Greenhouse Evaluation of Seed and Drench Treatments for Organic Management of Soilborne Pathogens of Spinach

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ABSTRACT

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The efficacy of 14 seed and drench treatments for control of soilborne damping-off pathogens in organic production of spinach was evaluated in a greenhouse study. The efficacy of each treatment was compared with nontreated seed and seed treated with a conventional fungicide for control of *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, and *Rhizoctonia solani*. Two experimental seed treatments, GTG I and GTG II (each comprised of a proprietary organic disinfectant and the latter also containing *Trichoderma harzianum* T22), provided equivalent control to the conventional fungicide, mefenoxam, against *P. ultimum* in one trial and significant reduction of damping-off in the second trial. Natural II and Natural X (*Streptomycete* products), and Subtilex (*Bacillus subtilis*) seed treatments each suppressed damping-off significantly in one of the two trials. For *R. solani*, GTG I and Natural II seed treatments reduced damping-off as effectively as a drench with the fungicide Terraclor (pentachloronitrobenzene). A soil drench with Prestop (*Gliocladium catenulatum*) suppressed postemergence wilt caused by *F. oxysporum* in both trials; a compost tea drench and seed treatment with Yield Shield (*Bacillus pumilis*) each suppressed postemergence wilt in only one of two trials. GTG I and GTG II significantly increased seed germination compared to nontreated seed. No treatment was effective against all three pathogens, and some treatments exacerbated damping-off.

In 1990, the U.S. Congress passed the Organic Foods Production Act (OFPA) as part of the U.S. Farm Bill in an effort to establish consistent organic production standards nationwide by implementing federally mandated organic standards. Disease management in organic production systems can be particularly challenging because organic producers do not have the option to utilize all of the conventional agricultural disease management tools. Thus, organic producers rely on methods of disease management such as crop rotation, cover cropping, and approved organic pesticides, including formulations of biological control organisms approved for organic production standards (24).

The demand for organically produced seed has increased since the rules of the United States Department of Agriculture (USDA) National Organic Program (NOP) have required the use of organic seed in organic production if organic seed is available that meets the desired characteristics of a grower's markets (14). Concern over losses due to seedborne and soilborne pathogens has also increased because of the limited effective options available for seed treatments that satisfy organic standards (14). Seed treatments can be inexpensive and an effective means of plant disease management (42). Biological control agents that reduced damping-off caused by *Pythium* spp. on a variety of crops include, for example, *Enterobacter cloaceae*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Rhizobium leguminosarum* bv. *viceae*, *Trichoderma* spp., and compost teas (20,21,33,37,43). Similarly, *Pseudomonas aeruginosa*, *P. fluorescens*, *Pseudomonas putida*, *Trichoderma harzianum*, *Gliocladium* spp., and *Bacillus amyloliquefaciens* reduced damping-off caused by *Rhizoctonia* spp. on various crops (4,7,15, 17,28,29,39); and *Burkholderia cepacia*, *E. cloaceae*, nonpathogenic *Fusarium* spp., *Gliocladium virens*, *Glomus intraradices*, *Glomus mosseae*, *P. fluorescens*, *P. putida*, *Rhizobium leguminosarum*, and *Trichoderma* spp. reduced wilts caused by *Fusarium* spp. (2,12,13,26,27,36). Although many commercial biological seed and drench treatments have been developed to protect seed and seedlings against soilborne plant pathogens, there is a need for seed treatments approved for use in organic production that are effective against a diversity of soilborne pathogens. Various products have been developed for organic production for which the registrants claim efficacy against particular soilborne pathogens, although results of independent studies in which these claims have been evaluated sometimes have been highly variable (16).

The purpose of this research was to provide an objective evaluation of seed and drench treatment products that have received U.S. Environmental Protection Agency (EPA) registration for use in organic production in the United States, as well as products that have the potential for approved use in organic production, for control of soilborne seedling blight or damping-off diseases of vegetables. Three pathogens were selected for evaluating these treatments on the basis of individual and collective impacts on seedling blight and damping-off complexes of many small-seeded vegetables: *Rhizoctonia solani* Kühn, which causes seed rot and damping-off (40); *Fusarium oxysporum* f. sp. *spinaciae* (Sherb.) Snyd. & Hans., the cause of seedling blight and vascular wilt of spinach (5); and *Pythium ultimum* Trow, the cause of pre- and postemergence damping-off of seedlings (18). Spinach (*Spinacia oleracea*) is susceptible to many prevalent soilborne pathogens that cause seedling blights and damping-off, and is a common crop for organic leafy vegetable markets (8). Therefore, spinach was used in this study as a model small-seeded vegetable crop for investigating efficacy of the selected seed and drench treatments for control of soilborne pathogens in organic production systems. The specific objectives were to evaluate seed and drench treatments on spinach in a greenhouse against each pathogen, determine if any of the seed treatments affect the incidence of necrotrophic fungi on spinach seed, and determine if any of the seed treatments affect spinach seed germination.

MATERIALS AND METHODS

Pathogen isolates. The isolate of *P. ultimum* used in this study, '030141', was originally obtained from wheat seedlings in the Palouse Region of Washington State by T. Paulitz (USDA ARS, Pullman, WA); its ability to cause disease on spinach seedlings was verified prior to this study (L. J. du Toit, *unpublished*). The isolate was preserved on potato dextrose agar (PDA) and water agar (WA) slants in test tubes, and stored at 4°C with transfers to new slants every 6 months.

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The isolate of *R. solani* used in this study, VSP 05-01B, was originally obtained from an onion plant in Washington State in 2005 and verified as pathogenic on spinach (L. J. du Toit, *unpublished*). The isolate was identified as belonging to anastomosis group 4 (AG4) and hyphal group II (HGII) by C. Pagani at North Carolina State University (Raleigh), on the basis of morphological characterization, nuclear condition, anastomosis grouping, and DNA sequencing of the internal transcribed spacer (ITS) regions 1 and 2 and the 5.8S rDNA region (40). For long-term preservation of the isolate, 250 g of rye seed was soaked in 0.5 liter of deionized water for 24 h, autoclaved at 120°C and 103 kPa for 90 min, cooled for 24 h, and autoclaved a second time for 90 min at 120°C at 103 kPa. Ten plugs (each 5 mm in diameter) were taken from the edge of actively growing colonies of the *R. solani* isolate on PDA, added to the rye seed, and incubated on a lab bench for 2 weeks for the fungus to colonize the rye seed. The flasks were shaken intermittently to promote colonization of the seed. The colonized seed was then dried for 1 week in a fume hood and stored in a refrigerator. Isolates of the pathogen were preserved by macerating the colonized rye seed using a coffee grinder, and storing the macerated tissue at 4°C. The isolates were also stored on colonized filter disks at –20°C. For the latter, four 1.5 cm diameter sterile filter disks (VWR Scientific Products, West Chester, PA) were arranged on PDA in a 6 cm diameter petri dish, and a colonized plug (5 mm diameter) taken from the edge of an actively growing PDA culture of the isolate was placed in the middle of the filter disks. Once the filter disks were covered with mycelium of the fungus, the disks were removed, placed in sterile coin envelopes (5.7 × 8.9 cm) (Westvaco Envelope Division, Springfield, MA), dried overnight in a laminar flow hood, and stored with desiccant at –20°C (35).

Isolate '001' of *F. oxysporum* f. sp. *spinaciae* was obtained from a wilted spinach plant in a spinach seed crop trial at the Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU Mount Vernon NWREC) in Mount Vernon in 2001 and was verified as pathogenic on spinach prior to this study (L. J. du Toit, *unpublished*). For long-term storage, a culture in 50 ml of Kerr's broth (23) in a flask was placed on a shaker for 5 days for production of microconidia. Aliquots (3 ml) of the microconidial suspension were dispensed into 20 ml vials each containing 15 g sterile soil/sand mixture (1:1 ratio), and shaken vigorously to disperse spores in the soil. The vial was kept at room temperature (approximately 24°C) for 2 days and then at 4°C for long-term preservation of the pathogen. The isolate was also stored on filter disks as described for *R. solani*.

Inoculum concentration trials. To optimize trials, inoculum concentration assays were carried out in a greenhouse for each pathogen to determine the concentration of inoculum that would achieve approximately 50% damping-off or wilt when nontreated seed was planted under the conditions of these trials. To produce inoculum for the assays, nonsterile Puget silt loam field soil (Klungland and McArthur, 1989) from the WSU Mount Vernon NWREC was sieved (1 mm size mesh) and then air-dried for 2 days on a greenhouse bench. Ground oatmeal (Quaker, Chicago, IL) was added (1% by weight) to the dried, sieved soil and mixed thoroughly in a PK Blendmaster soil blender (Paterson-Kelley Co. Division of Harsco Corp., East Stroudsburg, PA) for 10 min. During the last 5 min of mixing, deionized water (15% wt/wt) was added to the soil/oatmeal mix in the blender. Next, 500 g of this mixture was added to a 0.95 liter glass jar. The jar was topped with an autoclavable plastic lid used for mushroom spawning (Fungi Perfecti, Olympia, WA), with a 1.27 cm diameter hole drilled into the lid and a 70 mm diameter synthetic filter disk (Fungi Perfecti) placed beneath the lid. The lid was covered with two layers of aluminum foil and the jar and contents autoclaved for 50 min at 120°C and 103 kPa. Each jar was then autoclaved again approximately 24 h later for 50 min. The soil/oatmeal mixture was stored at room temperature until used to produce inoculum.

