yield of the metalaxyl plus TBZ-treated seed (Table 5). Thiabendazole alone was ineffective in improving seed yield over that of the wet control. *P. fluorescens* strain Q29z-80 resulted in yield statistically equivalent to that of the metalaxyl plus TBZ treatment. *P. oxalicum* seed treatment resulted in only 16% less seed weight than *P. fluorescens* strain Q29z-80 (Table 5). The emergence of seeds treated with *P. oligandrum* was not significantly different from that of seeds treated with *P. fluorescens* strain Q29z-80 or *P. oxalicum*, but yield from this treatment was only about half of that of the metalaxyl plus TBZ treatment. Yields obtained with *Rhizobium* sp. (*Cicer*) and *P. fluorescens* strain W4F1080 were not significantly better than that from the wet control treatment. Yield from the dry control was significantly greater than that from the wet control (Table 5).

**Seed treatment experiments in the growth chamber.** The results of two separate growth chamber experiments were similar and the values were averaged. None of the seed treatments was detrimental to seed germination and seedling emergence when the treated seeds were sown in soil that had been steamed to eliminate *Pythium*. In soil naturally infested with *P. ultimum* (90 cfu/g), almost no seedlings emerged from seeds treated with fosetyl-Al, *P. oligandrum*, *P. fluorescens* strain W4F1080, methylcellulose, TBZ, *Rhizobium* sp. (*Cicer*), or treated dry or wet controls (Table 6). Fifty and 70% of seedlings emerged when seeds were treated with *P. oxalicum* or *P. fluorescens* strain Q29z-80, respectively. The best emergence (greater than four out of five seeds average) occurred in the steamed soil control, metalaxyl, metalaxyl plus TBZ, benalaxyl, and captan treatments (Table 6). Plant height and fresh weight were not affected significantly by any of the fungicide treaments. With *P. oxalicum* and *P. fluorescens* strain Q29z-80, however, there was a significant reduction in plant height and fresh weight of

**Table 3.** Influence of *Pythium irregulare* isolates Pi-1, Pi-2, and Pi-3, *P. ultimum* var. *sporangiiferum* isolate Pus-C1, and *P. ultimum* var. *ultimum* isolates Puu-C and Puu-W on emergence of chickpeas grown in a growth chamber[w] at 10, 15, 20, and 25 C and in an outdoor lath house in sterilized, reinfested field soil

| | Emergence (no. of seedlings)[x] | | | | |
|---|---|---|---|---|---|
| **Isolate** | **25 C** | **20 C** | **15 C** | **10 C** | **Lath house[y]** |
| Control | 5.0 a[z] | 5.0 a | 4.7 a | 5.0 a | 4.7 a |
| Pi-1 | 5.0 a | 3.7 b | 1.3 b | 1.0 b | 1.3 cd |
| Pus-C1 | 0 b | 0 c | 0 c | 0 c | 0 d |
| Puu-C | 0 b | 0 c | 0 c | 0 c | 0 d |
| Pi-2 | NT | NT | NT | NT | 3.3 ab |
| Pi-3 | NT | NT | NT | NT | 2.0 bc |
| Puu-W | NT | NT | NT | NT | 0 d |
| LSD (0.05) | 0.57 | 0.57 | 0.57 | 0.56 | 1.6 |

[w] Soil from Central Ferry, Washington, was treated with live steam (100 C) for 60 min and reinfested with 500 colony-forming units per gram (cfu/g) of air-dried soil of each *Pythium* spp., respectively.
[x] Values are average of two experiments, each with three replications (pots) of five seeds per pot. NT = not tested.
[y] Average daily temperature was 16 C.
[z] Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

**Table 4.** Emergence and yield of chickpeas grown in fumigated, *Pythium*-reinfested soil or natural soil at Central Ferry, Washington

| **Treatment[w]** | **Emergence[x]** | **Yield (g)[y]** |
|---|---|---|
| Fumigated only (methyl bromide) | 85 a[z] | 1,350 a |
| Fumigated plus autoclaved inoculum | 87 a | 1,344 a |
| *P. irregulare* (Pi-1) | 88 a | 1,295 a |
| Control (natural soil) | 40 b | 807 b |
| *P. ultimum* var. *sporangiiferum* (Pus-C1) | 10 c | 336 c |
| *P. ultimum* var. *ultimum* (Puu-C) | 9 c | 293 c |
| LSD (0.05) | 16 | 228.5 |

[w] Plots were fumigated with methyl bromide (70 g/m$^2$) and covered with black polyethylene tarps removed 6 days after treatment. Initial population density of *Pythium ultimum* in natural soil at the time of sowing was 90 colony-forming units per gram (cfu/g) of air-dried soil. Fumigated soil was reinfested with *Pythium* spp. either in steamed soil as oospores (500 cfu/g) or sand-cornmeal inoculum mixed with steamed soil (38% w/w), placed in the seed furrow (1.0 kg per 5-m row).
[x] 100 seeds were planted in each 5-m row. Numbers represent mean emergence from eight single-row plots of the two types of inoculum 29 days after seeding.
[y] Yield data expressed as average seed weight per 5-m row.
[z] Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

emerged seedlings when compared to those from the metalaxyl plus TBZ treatment.

## DISCUSSION

*P. u.* var. *sporangiiferum*, *P. u.* var. *ultimum*, and *P. irregulare* were the only species pathogenic to chickpea among 25 isolates of *Pythium* obtained from chickpea seeds and roots and chickpea field soils of eastern Washington, northern Idaho, and southern Spain. The two varieties of *P. ultimum* were the most prevalent species isolated from chickpea tissues and soil in the Pacific Northwest, whereas in southern Spain, *P. irregulare* was more frequently isolated from the substrates. *P. ultimum* from either wheat or chickpea was the most pathogenic species on chickpea regardless of inoculum density or environmental conditions, and it resulted exclusively in seed rot and preemergence damping-off. In the field trial at Central Ferry, *P. ultimum* reduced emergence and yield by 90 and 75%, respectively. Both varieties of *P. ultimum* were pathogenic, differing only in the ability of *P. u.* var. *sporangiiferum* to produce zoospores (25). Production of zoospores varied among isolates C1 and C2 of *P. u.* var. *sporangiiferum*. This is the first report of *P. ultimum* as a pathogen of chickpea in Spain. Zoospore-producing isolates of this species have not been reported previously from chickpea, although *P. ultimum* is an important pathogen of this crop in several countries (19).

*P. irregulare* was only moderately pathogenic, and disease severity was highly influenced by the type and density of inoculum and environmental conditions. In steamed field soil, isolates of *P. irregulare* were more pathogenic at higher inoculum densities and lower temperatures, resulting in reduced emergence and stunting. The fungus was weakly pathogenic in nontreated greenhouse potting mix, and it was not pathogenic under field conditions. *P. irregulare* has not been reported on chickpea previously, although it is pathogenic to a large number of legumes as well as other crop plants, especially to seedlings at low temperatures (8,10, 25). Like other *Pythium* spp., pathogenicity of *P. irregulare* is highly influenced by inoculum density and environmental conditions (8,10,22). The soil temperatures at Central Ferry (17–20 C at the time of seeding) favored seed germination and seedling emergence resulting in lack of damage, in contrast to damage by this species at constant low temperatures in growth chamber studies. Besides other environmental factors, such as solar radiation and soil moisture, antagonists present in fumigated but not in steamed field soil may also contribute to differences in the pathogenicity of *P. irregulare* in field and growth chamber experiments. Nonetheless, *P. irregulare* may cause significant damage to chickpeas seeded into cool, moist soils as may occur in winter plantings in southern Spain and spring plantings in the Palouse region.