Three- to four-day-old cultures of *P. ultimum*, *R. solani*, or *F. oxysporum* f. sp. *spinaciae* grown on PDA were used to inoculate the soil/oatmeal mix individually. After five colonized agar plugs, each approximately 9 mm^3, were placed in each jar, the jars were shaken manually. The jars were then placed on a lab bench at room temperature for 4 weeks until the soil/oatmeal mix was thoroughly colonized by the pathogen, and then stored at 4°C until used in the greenhouse trials. The concentration of inoculum in the soil/oatmeal mix for each pathogen was quantified using soil-dilution plating. Each jar was shaken vigorously. A 10 g sample of inoculum was then added to a 200 ml flask containing 90 ml of 0.1% WA (sloppy agar) to suspend the inoculum, and the flask was placed on a rotating platform shaker for 12 min. Tenfold dilutions of each suspension were carried out to 10^{-4} using sloppy agar. Dilutions of *F. oxysporum* f. sp. *spinaciae* inoculum were plated onto Komada's agar medium (25) and incubated at room temperature on a laboratory bench. *F. oxysporum* colonies were counted after 4 days to determine the number of CFU/g inoculum. The *P. ultimum* inoculum dilution series was plated similarly onto a *Pythium*-selective medium (PSM) (30). The plates were incubated in the dark at room temperature because rose Bengal and rifampicin in the medium are light-sensitive. Colonies of *P. ultimum* were counted after 40 to 48 h to calculate CFU/g inoculum. The *R. solani* inoculum dilution series was plated onto WA and incubated at room temperature on a lab bench. *R. solani* colonies were counted 24 h after plating to determine CFU/g inoculum.

Approximately 1,200 g of wetted Sunshine Growers Organic potting mix (Sun Gro Horticulture) was used in each 30.5 × 30.5 × 6.4 cm flat for inoculum concentration assays. Inoculum concentrations of 0, 500, 1,000, 5,000, and 10,000 CFU/g wetted potting mix were evaluated for damping-off or wilt caused by each of the three pathogens. However, the concentrations proved too low for *F. oxysporum* f. sp. *spinaciae* and *R. solani* (data not shown), so the trials were repeated for these two pathogens using 0, 5,000, 10,000, 50,000, and 100,000 CFU/g, as well as 500,000 CFU/g for *F. oxysporum* f. sp. *spinaciae*. Similarly, the treatment of 50 CFU/g was not included in the second *P. ultimum* trial. Because of excessive wilt at 100,000 and 500,000 CFU/g in the first *F. oxysporum* f. sp. *spinaciae* trial, these concentrations were not included in the second trial. For each pathogen, the potting medium was mixed with the appropriate volume of soil/oatmeal inoculum for 5 min using a Gustafson Batch Lab Treater (Gustafson Equipment, Bayer CropScience, Shakopee, MN). The inoculated potting mix was then portioned into flats (1,200 g/flat). Thirty-six seeds of the spinach hybrid 'Lazio' (Pop Vriend Seeds BV, Andijk, the Netherlands) were planted per flat (six rows of six seeds planted at a 5 cm spacing within and between rows). Lazio was selected due to the popularity of this hybrid in the fresh market, susceptibility of the hybrid to damping-off pathogens, and resistance to 10 races of the spinach downy mildew pathogen (22). A separate trial was carried out for each pathogen in a greenhouse at approximately 25 ± 5°C using a randomized complete block design with five replications of the five or six inoculum concentrations. Each trial was repeated once.

The incidence of seedling emergence and the incidence of postemergence damping-off or wilt were counted in each flat at 3- to 4-day intervals for 32 to 56 days, depending on how rapidly symptoms developed and progressed. In the repeated trial for each pathogen, ratings were completed every 7 days. Flats were fertilized weekly with an OMRI-listed organic fertilizer (Alaska Fish Fertilizer, 5:1:1, Lilly Miller Brands, Walnut Creek, CA) at 7.9 ml/liter water. The OMRI-listed insecticides Entrust (spinosad, Dow AgroSciences, Indianapolis, IN) and AzaDirect (azadirachtin, Gowan Company, Yuma, AZ) were applied to the seedlings at 0.17 g/liter water and 1.6 ml/liter water, respectively, as necessary for thrips and aphid

management, respectively. Isolations were carried out in each trial on randomly selected seedlings to confirm the pathogen responsible for damping-off or wilt. For each trial, five symptomatic and three asymptomatic seedlings (including roots) were rinsed under running tap water and the foliage above the crown removed. The crown and roots were surface-sterilized in 0.6% NaOCl for 60 s, triple rinsed in sterile water, dried on sterile paper towel, cut into 5 mm long pieces, plated onto PDA and WA, and incubated on a lab bench for 3 to 5 days. Fungi and oomycetes that developed were identified microscopically to genus. In the repeated trial for each pathogen, all of the emerged seedlings in each flat were cut at the soil line at the final rating. The aboveground foliage was dried at 32°C (Model 1370F forced air oven, VWR Scientific Products, West Chester, PA) for 3 days.

Seed and drench treatment trials. On the basis of the inoculum concentration trials, the concentration of inoculum that resulted in approximately 50% mortality of spinach seedlings 5 to 7 weeks after planting was selected for evaluating seed and drench treatments: 1,000 CFU/g for *P. ultimum*, 10,000 CFU/g for *F. oxysporum* f. sp. *spinaciae*, and 50,000 CFU/g for *R. solani*. Excessive preemergence damping-off was observed in the first *R. solani* trial, so the concentration was reduced to 25,000 CFU/g for the second *R. solani* trial. Fourteen biological and/or organic seed or drench treatments and a conventional fungicide seed or drench treatment were evaluated in greenhouse assays against each of the three pathogens. Details of the treatments are shown in Table 1. The two control treatments included nontreated seed planted in inoculated potting mix and nontreated seed planted in noninoculated potting mix. Each trial was arranged as a randomized complete block design with five replications. For each experimental unit, six rows of six seed (cv. Lazio) were planted into a 30.5 × 30.5 × 6.4 cm tall flat with 5 cm within and between rows. Each trial for each pathogen was repeated.

Each treatment was applied at the highest rate recommended by the label or the manufacturer. GTG I, GTG II, Natural II, and Natural X seed treatments were applied by the respective companies. All other products were applied at the WSU Mount Vernon NWREC. The compost tea, brewed by C. Crosby at the WSU Crop and Soil Sciences Department (Pullman, WA),

Table 1. Seed and drench treatments evaluated in greenhouse trials for efficacy against damping-off and vascular wilt of spinach caused by *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *spinaciae*[u]

Treatment[v]	Active ingredient (concentration in product)	Registrant or manufacturer	Method of application	Rate of product application[w]	OMRI-listed[x]
Compost tea	High bacterial diversity compost tea[y]	Washington State University, Pullman	Drench immediately after planting	646 liters/100 m^3 potting mix	Yes
GTG I	Proprietary organic disinfectant (unknown rate)	Germain's Technology Group, Gilroy, CA	Seed treatment	Proprietary	Not yet applied
GTG II	Proprietary organic disinfectant + *Trichoderma harzianum* T-22 (unknown rates)	Germain's Technology Group	Seed treatment	Proprietary	Not yet applied
Kodiak Concentrate Biological Fungicide	*Bacillus subtilis* (1.37%)	Bayer CropScience, Research Triangle Park, NC	Slurry seed treatment	31.2 g/100 kg seed	Yes
Micro 108 Seed Inoculant + Actinovate AG	*Streptomyces lydicus* (10^8 CFU/g) + *S. lydicus* (10^7 CFU/g)	Natural Industries, Houston, TX	Dry seed coating + drench immediately after planting	1.76 kg/100 kg seed + 1.29 kg/100 liters water	Yes
Mycostop Mix Biofungicide	*Streptomyces griseoviridis* (4%)	Verdera Oy, Luoteisrinne, Finland	Dry seed coating	625.7 g/100 kg seed	Yes
Natural II	Actinomycete (0.6%)	Agricoat LLC, Soledad, CA	Seed treatment	750.7 g/100 kg seed	No
Natural X	Actinomycete (0.6%)	Agricoat LLC	Seed treatment	750.7 g/100 kg seed	No
PGPR Galaxy	Bacterial mixture[z]	Holmes ENVIRO, LLC, Philomath, OR	Slurry seed treatment	223 ml/100 kg seed	Yes
Prestop Biofungicide Powder	*Gliocladium catenulatum* (32%)	Verdera Oy	Drench immediately after planting	180 g/100 liters water	No
SoilGard 12G Microbial Fungicide	*Gliocladium virens* (12%)	Certis USA, Columbia, MD	Drench >24 h before planting	239.7 g/100 liters water	Yes
Subtilex Biological Fungicide	*Bacillus subtilis* (2.75%)	Becker Underwood, Ames, IA	Slurry seed treatment	15.6 g/100 kg seed	No
T-22 Planter Box Biological Fungicide	*Trichoderma harzianum* T-22 (1.15%)	BioWorks, Victor, NY	Dry seed coating + drench 4 days after planting	250 g/100 kg seed	Yes
Yield Shield Concentrate Biological Fungicide	*Bacillus pumilis* (0.28%)	Bayer CropScience	Slurry seed treatment	6.26 g/100 kg seed	Yes
Apron XL LS	Mefenoxam (33%)	Syngenta Crop Protection, Greensboro, NC	Slurry seed treatment	20.8 ml/100 kg seed	No
Mertect 340F	Thiabendazole (42.3%)	Syngenta Crop Protection	Slurry seed treatment	122.4 ml/100 kg seed	No
Terraclor 75% WP	Pentachloronitrobenzene (75%)	Crompton Uniroyal Chemical, Middlebury, CT	Drench immediately after planting	59.9 g/100 liters water	No
Nontreated seed	–	–	–	–	–

[u] Each trial was a randomized complete block design with four or five replications. Potting mix was inoculated with the selected pathogen at a concentration determined by inoculum concentration trials, and planted with the spinach hybrid Lazio.

[v] Products were evaluated separately against each pathogen. Some products were not approved by the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) for use in organic systems in 2007 (see OMRI website for a list of approved products). Micro 108 Seed Inoculant + Actinovate AG, Mycostop Mix Biofungicide, T-22 Planter Box Biological Fungicide, and Apron XL LS were approved by the Washington State Department of Agriculture for use on certified organic spinach crops in Washington in 2007 when this study was completed. Apron XL LS, Mertect 340F, and Terraclor 75% WP were conventional treatments for *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., respectively. Nontreated seed was also a control treatment.

[w] Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar size seed or according to registrant recommendations. Drenches were applied according to the label based on surface area or volume of potting medium treated.

[x] Not yet applied = registrant had not applied for OMRI approval by March 2007.

[y] Ingredients of the compost tea: vermicompost (50 ml/liter), seaweed powder (1 ml/liter), liquid humic acids (2 ml/liter), and Azomite rock dust (3 g/liter) (37). The compost tea was brewed by C. Crosby at Washington State University, Pullman, where the tea was aerated for 24 h prior to shipment to the WSU Mount Vernon NWREC, where the tea was applied at 0.6 liter/1,200 g potting mix/flat.

[z] PGPR Galaxy contains *Bacillus azotofixans*, *Azotobacter chroococcum*, *Pseudomonas putida*, and *Pseudomonas fluorescens* (each at 304 × 10^9 cells/liter).

was developed for high bacterial diversity, and was brewed with vermicompost, seaweed powder, liquid humic acids, and azomite rock dust as described by Scheuerell and Mahaffee (37). The compost tea was brewed the day prior to planting each trial and shipped overnight to the WSU Mount Vernon NWREC.

The number of emerged seedlings and the number of damped-off or wilted seedlings were counted weekly for 4 to 7 weeks, depending on the pathogen. *P. ultimum* and *R. solani* each caused significant preemergence damping-off, followed by postemergence damping-off within 3 weeks of emergence. Therefore, trials with *P. ultimum* and *R. solani* were terminated 4 to 5 weeks after planting. Trials with *F. oxysporum* f. sp. *spinaciae* were continued up to 7 weeks since this pathogen primarily caused postemergence wilt 4 to 7 weeks after planting. Trials with *P. ultimum* and *R. solani* were completed at 25 ± 5°C, whereas the *F. oxysporum* f. sp. *spinaciae* trials were carried out at 28 ± 3°C to enhance expression of vascular wilt as a result of increased transpirational demand of seedlings at higher temperatures.

The plants in each trial were fertilized weekly with the OMRI-listed organic fish fertilizer as described for the inoculum concentration trials, but mixed with a seaweed extract fertilizer (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) applied at 2.5 g/liter water. The seedlings in each flat were cut at the soil line at the final rating to determine total dry foliar biomass/flat. Isolations onto PDA and WA were completed from five symptomatic and three asymptomatic seedlings randomly selected from each trial, as described for the inoculum concentration trials.

Because the pH of the potting medium may impact biological control organisms (16), and aggressiveness of plant pathogens may be affected by pH (10), the pH of the potting mix in a subset of the trials was measured after the mix was moistened and immediately before planting. For each pH assessment, three 10 g samples of potting mix were sampled randomly from the flats. Each sample was added to 30 ml deionized water, stirred for 30 s, left standing for 10 min, and the process repeated twice before the pH was measured using a Symphony pH meter (VWR).

Health assay of treated seeds. A modified freeze-blotter seed health assay was carried out for each of the seed treatments to determine the incidence of seed infected with various necrotrophic fungi, as described by du Toit et al. (11) with slight modifications. All seed treatments were assessed in the seed health assays, including two conventional seed treatments (Apron XL LS and Mertect 340F) and nontreated seed. Four replications of 100 seeds were assayed per treatment. The seeds were arranged on sterile Steel blue germination blotters (8.25 cm diameter, Anchor Paper Co., St. Paul, MN) moistened with 5 ml sterile water in 10 cm diameter plastic petri plates (20 seeds per plate). After the plates were sealed with Parafilm, the seeds were left to imbibe for 25 h. The plates were then moved to a –20°C freezer for 25 h to kill the embryos, thawed at room temperature for 15 to 30 min, and placed in an incubator (Model I30BLL, Percival Scientific, Perry, IA) maintained at 24°C for 14 days with a 12-h/12-h day/night cycle (near-UV and fluorescent white light by day). The seeds were examined microscopically (×10 to ×100 magnification) 5, 9, and 14 days after plating. Necrotrophic fungi developing on the seed were identified based on morphological characteristics. The seed health assay was repeated using a different commercial seed lot of Lazio spinach that was also used in the second greenhouse seed and drench treatment trial for each pathogen, because there was insufficient seed of the first lot remaining after the first set of greenhouse trials.

Germination assay of treated seeds. For each of the two seed lots used in the seed and drench treatment trials, 100 seeds per treatment were subjected to a germination assay based on the protocol of the Association of Official Seed Analysts (44). Fifty seeds were placed between two layers of seed germination blotters (25.4 × 38.1 cm, 38# regular weight; Anchor Paper Co.) moistened with deionized water. For each replication of each treatment, each of two sets of blotters containing 50 seeds was rolled up in a sheet of waxed paper (61 × 91.4 cm; Anchor Paper Co.) and placed upright in a plastic bag in a seed germinator (Stults Scientific Engineering Corp., Springfield, IL) at 15°C with no light. The incidence (%) of seed germinated after 7, 14, and 21 days was counted. At the final rating, the incidence of seeds with abnormal germination and the incidence of rotten seeds were counted. In addition, a 5 day reading was included in the second assay to determine if any treatments caused significantly earlier germination compared with nontreated seed.

Statistical analyses. Analyses of variance (ANOVAs) and means comparisons using Fisher's protected least significant difference (LSD at $P < 0.05$) were carried out using PROC GLM of SAS (Version 9.1, SAS Institute, Cary, NC) for each of the dependent variables in the seed health assays, germination assays, inoculum concentration trials, and seed and drench treatment trials. Friedman's nonparametric rank test was used when the data and transformations (logarithmic, square root, or arcsine square root) did not meet assumptions for parametric analyses, i.e., normally distributed residuals with homogeneous variances (41). For each trial, the percentage of preemergence damping-off per flat was calculated by subtracting the percentage of nonemerged seedlings for the nontreated seed in noninoculated medium [= 36 – (number of emerged seedlings)] from the percentage of nonemerged seedlings in that flat. Postemergence damping-off or wilt was calculated as a percentage of emerged seedlings. Total aboveground dry biomass per flat was determined as described above, as well as the area under emergence progress curve (AUEPC), area under preemergence disease progress curve ($AUDPC_{pre}$), area under postemergence disease progress curve ($AUDPC_{post}$), and area under total disease progress curve ($AUDPC_{total}$). The area under emergence or disease progress curves is a cumulative measurement calculated as an average of emergence or disease ratings over time: $[(\Sigma(y_i + y_{i+1}/2)(t_i - t_{i+1})]$, in which y_i = number of emerged or diseased seedlings at the ith rating, y_{i+1} = number of emerged or diseased seedlings at the $(i + 1)$ rating, t_i = number of days at the ith rating, and t_{i+1} = number of days at the $(i + 1)$ rating (38). Data were analyzed separately for repeated trials because of different inoculum concentrations evaluated in the repeated inoculum concentration trials, and because of significant trial main effects and interaction terms in the combined ANOVAs.

RESULTS

Inoculum concentration trials. The soil/oatmeal inoculation method for *P. ultimum*, *F. oxysporum* f. sp. *spinaciae*, and *R. solani* produced damping-off or wilt of spinach seedlings (Fig. 1). Isolations from symptomatic seedlings confirmed the presence of the appropriate pathogen. The pathogens were not isolated from asymptomatic seedlings.

P. ultimum caused pre- and postemergence damping-off at similar incidences. In trial 1, emergence in noninoculated flats was 92.3 ± 2.4% (mean ± standard error) by 32 days after planting (dap) versus 60.8 to 68.3% at 500 to 5,000 CFU/g, respectively (Fig. 1A) (not significantly different among these concentrations at $P < 0.05$). Concentrations of 100 to 5,000 CFU/g resulted in 30 to 65% damping-off by 32 dap (Fig. 1A). However, total preemergence and postemergence damping-off at 500, 1,000, and 5,000 CFU/g were not significantly different (Fig. 1A). Total damping-off at 50 CFU/g was only 8.7 ± 2.5%. In trial 2, emergence in noninoculated flats by 35 dap averaged 91.7 ± 2.5%, and ranged from 56.1 to 65.0% in inoculated flats (Fig. 1B). The incidence of damping-off did not differ significantly at 100 to 5,000 CFU/g by 35 dap (50.8 to 74.2%; Fig. 1B). Total, preemergence, and postemergence damping-off did not differ significantly at 500 to 5,000 CFU/g (Fig. 1B). Biomass of noninoculated seedlings was 2.37 ± 0.2 g and declined with increasing inoculum concentration (1.12 ±

0.1 and 0.65 ± 0.1 g at 100 and 500 CFU/g, respectively), but was not significantly different from 500 to 5,000 CFU/g (0.62 ± 0.1 and 0.46 ± 0.1 g at 1,000, and 5,000 CFU/g, respectively) (data not shown).

Preemergence damping-off caused by *R. solani* occurred at a greater incidence than postemergence damping-off at the highest inoculum concentrations (Fig. 1C and D, respectively). Inoculum concentration significantly ($P < 0.05$) affected emergence as well as preemergence, postemergence, and total damping-off weekly in both trials, except postemergence wilt 7 dap (data not shown). In trial 1, emergence in noninoculated flats reached 80.6% 32 dap versus 85.6, 70.6, 57.8, and 12.8% at 5,000, 10,000, 50,000, and 100,000 CFU/g, respectively. Similarly, preemergence and postemergence damping-off was greatest at 100,000 CFU/g (67.8 and 34.0%, respectively). Total damping-off 32 dap ranged from 7.0 to 88.0% at 5,000 to 100,000 CFU/g (Fig. 1C), with no significant difference at 5,000 CFU/g versus noninoculated flats (1.3%). Total damping-off at 50,000 CFU/g (40.5%) was not significantly different than at 10,000 CFU/g (30.8%) but less than at 100,000 CFU/g (88.4%) (Fig. 1C). In trial 2, emergence in noninoculated flats, which reached 92.2% by 35 dap, was greater than in inoculated flats each week (data not shown). Final emergence was not significantly different from 10,000 to 50,000 CFU/g (68.9 to 75.6%), but was significantly less at 75,000 CFU/g (40.6%) (Fig. 1D). Preemergence damping-off was not significantly different from 10,000 to 50,000 CFU/g (16.1 to 22.8%) by 35 dap, but was greatest at 75,000 CFU/g (51.1% by 35 dap) (Fig. 1D). In contrast, postemergence damping-off was not significantly different at 25,000 to 75,000 CFU/g (18.4 to 20.2%). Total damping-off 35 dap was not significantly different at 25,000 and 50,000 CFU/g (35.2 to 43.0%, respectively), but was greater at 75,000 CFU/g (69.5%) (Fig. 1D). Dry spinach biomass declined significantly with increasing inoculum concentration, but was not significantly different at 10,000 to 50,000 CFU/g (2.68 to 3.22 g). Biomass was greatest in the noninoculated flats (4.08 g) and least at 75,000 CFU/g (1.62 g) (data not shown).