Under conditions favorable to disease development, ungerminated chickpea seeds were susceptible to *P. ultimum* and *P. irregulare*; however, 10-day-old chickpea seedlings were resistant. This phenomenon is well-known in other plant species and several *Pythium* spp., including *P. ultimum* and *P. irregulare* (7,8,22), and has been described with seedlings of chickpea and *P. ultimum* (11). We did not observe any macroscopic differences between healthy and inoculated roots of 60-day-old chickpea plants in greenhouse experiments when 10-day-old seedlings were inoculated with *P. ultimum* and *P. irregulare*.

Several fungicide seed treatments provided excellent control of seed rot caused by *Pythium* spp. in naturally infested soil

**Table 5.** Influence of fungicide and biological seed treatments on emergence and yield of chickpeas grown in naturally infested[v] soil at Central Ferry, Washington

| Seed treatment | Rate[w] | Emergence (%)[x] | Yield (g)[y] |
|---|---|---|---|
| Metalaxyl + Thiabendazole | 0.5 + 0.27** | 97 a[z] | 1231 a |
| Metalaxyl | 0.5** | 95 a | 1011 abc |
| Captan | 3.0** | 92 a | 1085 ab |
| Benalaxyl | 0.5** | 89 a | 1038 ab |
| Control (methyl bromide) | ... | 85 a | 1110 ab |
| *Pythium oligandrum* | $7.2 \times 10^4$ oospores* | 52 b | 626 de |
| Fosetyl-Al | 0.5** | 52 b | 701 cde |
| *Pseudomonas fluorescens* (Q29z-80) | $1.0 \times 10^8$ cfu* | 51 b | 960 abcd |
| *Penicillium oxalicum* | $6 \times 10^7$ conidia* | 42 b | 806 bcde |
| *Pseudomonas fluorescens* (W4F1080) | $1.0 \times 10^8$ cfu* | 11 c | 240 fg |
| *Rhizobium* sp. (*Cicer*) | 5 mg peat inoculum* | 5 c | 89 g |
| Control (methylcellulose) | 1% (0.25 ml)* | 9 c | 324 fg |
| Thiabendazole | 3.0** | 2 c | 83 g |
| Control (dry) | ... | 19 c | 521 ef |
| Control (wet) | 0.1 ml $H_2O$* | 2 c | 57 g |
| LSD (0.05) | | 21 | 326 |

[v] Initial population density of *Pythium ultimum* at time of seeding was 90 colony-forming units per gram (cfu/g) of air-dried soil.
[w] Asterisks represent application rate. * = concentration per gram of seed, ** = gai/kg of seed.
[x] 100 seeds were planted in each 5-m row. Numbers represent mean emergence from five single-row plots 22 days after seeding.
[y] Yield data expressed as average seed weight in grams per 5-m row.
[z] Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

**Table 6.** Influence of fungicide and biological seed treatments on emergence and yield of chickpeas grown in naturally infested[w] soil at Central Ferry, Washington

| Seed treatment[x] | Emergence[y] | Mean plant height (cm) | Mean fresh weight (mg/plant) |
|---|---|---|---|
| Control (steamed soil) | 4.8 a[z] | 15.4 ab | 762 abc |
| Metalaxyl + Thiabendazole | 4.8 a | 16.1 a | 910 a |
| Metalaxyl | 4.7 a | 16.5 a | 922 a |
| Benalaxyl | 4.5 a | 16.2 a | 889 a |
| Captan | 4.2 ab | 14.2 abc | 871 ab |
| *Pseudomonas fluorescens* (Q29z-80) | 3.5 b | 12.0 c | 526 c |
| *Penicillium oxalicum* | 2.5 c | 12.9 bc | 638 bc |
| Fosetyl-Al | 0.7 d | 0 | 0 |
| *Pythium oligandrum* | 0.5 d | 0 | 0 |
| *Pseudomonas fluorescens* (W4F1080) | 0.2 d | 0 | 0 |
| Control (dry) | 0 d | 0 | 0 |
| Control (wet) | 0 d | 0 | 0 |
| Control (methylcellulose) | 0 d | 0 | 0 |
| Thiabendazole | 0 d | 0 | 0 |
| *Rhizobium* sp. (*Cicer*) | 0 d | 0 | 0 |
| LSD (0.05) | 0.7 | 2.9 | 245 |