Inoculation with *F. oxysporum* f. sp. *spinaciae* did not significantly affect emergence or preemergence damping-off, but inoculum concentration significantly affected postemergence wilt and total wilt from 21 to 32 dap in trial 1 (Fig. 1E), and 28 to 56 dap in trial 2 (Fig. 1F). In trial 1, emergence 32 dap ranged from 83.3 to 89.4% across all treatments (Fig. 1E). Postemergence wilt was first observed 14 dap, but a majority of wilt did not develop until 21 to 32 dap. Postemergence wilt 32 dap was not significantly different at 50,000 to 500,000 CFU/g (91.8 to 100%), but was less at 10,000 CFU/g (52.1%), and significantly less than in noninoculated flats

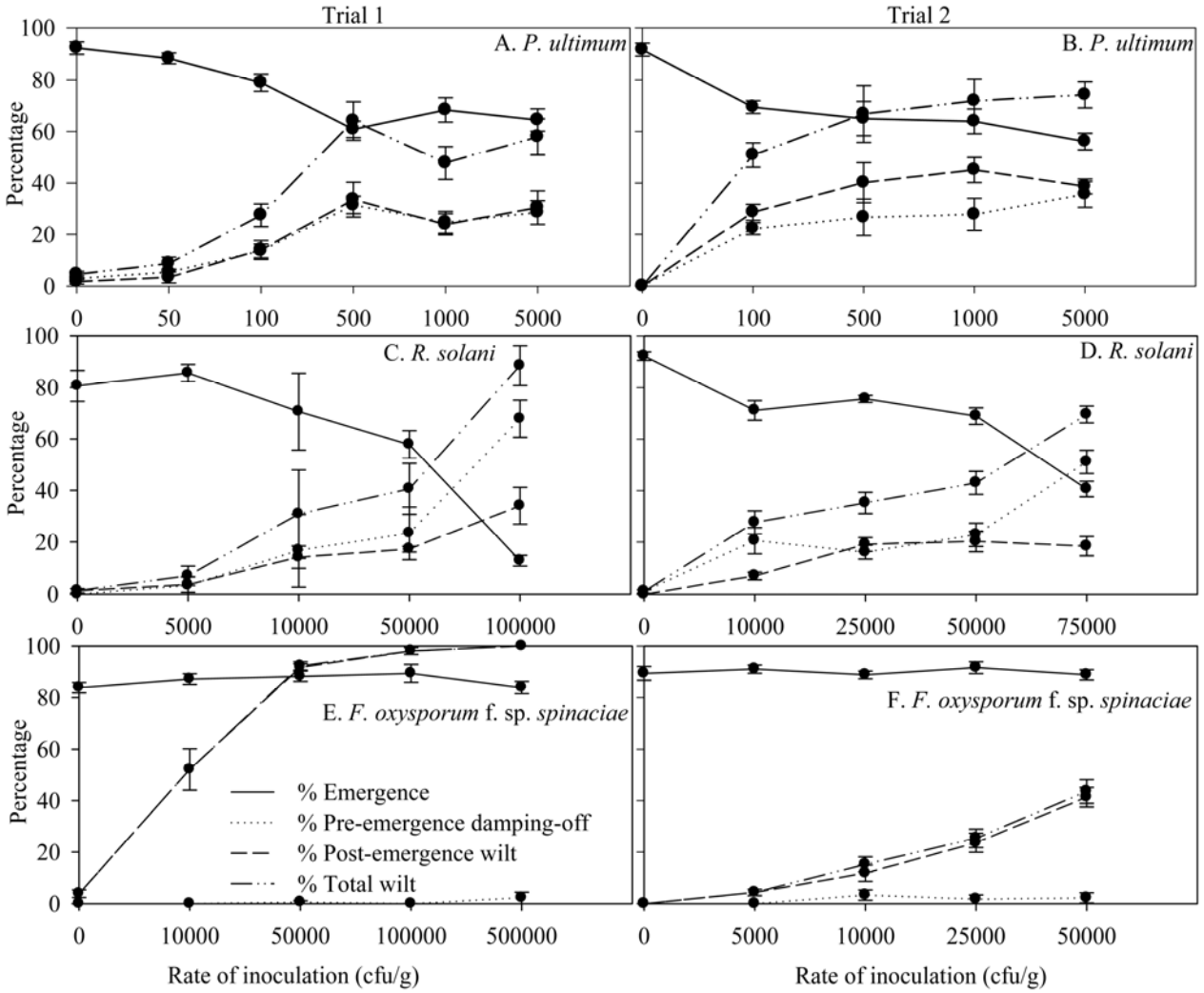

Fig. 1. Emergence, preemergence damping-off, postemergence wilt, and total disease (pre- and postemergence) caused by *Pythium ultimum* **(A and B)**, *Rhizoctonia solani* **(C and D)**, and *Fusarium oxysporum* f. sp. *spinaciae* **(E and F)** in greenhouse trials evaluating the effect of inoculum concentration on spinach seedlings. Emergence and disease were measured weekly, with results shown for the final rating. For *P. ultimum*, trial 1 **(A)** was completed over 32 days, and trial 2 **(B)** over 35 days, each at 25 ± 5°C. For *R. solani*, trial 1 **(C)** was completed over 32 days, and trial 2 **(D)** over 35 days, each at 25 ± 5°C. For *F. oxysporum* f. sp. *spinaciae*, trial 1 **(E)** was completed over 32 days at 25 ± 5°C, and trial 2 **(F)** over 56 days at 28 ± 5°C. Each data point is the mean ± standard error of five replications.

(3.9%). In trial 2, emergence 56 dap was not significantly different among inoculum concentrations (88.9 to 91.7%, Fig. 1F). Preemergence damping-off was only 1.4% across all treatments. Postemergence and total wilt averaged 41.3 and 43.5%, respectively, at 50,000 CFU/g, and only 11.8 and 15.2%, respectively, at 10,000 CFU/g. Seedling biomass at 0, 5,000, and 10,000 CFU/g was not significantly different (19.59 to 21.75 g), but was less than at 25,000 and 50,000 CFU/g (16.95 and 14.92 g, respectively) (data not shown).

Damping-off caused by *P. ultimum* was 43.5 ± 6.4% in trial 1 and 71.9 ± 8.4% in trial 2 for flats inoculated at 1,000 CFU/g (Fig. 1A and B). Therefore, 1,000 CFU/g was selected as the inoculation rate for the *P. ultimum* seed and drench treatment trials. For *R. solani*, 50,000 CFU/g was selected because this concentration resulted in 40.5 ± 9.9% damping-off in trial 1 and 43.0 ± 4.5% in trial 2 (Fig. 1C and D). Wilt caused by *F. oxysporum* f. sp. *spinaciae* at 10,000 CFU/g was 52.1 ± 8.0% in trial 1 but only 15.2 ± 2.9% in trial 2 (Fig. 1E and F).

***P. ultimum* seed and drench treatment trials.** Inoculation of potting medium with 1,000 CFU/g of *P. ultimum* differentiated efficacy of the seed and drench treatments in both trials (Table 2). The treatments had a significant effect on emergence, preemergence damping-off, and postemer-

Table 2. Greenhouse evaluation of seed and drench treatments for management of damping-off caused by *Pythium ultimum* in organic spinach[u]

Trial and treatment[w]	Seed or drench application[x]	% Emergence (28 or 35 days)	% Damping-off (28 or 35 days)[v]		Total dry weight (g, 28 or 35 days)[y]
			Preemergence	Postemergence	
Trial 1					
Compost tea	Drench	80.0 b	11.1 e	16.6 e	4.09 c
GTG I	Seed treatment	95.6 a	0.0 f	3.5 fg	5.39 ab
GTG II	Seed treatment	95.6 a	0.6 f	6.4 f	6.06 a
Kodiak Concentrate Biological Fungicide	Seed treatment	79.5 b	11.1 e	43.5 cd	2.61 d
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	67.2 de	23.3 bc	41.3 d	2.30 def
Mycostop Mix Biofungicide	Seed treatment	67.8 de	22.8 bc	59.4 ab	1.87 f
Natural II	Seed treatment	93.9 a	1.7 f	0.6 g	4.49 bc
Natural X	Seed treatment	90.6 a	2.8 f	7.4 f	4.03 c
PGPR Galaxy	Seed treatment	71.1 bcde	19.5 bcd	51.8 abcd	2.30 def
Prestop Biofungicide Powder	Drench	47.8 f	42.8 a	57.3 ab	0.85 g
SoilGard 12G Microbial Fungicide	Drench	64.4 e	26.1 ab	41.4 d	2.41 def
Subtilex Biological Fungicide	Seed treatment	75.0 bcd	15.6 cde	50.6 abcd	2.56 de
T-22 Planter Box Biological Fungicide	Seed treatment + drench	69.4 cde	21.1 bcd	54.7 abc	1.99 def
Yield Shield Concentrate Biological Fungicide	Seed treatment	78.3 bc	12.2 e	61.3 a	1.95 ef
Apron XL LS	Seed treatment	94.4 a	0.0 f	2.4 fg	5.07 b
Nontreated seed in inoculated medium	–	77.8 bc	12.8 de	48.5 bcd	2.28 def
Nontreated seed in noninoculated medium	–	90.6 a	0.0 f	3.7 fg	4.55 bc
LSD ($P < 0.05$)[z]		9.09	Rank	Square root	Log
Trial 2					
Compost tea	Drench	62.8 de	28.9 ab	47.2 abcde	1.24 g
GTG I	Seed treatment	92.2 a	0.6 e	15.8 gh	4.37 ab
GTG II	Seed treatment	95.6 a	0.0 e	29.5 fgh	3.70 bc
Kodiak Concentrate Biological Fungicide	Seed treatment	72.2 bc	19.4 cd	31.1 defg	3.09 cd
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	73.9 b	17.8 d	34.4 bcdefg	2.65 cde
Mycostop Mix Biofungicide	Seed treatment	73.3 b	18.3 d	36.7 bcdef	2.80 cd
Natural II	Seed treatment	54.5 e	37.2 a	64.1 a	1.24 g
Natural X	Seed treatment	68.3 bc	23.3 cd	32.2 efg	2.70 cde
PGPR Galaxy	Seed treatment	71.1 bc	20.6 cd	30.1 efg	2.78 cd
Prestop Biofungicide Powder	Drench	55.6 de	36.1 ab	54.1 ab	1.34 fg
SoilGard 12G Microbial Fungicide	Drench	48.3 e	43.3 a	51.6 abcd	1.40 fg
Subtilex Biological Fungicide	Seed treatment	85.6 a	6.7 e	11.2 hi	3.06 cd
T-22 Planter Box Biological Fungicide	Seed treatment + drench	72.8 b	18.9 d	57.8 a	1.76 efg
Yield Shield Concentrate Biological Fungicide	Seed treatment	66.1 cd	25.6 bc	53.2 abc	2.16 def
Apron XL LS	Seed treatment	91.1 a	1.7 e	3.7 ij	4.61 ab
Nontreated seed in inoculated medium	–	72.2 bc	19.4 cd	33.0 cdefg	2.82 cd
Nontreated seed in noninoculated medium	–	91.7 a	0.0 e	1.8 j	5.20 a
LSD ($P < 0.05$)[z]		Rank	Rank	Arcsin	Arcsin

[u] A randomized complete block design with five replications was used for each trial. The number of emerged seedlings and number of wilted seedlings was recorded weekly for 4 or 5 weeks. Results are shown for the final rating (35 and 28 days after planting for trials 1 and 2, respectively).

[v] Preemergence damping-off = percent nonemerged seedlings in each flat compared to the noninoculated control flat in each replication. Postemergence damping-off = percent emerged seedlings in each flat that died or exhibited damping-off. Total damping-off = pre- + postemergence damping-off.

[w] Not all products were EPA-registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 1 for details of the treatments. Apron XL LS (mefenoxam) was included as a conventional fungicide seed treatment for control of *P. ultimum*. For both control treatments, the seed was not treated. For all treatments except nontreated seed planted into noninoculated medium, seed was planted into potting mix inoculated with *P. ultimum* at 1,000 CFU/g (wt/wt).

[x] Refer to Table 1 for details on method of drench and/or seed treatment for each product.

[y] Biomass = aboveground dry weight of all emerged seedlings in each flat at the final rating.

[z] LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Log', 'square root', 'arcsin', or 'rank': original means are presented but means separation by LSD was based on transformation (logarithmic, square root, or arcsin square root transformation) or Friedman's nonparametric rank test, respectively, because of heterogeneous variances and/or non-normal distribution of residuals.

gence damping-off in each trial for all weekly ratings compared with nontreated seed in noninoculated medium ($P < 0.05$), with the exception of postemergence damping-off 7 dap. The treatments had a similar effect on AUEPC, $AUDPC_{pre}$, $AUDPC_{post}$, and $AUDPC_{total}$ (data not shown).

In flats planted with seeds treated with GTG I and GTG II, seedlings first emerged 4 dap, whereas seedlings did not emerge until 5 dap or later for other treatments (data not shown). In trial 1, emergence 35 dap for the GTG I, GTG II, Natural II, and Natural X seed treatments was not significantly different than that of Apron XL LS seed treatment or nontreated seed in noninoculated flats, but was significantly greater than that of nontreated seed in inoculated flats (Table 2). In contrast, *P. ultimum* significantly reduced seedling emergence in flats with the Prestop, Micro 108, Mycostop Mix, and SoilGard 12G treatments compared with nontreated seeds. Preemergence damping-off 35 dap in the GTG I, GTG II, Natural II, and Natural X treatments was similar to that of Apron XL LS seed treatment (Table 2), but was significantly greater in the Micro 108, Mycostop Mix, Prestop, and SoilGard treatments compared with nontreated seed in inoculated flats. Postemergence damping-off was first observed 7 dap. *P. ultimum* caused significantly less postemergence damping-off (<8.0%) for seed treated with GTG I, GTG II, Natural II, and Natural X than for nontreated seed in inoculated flats, and was similar to that of seed treated with Apron XL LS (Table 2). The compost tea drench also significantly reduced postemergence damping-off compared with noninoculated seed in inoculated flats. In contrast, *P. ultimum* caused greater postemergence damping-off 35 dap in flats with Yield Shield, Mycostop Mix, Prestop, T-22 Planter Box, PGPR Galaxy, and Subtilex (all >50%) (Table 2). Spinach biomass 35 dap was greatest in flats with GTG I and GTG II seed treatments (Table 2). GTG II resulted in significantly greater biomass compared with that of nontreated seed and Apron XL LS seed treatment. Flats with GTG I and Natural II treatments had spinach biomass similar to flats with Apron XL LS. *P. ultimum* significantly reduced seedling biomass only in flats drenched with Prestop compared with nontreated seed in inoculated flats.

In trial 2, total damping-off 28 dap for nontreated seed planted in inoculated flats was 52.6 ± 4.8% (Table 2). Emergence was first observed 4 dap in flats with GTG I or GTG II seed treatments, and after at least 5 dap for other treatments. Emergence of seedlings in the GTG I, GTG II, and Subtilex treatments was statistically similar to that of Apron XL LS–treated seed, as well as nontreated seed in noninoculated flats. However, emergence in flats with compost tea, Natural II, Prestop, and SoilGard 12G was significantly less compared with that of nontreated seed in inoculated flats (Table 2). Preemergence damping-off 28 dap in flats with GTG I, GTG II, and Subtilex treatments was similar to that of nontreated seed in noninoculated flats and Apron XL LS–treated seed in inoculated flats. In contrast, preemergence damping-off 28 dap was significantly greater in flats with compost tea, Natural II, Prestop, and SoilGard 12G treatments compared with that of nontreated seed (Table 2). Postemergence damping-off in trial 2 was first observed 7 dap. By 28 dap, postemergence damping-off in flats with the Subtilex seed treatment was similar to that of Apron XL LS–treated seed. In contrast, postemergence damping-off 28 dap was significantly greater in flats with Natural II, Prestop, and T-22 Planter Box treatments. Spinach biomass from GTG I–treated seed was not significantly different from that of nontreated seed in noninoculated flats or Apron XL LS–treated seed (Table 2). In addition, biomass of seedlings from seed treated with GTG II (3.70 ± 0.67 g) was similar to that in the Apron XL LS treatment. Spinach biomass in flats with compost tea, Natural II, Prestop, SoilGard 12G, and T-22 Planter Box was significantly less than that of nontreated seed in inoculated flats.

***R. solani* seed and drench treatment trials.** The 50,000 CFU/g rate of inoculation in the first *R. solani* trial resulted in 74.6 ± 3.9% total damping-off 28 dap for nontreated seed in inoculated flats, which was greater than the targeted 50% damping-off. However, for trial 2, with 25,000 CFU/g, total damping-off for nontreated seed in inoculated flats was only 6.4 ± 2.8% by 28 dap. Seed and drench treatments had a significant ($P < 0.05$) effect on emergence, preemergence damping-off, and postemergence damping-off for all weekly ratings in trial 1, except postemergence damping-off 7 dap. In trial 2, the treatments significantly affected emergence as well as preemergence damping-off for all weekly ratings, but not postemergence damping-off. The treatments also significantly affected AUEPC, $AUDPC_{pre}$, and $AUDPC_{total}$, in each trial and $AUDPC_{post}$ in trial 1 but not in trial 2 (data not shown). The treatments significantly affected spinach biomass (Table 3).

Due, in part, to a low incidence of damping-off in trial 2, limited similarities were evident between the two trials with *R. solani* (Table 3). In both trials, emergence was first observed 4 dap in flats planted with seed treated with GTG I or GTG II, but not until at least 5 dap for the other treatments. In trial 1, final emergence in flats with nontreated seed was 91.0 ± 2.1% in noninoculated flats versus 23.6 ± 8.1% in inoculated flats. Only GTG I and Natural II treatments resulted in final emergence ratings similar to that of the Terraclor-drenched flats, all of which were significantly greater than that of nontreated seed in inoculated flats. Compost tea, GTG II, Natural X, Prestop, and Subtilex treatments significantly increased emergence compared with nontreated seed in inoculated flats. Preemergence damping-off was similar in flats with GTG I, Natural II, and Terraclor treatments, all of which had significantly less preemergence damping-off than inoculated flats with nontreated seed (Table 3). In addition, preemergence damping-off in flats with compost tea, GTG II, Natural X, Prestop, and Subtilex was significantly less than in flats with nontreated seed. In contrast, preemergence damping-off was significantly greater in Yield Shield–treated flats. Postemergence damping-off in trial 1 was first observed 6 dap, and by 28 dap was greater in flats with Mycostop Mix, SoilGard 12G, Subtilex, and T-22 Planter Box treatments compared with nontreated seed in inoculated flats (Table 3). Postemergence damping-off was not recorded in flats with Yield Shield, as no seedlings emerged. No treatment significantly reduced postemergence damping-off compared with nontreated seed or the Terraclor drench. Spinach biomass in trial 1 was greatest for seedlings that developed from seed treated with GTG I or Natural X, similar to Terraclor-drenched flats and nontreated seed in noninoculated flats (Table 3). Seedlings from flats with compost tea, GTG II, Natural II, and T-22 Planter Box had significantly greater biomass than that of nontreated seed in inoculated flats.