[w] Initial population density of *Pythium ultimum* at time of seeding was 90 colony-forming units per gram of air-dried soil.
[x] See Table 5 for application rates.
[y] Emergence data are the average of two experiments with three replicate pots, each with five seeds. Final emergence counts were taken after 15 days, at which time the test was terminated.
[z] Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD ($P = 0.05$).

both in the field and growth chamber. With respect to seedling emergence and yield, metalaxyl, captan, benalaxyl and metalaxyl plus TBZ were at least as effective as fumigation with methyl bromide. In contrast, seed dressings with fosetyl-Al or thiabendazole had little or no effect on controlling the disease. Fosetyl-Al is not generally used as a seed treatment to control diseases caused by *Pythium* (3), and thiabendazole is recommended as a seed treatment to control seedborne *Ascochyta rabiei* (Pass.) Labr., an important pathogen of chickpea (13).

Moderate protection against Pythium seed rot in the field was obtained with three biological seed treatments. *P. fluorescens* strain Q29z-80 provided the best yield in the field trial and was statistically equivalent to the fungicide seed treatments. In the growth chamber study, *P. fluorescens* strain Q29z-80 was equivalent to captan, with respect to emergence and plant height, and with the steamed soil control with respect to plant fresh weight. In a 3-yr study at two locations in the Palouse region, Kaiser et al (14) reported that the bacterial seed treatment did not perform as well as chemical control measures or seed treatment with *P. oxalicum* in preventing seed rot and preemergence damping-off. They attributed the variability to environmental conditions over the period of the study. In the current study, *P. fluorescens* strain Q29z-80 was at least as effective as *P. oxalicum* in controlling seed rot and preemergence damping-off in naturally infested field soil. Although emergence of chickpea seed treated with *P. fluorescens* strain Q29z-80 and *P. oxalicum* was about half that of chickpea seeds treated with fungicides, yields for both biological treatments were statistically equivalent to most of the fungicide seed treatments. Chickpeas usually compensate for fewer plants per area by growing larger and producing more seeds per plant (12).

Seed treatment with *P. oligandrum* also provided protection from Pythium seed rot in field studies. Seeds treated with oospores of the fungus emerged, and plants yielded at rates similar to that of *P. fluorescens* strain Q29z-80 and *P. oxalicum*-treated seeds but yielded somewhat less than that of the chemical control measures. This biocontrol agent was ineffective in preventing seed rot and preemergence damping-off in controlled temperature experiments. This discrepancy may be attributed to more favorable environmental conditions for disease in the controlled experiments as indicated by differences in emergence of untreated chickpea seeds in natural soil (about 10% in the field experiment vs. 0% in controlled experiments). Soil factors, especially soil moisture, may be implicated in the difference observed in control of seed rot of field-sown chickpeas and those planted in the growth chamber. Soil in the growth chamber experiments was saturated before seeds were placed on the soil surface and then covered with air-dried soil. In the field, soil moisture was less than field capacity at the time of seeding and may have been more favorable for oospore germination and mycelial growth of *P. oligandrum* in the spermosphere. In order for biocontrol by *P. oligandrum* to be effective, the oospores must germinate and occupy the host seed spermosphere before *P. ultimum* (17).

The isolate of *P. oligandrum* used in this study was not pathogenic to chickpea, because treated seeds emerged and grew well in steamed soil. Control of damping-off of sugar beet seedlings caused by *P. ultimum* has been obtained with *P. oligandrum* oospores placed on seed (12,500 per seed) (17). In our study, about three times the number of oospores used by Martin and Hancock (17) was placed on chickpea seeds (36,000 per seed), but chickpea seeds are at least five times larger than sugar beet seeds. It is possible that the quantity of oospores placed on chickpea seed was adequate to provide effective control in the field where disease was somewhat less than that encountered in growth chamber tests.