In trial 2, emergence of nontreated seed was not affected by inoculum (Table 3). In addition, no treatment significantly increased emergence compared with the control treatments, but emergence in flats with compost tea, Prestop, SoilGard 12G, and T-22 Planter Box was significantly less than emergence from nontreated seed in inoculated flats. Preemergence damping-off 28 dap was significantly greater in flats with compost tea, PGPR Galaxy, Prestop, and SoilGard 12G compared with nontreated seed. No significant differences in postemergence damping-off were detected among treatments because of low disease pressure. No treatment significantly improved spinach biomass compared with biomass from flats with nontreated seed, but biomass from flats with compost tea, Kodiak, Micro 108, Natural X, Prestop, SoilGard 12G, Subtilex, Yield Shield, and Terraclor treatments was reduced (Table 3).

***F. oxysporum* f. sp. *spinaciae* seed and drench treatment trials.** Total damping-off for nontreated seed planted in inoculated flats was 42.9 ± 7.3% by 42 dap in trial 1, and 29.9 ± 6.9% in trial 2 (Table 4). Emergence was first observed 4 dap in flats planted with seed treated with GTG I or GTG II, but not until at least 5 dap for the other treatments. In trial 1, emergence 42 dap was similar for the nontreated seed in inoculated versus noninoculated flats (Table 4). By 42 dap, none of the treat-

ments significantly increased emergence compared with flats with Mertect 340F seed treatment or nontreated seed. T-22 Planter Box, Micro 108, Prestop, SoilGard 12G, and Yield Shield treatments reduced emergence significantly compared with nontreated seed. Preemergence damping-off in trial 1 was low but significantly greater in flats with Micro 108, SoilGard 12G, T-22 Planter Box, and Yield Shield treatments compared with flats with nontreated seed (Table 4). Preemergence damping-off in flats with all other treatments was similar to that of Mertect 340F and nontreated seed. Postemergence wilt in trial 1 was first observed 28 dap. By 42 dap, Prestop and Yield Shield flats significantly reduced postemergence wilt, whereas postemergence wilt was significantly greater in flats with GTG I, GTG II, Kodiak, PGPR Galaxy, SoilGard 12G, and T-22 Planter Box treatments (Table 4). Spinach dry biomass in trial 1 was greater in flats with compost tea, Prestop, Subtilex, or Yield Shield treatments compared with nontreated seed and Mertect 340F–treated seed (Table 4). Spinach biomass was significantly less in the flats with SoilGard 12G and T-22 Planter Box treatments. Seed and drench treatments had a significant effect ($P < 0.05$) on AUEPC, $AUDPC_{pre}$, $AUDPC_{post}$, and $AUDPC_{total}$, similar to final emergence and wilt ratings (data not shown).

In trial 2, only Micro 108 and Prestop treatments significantly reduced emergence compared with that of nontreated seed. Preemergence damping-off 49 dap was significantly greater in the Micro 108 and Prestop flats compared with nontreated seed (Table 4). Preemergence damping-off in flats with other treatments was similar to that of nontreated seed or the Mertect 340F–treated seed. Postemergence wilt was first observed 14 dap in

Table 3. Greenhouse evaluation of seed and drench treatments for management of damping-off caused by *Rhizoctonia solani* in organic spinach[u]

Treatment[w]	Seed or drench application[x]	% Emergence (28 days)	% Damping-off (28 days)[v]		Total dry weight (g, 28 days)[y]
			Preemergence	Postemergence	
Trial 1					
Compost tea	Drench	44.4 cd	46.5 fg	15.7 abcde	1.78 bcdef
GTG I	Seed treatment	63.9 ab	27.1 hi	11.7 bcdef	3.89 a
GTG II	Seed treatment	41.0 cd	50.0 fg	16.5 abcd	1.75 bcd
Kodiak Concentrate Biological Fungicide	Seed treatment	22.9 fgh	68.1 bcd	17.1 abcd	0.97 fghij
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	27.1 fgh	63.9 bcd	11.8 bcdef	0.81 ghij
Mycostop Mix Biofungicide	Seed treatment	20.1 ghi	70.8 abc	22.9 abc	0.60 ijk
Natural II	Seed treatment	65.6 abc	27.2 ghi	8.5 cdef	2.17 bcde
Natural X	Seed treatment	41.7 bcd	48.2 fgh	13.4 bcdef	2.03 abc
PGPR Galaxy	Seed treatment	16.0 hi	75.0 ab	11.3 cdef	0.53 jk
Prestop Biofungicide Powder	Drench	34.7 de	56.3 ef	7.1 def	1.31 efgh
SoilGard 12G Microbial Fungicide	Drench	32.6 def	58.3 def	22.2 abc	1.04 fghi
Subtilex Biological Fungicide	Seed treatment	38.9 de	52.1 ef	26.2 ab	1.19 defgh
T-22 Planter Box Biological Fungicide	Seed treatment + drench	27.8 efg	63.2 cde	28.9 a	1.47 cdefg
Yield Shield Concentrate Biological Fungicide	Seed treatment	0.0 i	91.0 a	0.0 f	0.00 k
Terraclor 75% WP	Drench	81.3 a	10.4 i	1.7 ef	3.54 a
Nontreated seed in inoculated medium	–	23.6 fgh	67.4 bcd	7.2 def	0.76 hijk
Nontreated seed in noninoculated medium	–	91.0 a	0.0 i	1.5 ef	2.57 ab
LSD ($P < 0.05$)[z]		Rank	Rank	14.54	Rank
Trial 2					
Compost tea	Drench	65.0 f	15.0 a	4.7 a	1.92 i
GTG I	Seed treatment	79.4 abc	5.0 bcde	3.4 a	4.74 ab
GTG II	Seed treatment	78.3 abcd	3.9 cde	7.6 a	4.78 ab
Kodiak Concentrate Biological Fungicide	Seed treatment	80.0 abc	5.0 cde	4.9 a	4.08 bcde
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	78.9 abc	5.6 abcde	7.4 a	3.92 cde
Mycostop Mix Biofungicide	Seed treatment	85.6 a	0.6 e	4.0 a	4.18 abcd
Natural II	Seed treatment	81.1 abc	3.3 cde	3.4 a	4.42 abc
Natural X	Seed treatment	73.9 bcdef	5.0 abcde	6.5 a	3.52 def
PGPR Galaxy	Seed treatment	75.6 abcde	9.5 abc	7.0 a	4.24 abcd
Prestop Biofungicide Powder	Drench	66.1 ef	12.8 ab	6.2 a	2.47 hi
SoilGard 12G Microbial Fungicide	Drench	68.3 def	10.0 abc	7.4 a	3.10 fgh
Subtilex Biological Fungicide	Seed treatment	75.0 bcdef	6.1 bcde	8.4 a	3.77 cdef
T-22 Planter Box Biological Fungicide	Seed treatment + drench	72.2 cdef	8.9 abcd	3.4 a	4.27 abcd
Yield Shield Concentrate Biological Fungicide	Seed treatment	80.6 abc	2.8 bcde	11.5 a	3.85 cdef
Terraclor 75% WP	Drench	81.1 abc	2.3 bcde	0.7 a	2.70 ghi
Nontreated seed in inoculated medium	–	82.8 ab	1.7 de	4.7 a	4.93 a
Nontreated seed in noninoculated medium	–	78.3 abcd	0.0 e	1.6 a	3.28 efg
LSD ($P < 0.05$)[z]		10.36	Rank	NS	0.806

[u] A randomized complete block design with four or five replications was used (trials 1 and 2, respectively). The number of emerged seedlings and number of wilted seedlings was recorded at weekly intervals for four weeks. Results are shown for the final rating (28 days).

[v] Preemergence damping-off = percent nonemerged seedlings in each flat compared to the noninoculated control flats in each replication. Postemergence damping-off = percent emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off = pre- + postemergence damping-off.

[w] Not all products were EPA-registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 1 for details of the treatments. Terraclor 75% WP (pentachloronitrobenzene) was included as a conventional fungicide drench for control of *R. solani*. For both control treatments, the seed was not treated. For all treatments except nontreated seed planted into noninoculated medium, seed was planted into potting mix inoculated with *R. solani* at 50,000 or 25,000 CFU/g (wt/wt) in trials 1 and 2, respectively.

[x] Refer to Table 1 for details on method of drench and/or seed treatment for each product.

[y] Biomass = aboveground dry weight of all emerged seedlings in each flat at the final rating.

[z] LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Rank': original means are presented but means separation by LSD was based on Friedman's nonparametric rank test because of heterogeneous variances and/or non-normal distribution of residuals.

trial 2 versus 28 dap in trial 1. Postemergence wilt 49 dap was significantly less in flats with compost tea and Prestop treatments compared with nontreated seed in inoculated flats (Table 4). Biomass of seedlings that developed from GTG I–treated seed was similar to that of seedlings that grew from nontreated seed in noninoculated flats and Mertect 340F–treated seed, and was significantly greater than that of nontreated seed in inoculated flats (Table 4). In contrast, Micro 108 significantly reduced spinach biomass compared with that of nontreated seed.

Potting mix pH from treatment trials. The pH of the potting mix averaged 6.46 prior to planting the second *P. ultimum* trial and 6.40 prior to the second *F. oxysporum* f. sp. *spinaciae* trial. The pH at the end of the *F. oxysporum* f. sp. *spinaciae* trial also averaged 6.40.

Seed health assays of treated seed. For the seed health assay of the first Lazio seed lot (trial 1), the incidence of *Verticillium* spp. detected was reduced significantly by GTG I (10.5%), GTG II (0.8%), Mycostop Mix (13.0%), Natural II (4.8%), Natural X (20.3%), and Mertect 340F (0.8%) compared with nontreated seed (49.8%) (Table 5). In contrast, the incidence of *Verticillium* spp. was only 1.3% for the second seed lot (trial 2). In that seed lot, T-22 Planter Box and Mertect 340F reduced the incidence of *Verticillium* spp. to 0%. Most isolates of *Verticillium* observed resembled *V. dahliae*, a wilt pathogen of spinach (11). The prevalence of seedborne *Stemphylium botryosum*, causal agent of Stemphylium leaf spot of spinach (19), was significantly greater on seed treated with GTG I for the first seed lot (17.5%), and on seed treated with GTG I (11.3%) and GTG II (9.5%) for the second lot compared with nontreated seed. In comparison, *S. botryosum* was less prevalent on seed of the first lot treated with Mycostop Mix, Natural II, and Apron XL LS, but only by Mycostop Mix for the second lot. The prevalence of

Table 4. Greenhouse evaluation of seed and drench treatments for management of seedling wilt caused by *Fusarium oxysporum* f. sp. *spinaciae* in organic spinach[u]

Treatment[w]	Seed or drench application[x]	% Emergence (42 or 49 days)	% Damping-off (42 or 49 days)[v]		Total dry weight (g, 42 or 49 days)[y]
			Preemergence	Postemergence	
Trial 1					
Compost tea	Drench	90.6 abcde	5.0 bcdefg	28.4 gh	6.64 a
GTG I	Seed treatment	93.3 abcd	2.2 defg	56.5 abc	4.03 efg
GTG II	Seed treatment	93.8 abc	1.1 fg	54.0 abcd	3.50 fgh
Kodiak Concentrate Biological Fungicide	Seed treatment	90.6 abcde	3.3 cdefg	60.7 ab	3.21 ghi
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	83.3 ef	10.6 abcd	44.5 cdef	3.00 hij
Mycostop Mix Biofungicide	Seed treatment	92.8 abcd	2.8 cdefg	53.9 abcd	3.32 gh
Natural II	Seed treatment	88.3 bcde	5.6 abcdef	33.7 efgh	4.23 def
Natural X	Seed treatment	96.7 a	1.7 fg	43.3 cdefg	4.03 efg
PGPR Galaxy	Seed treatment	90.6 abcde	6.7 bcdef	55.8 abc	3.23 ghi
Prestop Biofungicide Powder	Drench	85.6 def	8.3 abcde	25.5 h	4.65 cde
SoilGard 12G Microbial Fungicide	Drench	84.4 ef	12.2 ab	66.5 a	2.40 ij
Subtilex Biological Fungicide	Seed treatment	91.1 abcde	3.9 cdefg	33.2 fgh	5.06 bcd
T-22 Planter Box Biological Fungicide	Seed treatment + drench	80.0 f	13.9 a	66.1 a	2.14 j
Yield Shield Concentrate Biological Fungicide	Seed treatment	86.7 cdef	8.9 abc	22.3 hi	5.81 ab
Mertect 340F	Seed treatment	92.8 abcd	3.3 cdefg	48.3 bcde	3.54 fgh
Nontreated seed in inoculated medium	–	95.6 ab	2.2 efg	40.7 defg	3.48 fgh
Nontreated seed in noninoculated medium	–	93.9 abc	0.0 g	10.2 i	5.17 bc
LSD ($P < 0.05$)[z]		7.95	Log	14.97	0.908
Trial 2					
Compost tea	Drench	74.4 abc	7.8 cde	11.7 de	12.95 bcd
GTG I	Seed treatment	73.9 abc	8.3 abc	19.1 cd	13.71 ab
GTG II	Seed treatment	67.8 cde	13.3 bc	20.3 bcd	12.37 cde
Kodiak Concentrate Biological Fungicide	Seed treatment	76.1 abc	8.9 abc	26.5 abc	11.60 ef
Micro 108 Seed Inoculant + Actinovate AG	Seed treatment + drench	57.8 e	20.6 ab	26.6 abc	10.51 f
Mycostop Mix Biofungicide	Seed treatment	73.3 bcd	6.7 cd	24.3 abc	12.63 de
Natural II	Seed treatment	83.9 ab	4.4 cde	25.6 abc	12.40 de
Natural X	Seed treatment	87.2 a	1.7 de	21.3 bcd	12.85 cde
PGPR Galaxy	Seed treatment	80.6 abc	5.6 cde	34.4 a	12.44 de
Prestop Biofungicide Powder	Drench	60.0 de	20.0 a	11.6 de	11.74 ef
SoilGard 12G Microbial Fungicide	Drench	73.9 abc	9.4 cd	31.8 ab	11.82 de
Subtilex Biological Fungicide	Seed treatment	72.2 bcd	11.1 cde	29.5 abc	12.71 cde
T-22 Planter Box Biological Fungicide	Seed treatment + drench	83.9 ab	1.7 cde	26.0 abc	13.27 bcd
Yield Shield Concentrate Biological Fungicide	Seed treatment	77.2 abc	6.7 cde	23.0 abcd	11.78 ef
Mertect 340F	Seed treatment	85.0 ab	3.3 cde	21.9 bcd	13.44 abc
Nontreated seed in inoculated medium	–	79.4 abc	3.9 cde	26.1 abc	12.85 cde
Nontreated seed in noninoculated medium	–	77.8 abc	0.0 e	0.7 e	14.90 a
LSD ($P < 0.05$)[z]		13.44	Rank	12.19	Rank

[u] A randomized complete block design with five replications was used for each trial. The number of emerged seedlings and the number of wilted seedlings was recorded weekly for 6 and 7 weeks in trials 1 and 2, respectively. Results are shown for the final rating (42 and 49 days for trials 1 and 2, respectively).

[v] Preemergence damping-off = percent nonemerged seedlings in each flat compared to the noninoculated control flat in each replication. Postemergence damping-off = percent emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off = pre- + postemergence damping-off.

[w] Not all products were EPA-registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 1 for details of the treatments. Mertect 340F (thiabendazole) was included as a conventional fungicide seed treatment for control of *F. oxysporum* f. sp. *spinaciae*. For both control treatments, the seed was not treated. For all treatments except nontreated seed planted into noninoculated medium, seed was planted into potting mix inoculated with *F. oxysporum* f. sp. *spinaciae* at 10,000 CFU/g (wt/wt).

[x] Refer to Table 1 for details on method of drench and/or seed treatment for each product.

[y] Biomass = aboveground dry weight of all emerged seedlings in each flat at the final rating.

[z] LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Log' or 'rank': original means are presented but means separation by LSD was based on logarithmic transformation or Friedman's nonparametric rank test, respectively, because of heterogeneous variances and/or non-normal distribution of residuals.

Fusarium spp. was similar between treatments on both lots (Table 5). The seed lot in trial 2 was severely infested with *Alternaria* spp. (96.3% on nontreated seed) compared with the lot in trial 1 (13.3%). GTG II and GTG I reduced the incidence of *Alternaria* spp. on this lot to 2.3 and 3.0%, respectively, and Mycostop Mix reduced the incidence of *Alternaria* spp. to 88.0% (Table 5).

In the seed lot used in trial 1, *Trichoderma* spp. were significantly more prevalent on seed treated with GTG II (99.3%) and T-22 Planter Box (48.5%) compared with nontreated seed (0%). In the seed lot in trial 2, *Trichoderma* spp. were detected in 54.0% of the seed treated with GTG II and 72.8% of the seed treated with T-22 Planter Box versus 0% for nontreated seed. These two treatments contained *T. harzianum* (Table 1). For the second seed lot, the incidence of actinomycetes observed on seed was significantly greater on seed treated with Mycostop Mix (91.0%) and Natural II (4.0%) compared with nontreated seed (1.8%). The active ingredients in Mycostop Mix and Natural II are actinomycetes (Table 1).

Germination assays of treated seed. After 7 days, only seed treated with GTG I and GTG II had significantly greater germination than the nontreated seed in trial 1 (Table 6). In contrast, Natural II and Natural X significantly reduced germination. Results were similar in trial 2. In the second trial, GTG I was the only treatment that resulted in significantly greater germination relative to that of nontreated seed after 14 days. After 21 days, seed treated with GTG I, GTG II, and Mycostop Mix had significantly greater germination compared with nontreated seed (Table 6). The incidences of abnormal germination, rotten seed, and nongerminated seeds were similar among treatments for each seed lot (data not shown).

DISCUSSION

The inoculum density of *P. ultimum* provided a consistent level of disease pressure and resulted in a similar incidence of pre- and postemergence damping-off that differentiated efficacy of the treatments under the conditions of the greenhouse trials. In both trials, drench treatments with Micro 108, Prestop, SoilGard 12G, and T-22 Planter Box increased damping-off compared with other treatments, as did the compost tea drench in one trial. The drench treatments may have exacerbated disease because of the additional liquid added to the potting medium compared with seed treatments since *P. ultimum* thrives under wet soil conditions (18). However, the compost tea used in this study significantly suppressed damping-off caused by *P. ultimum* in another study (37). For *P. ultimum*, two experimental seed treatments, GTG I and GTG II, provided consistent control of damping-off equivalent to that provided by the fungicide Apron XL LS (mefenoxam), while Natural II, Natural X, and Subtilex seed treatments suppressed damping-off in only one of the two trials. Although *T. harzianum* is one of the active ingredients in GTG II and was active against Pythium damping-off of cotton (20), *T. harzianum* was not in GTG I, which showed a similar level of control of *P. ultimum* as GTG II. This suggests the organic disinfectant in each of these products was the primary ingredient that reduced damping-off from *P. ultimum*. A second treatment evaluated in this study with the same T-22 strain of *T. harzianum* (T-22 Planter Box) did not significantly reduce damping-off caused by *P. ultimum*. Other biological control agents have been shown to effectively reduce damping-off caused by *Pythium* spp. on a variety of crops, including *Enterobacter cloaceae*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, and *Rhizobium leguminosarum* bv. *viceae* (21,33,43). Although the labels for Mycostop Mix, Prestop, SoilGard 12G, and T-22 Planter Box state efficacy of the products for suppressing damping-off by *Pythium* spp., these products were not effective against *P. ultimum* under the conditions of this trial.