Viability of oospores of *P. oligandrum* on seed did not appear to be a factor in the efficacy of the hyperparasite, because oospores remained germinable on chickpea seeds stored at 10 C for 7–8 wk after treatment (D. M. Ingram, *unpublished data*). Although hyphae of *P. ultimum* and *P. irregulare* were parasitized by *P. oligandrum* in dual cultures (D. M. Ingram, *unpublished data*), the evidence implicating parasitism as a mechanism of disease control is not conclusive and possibly other mechanisms, such as competition, are more important in protecting seeds from pathogens in the soil (17).

*P. fluorescens* strain W4F1080 from potatoes reduced tuber rot caused by *P. ultimum* (D. Rhodes, *unpublished data*), but it is ineffective in protecting chickpea seed from this pathogen. Biocontrol agents will probably be most effective when they are isolated from the host plant that the pathogen is attacking (26) or isolated from soils where rotations with the crops is practiced.

Buonassisi et al (10) demonstrated that a majority of the *Rhizobium* strains from bean (*Phaseolus vulgaris* L.) inhibited growth of *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyder & Hansen, but not isolates of *P. ultimum* or *P. sylvaticum* Campbell & Hendrix. The *Rhizobium* strain specific for chickpea used in our study did not improve emergence or yield in naturally infested soil in the field or growth chamber. Our results corroborate the failure of *Rhizobium* spp. to inhibit *Pythium* spp. in previous reports (1).

Seeds treated with water alone (wet) yielded significantly less than seeds treated dry. Although the seed treatment process is short (2–3 hr from wet to dry), imbibition may be initiated and the subsequent drying may lead to cracks in the seed coat. This increases the amount of *Pythium* damage to pea seeds (20,21), possibly because of increased leaching of nutrients from the seed through the ruptured seed coats, which stimulates growth of the pathogen enabling it to rapidly penetrate and colonize the seed.

In southern Spain, chickpeas traditionally have been cultivated as a spring crop. However, with the availability of new chickpea cultivars that are resistant to Ascochyta blight, more growers are switching to fall-winter plantings because of increased yields and decreased incidence of other important diseases, such as Fusarium wilt, which can be very damaging to spring-planted chickpeas. Kabuli-type chickpeas are grown almost exclusively in Spain. In the Pacific Northwest, kabuli chickpeas are very susceptible to seed rot and preemergence damping-off caused by *Pythium* spp. (11), and seeds must be treated with a fungicide, such as captan, to prevent serious losses from *Pythium*. In Spain, chickpea seeds generally are not treated with fungicides before seeding in the spring. However, some type of seed treatment probably will be required if Spanish growers start planting kabuli cultivars in the fall or winter, when weather conditions (cool and wet) will favor Pythium seed rot and preemergence damping-off. The results of our seed treatment tests with different fungicides and biocontrol agents provide valuable information on seed treatments that would be worthy of study to control the Pythium disease in kabuli chickpeas in Spain.

In the United States, the public is becoming increasingly concerned about the adverse effects of pesticides on the environment and human health. As a result, more stringent regulations and laws are being passed that will restrict or eliminate the use of certain pesticides. In the Pacific Northwest, control of Pythium seed rot and preemergence damping-off of chickpea is achieved mainly by treatment of seeds with captan, which is one of the few fungicides registered for use on this crop. However, the future of captan and other possible fungicides effective against *Pythium* spp. on chickpea is in doubt. Consequently, there is a pressing need to develop safe, effective, and economical alternative methods of controlling this important chickpea disease. We obtained very encouraging results in control of Pythium seed rot of chickpea with several biocontrol agents. Now might be an opportune time to devote more research toward developing biocontrol methods for commercial application before we no

longer have access to chemicals like captan.


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