Preemergence damping-off caused by *R. solani* was generally more prevalent than postemergence damping-off. It is well known that *R. solani* can rapidly increase inoculum and can infect seeds before and during germination, often causing low emergence (3,31) as observed in the greenhouse trials in this study. An inoculum density of 50,000 CFU/g resulted in 75% damping-off, while 25,000 CFU/g resulted in inadequate disease pressure. Quantifying *Rhizoctonia* spp. using soil dilution plating methods can be difficult (34), and inoculum density of the *R. solani* soil/oatmeal inoculum in this study was variable among batches of inoculum (data not shown). Nonetheless, in trial 1 seed

Table 5. Results of a freeze-blotter seed health assay of seed with treatments evaluated for management of damping-off or seedling blight in organic spinach[x]

	% Seed infected or infested (14 days after plating)							
	Trial 1				Trial 2			
Seed treatment[y]	*Stemphylium botryosum*	*Verticillium* spp.	*Fusarium* spp.	*Alternaria* spp.	*Stemphylium botryosum*	*Verticillium* spp.	*Fusarium* spp.	*Alternaria* spp.
Nontreated	4.00 bcd	49.75 a	2.25 abcd	13.25 ab	5.50 c	1.25 bc	0.00 a	96.25 ab
GTG I	17.50 a	10.50 c	1.75 abcd	5.00 de	11.25 a	10.25 a	0.00 a	3.00 d
GTG II	3.50 bcde	0.75 e	0.25 cd	0.50 f	9.50 ab	8.50 ab	0.75 a	2.25 d
Kodiak Concentrate Biological Fungicide	3.50 bcde	48.50 a	1.75 abc	10.25 ab	5.00 c	0.75 cd	0.00 a	98.00 ab
Micro 108 Seed Inoculant	4.50 bc	48.75 a	4.75 a	14.00 a	4.25 c	1.00 cd	0.00 a	98.50 a
Mycostop Mix Biofungicide	0.50 f	13.00 c	3.50 ab	2.00 ef	0.50 d	0.75 cd	0.00 a	88.00 c
Natural II	0.50 f	4.75 d	1.25 abcd	0.25 f	6.25 bc	1.00 cd	0.00 a	97.75 ab
Natural X	1.75 def	20.25 b	0.75 bcd	1.00 f	3.75 cd	0.50 cd	0.00 a	97.25 ab
PGPR Galaxy	4.50 bc	45.75 a	2.25 ab	9.25 bc	4.25 c	0.75 cd	0.00 a	98.00 ab
Subtilex Biological Fungicide	2.00 cdef	45.25 a	2.75 ab	7.25 cd	3.25 cd	0.75 cd	0.25 a	97.50 ab
T-22 Planter Box Biological Fungicide	1.75 def	41.50 a	3.00 a	10.50 ab	5.25 c	0.00 d	0.50 a	96.00 b
Yield Shield Concentrate Biological Fungicide	3.00 bcde	49.25 a	1.25 abcd	11.75 ab	3.00 cd	1.00 cd	0.00 a	98.50 ab
Apron XL LS	1.50 ef	40.75 a	2.00 ab	3.75 def	3.25 cd	0.50 cd	0.00 a	98.25 ab
Mertect 340F	6.00 b	0.75 e	0.00 d	11.5 ab	4.50 c	0.00 d	0.00 a	97.25 ab
LSD ($P < 0.05$)[z]	Log	Log	Rank	Rank	3.567	Rank	NS	Arcsin

[x] Four replications of 100 seeds/treatment assayed as described by du Toit et al. (11). A different commercial seed lot of the hybrid Lazio was evaluated in each trial.

[y] Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Apron XL LS (mefenoxam) and Mertect 340F (thiabendazole) were conventional fungicide seed treatments for control of *Pythium* spp. and *Fusarium* spp., respectively. Refer to Table 1 for details of the seed treatments.

[z] LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Log', 'arcsin', and 'rank': original means are presented but means separation is based on transformation (logarithmic or arcsin square root transformation) or Friedman's nonparametric rank test, respectively, because of heterogeneous variances and/or non-normal distribution of residuals. NS = not significantly different at $P < 0.05$ (41).

treatment with GTG I and Natural II resulted in emergence similar to the Terraclor fungicide drench. The active ingredients of GTG I and Natural II are an organic disinfectant and an actinomycete, respectively. Other biological control agents, including *P. aeruginosa* and *P. fluorescens*, *P. putida*, *T. harzianum*, *Gliocladium* spp., and *Bacillus amyloliquefaciens,* have been shown to effectively reduce damping-off caused by *Rhizoctonia* spp. on a variety of crops (4,7,15,17,28,29,39). However, treatments evaluated in this study with these active ingredients (GTG II, PGPR Galaxy, PreStop, SoilGard 12G, and T-22 Planter Box) did not reduce damping-off by *R. solani* even though the labels for Kodiak, Mycostop Mix, Prestop, SoilGard 12G, Subtilex, T-22 Planter Box, and Yield Shield state efficacy at suppressing *Rhizoctonia* spp. Marshall (29) demonstrated that seed treatment with *T. harzianum* resulted in a greater reduction in disease caused by *R. solani* at a soil pH of 3.5 versus 5.6. This suggests that products evaluated in this study that contained *Trichoderma* spp. may have been limited in efficacy by the pH of the potting medium, which ranged from 6.40 to 6.46.

Fusarium wilts are generally more severe in warm soils (6). For the inoculum density trials with *F. oxysporum* f. sp. *spinaciae*, postemergence wilt was the predominant symptom. In trial 1, 10,000 CFU/g achieved approximately 50% wilt for nontreated seed. However, in trial 2 this concentration resulted in 30% wilt from nontreated seed, which may have limited the ability to differentiate among seed and drench treatments. Additionally, these trials were carried out for >40 days, which may have impacted efficacy of the treatments, as many seed treatments have efficacy against specific pathogens for a limited duration after planting (16).

In this study, only PreStop Biofungicide consistently reduced the incidence of postemergence wilt caused by *F. oxysporum* f. sp. *spinaciae*, although flats drenched with this product had significantly more preemergence damping-off than flats planted with nontreated seed. The compost tea drench and Yield Shield seed treatment each suppressed wilt significantly in one of the two trials. Other biological control agents have been shown to effectively reduce wilt caused by *Fusarium* spp., including *P. fluorescens*, *P. putida*, *Glomus intraradices*, *G. mosseae*, *E. cloaceae*, *R. leguminosarum*, *Trichoderma* spp., *G. virens*, *Burkholderia cepacia*, and nonpathogenic *Fusarium* spp. (2,12,13,26,27,36). However, in this study, treatments with the active ingredients listed in those studies (GTG II, PGPR Galaxy, SoilGard 12G, and T-22 Planter Box) did not reduce wilt caused by *F. oxysporum* f. sp. *spinaciae*. In each trial, total wilt for the Mertect 340F fungicide seed treatment plots also was similar to that of nontreated seed, indicating that this fungicide seed treatment was not effective for management of Fusarium wilt under the conditions of these trials.

Seed treatments can protect against seedborne as well as soilborne pathogens (1,32,42). Seed health assays in this study indicated differences in the incidence of some seedborne necrotrophic fungi, particularly *Verticillium* spp. and *Alternaria* spp., in the two commercial seed lots. One of the seed lots was highly infested with *Alternaria* spp., which may have suppressed development of other fungi on the seed (*Verticillium* spp. and *S. botryosum*). This was apparent for seed treated with GTG I and GTG II, which had significantly less seed with *Alternaria* spp. but greater amounts of *Verticillium* spp. and *S. botryosum*. *Stemphylium* and *Verticillium* infections of spinach seed can be deep in the pericarp and even in the embryo (19), which might explain why the organic disinfectant in GTG I and GTG II did not eliminate these fungi from the seed. A greater proportion of seed were colonized by actinomycetes for the Mycostop Mix and Natural II seed treatments compared with any other treatment. Similarly, *Trichoderma* spp. were more prevalent on seed treated with GTG II and T-22 Planter Box, demonstrating the presence of the biological control agents contained in these products.

Seed treated with GTG I and GTG II had the earliest germination in all trials as well as the germination assays. Early germination and emergence could reduce the duration of susceptibility of spinach seedlings to infection by *P. ultimum* and *R. solani*, since these pathogens do not cause losses in more mature plants (8). Seed treated with Natural II had delayed germination, consistent with previous spinach seed trials with this product (9). GTG I, GTG II, and Mycostop Mix increased seed germination and reduced the incidence of seedborne *Alternaria* spp., which can cause seed rot on various crops (1), on one of the spinach seed lots.

Inconsistency in performance was observed in this study for many of the seed and drench treatments evaluated. This could be attributed to a number of factors, such as differences in incidence of seedborne fungi that may have affected efficacy

Table 6. Results of a germination assay of spinach seed with treatments evaluated for management of damping-off and seedling blight in organic spinach[x]

	% Seed germination						
	Trial 1			Trial 2			
Seed treatment[y]	7 days	14 days	21 days	5 days	7 days	14 days	21 days
Nontreated	50.25 cd	88.75 a	90.25 a	16.00 cde	80.50 abc	88.50 bcde	88.50 cdef
GTG I	70.00 a	92.00 a	92.50 a	38.00 a	78.00 cd	92.50 a	93.25 a
GTG II	64.50 ab	89.50 a	91.00 a	29.25 b	76.25 cd	90.75 abc	92.75 a
Kodiak Concentrate Biological Fungicide	52.50 cd	89.50 a	90.50 a	19.00 cd	78.00 cd	85.25 ef	85.50 fg
Micro 108 Seed Inoculant	51.75 cd	88.25 a	88.75 a	18.50 cd	82.75 abc	88.75 abcde	88.75 bcdef
Mycostop Mix Biofungicide	47.00 de	88.00 a	89.00 a	21.75 bc	85.25 ab	92.25 ab	92.50 ab
Natural II	34.75 f	88.75 a	90.75 a	10.50 e	80.50 abc	91.25 abc	91.50 abcd
Natural X	40.50 ef	89.00 a	90.25 a	16.75 cde	79.25 bcd	90.50 abc	90.50 abcde
PGPR Galaxy	53.75 cd	85.75 a	87.25 a	17.50 cde	78.50 cd	86.50 def	87.00 efg
Subtilex Biological Fungicide	57.00 bc	90.00 a	91.00 a	17.25 cde	81.00 abc	88.00 cdef	88.50 cdef
T-22 Planter Box Biological Fungicide	55.00 cd	89.25 a	90.25 a	12.25 de	86.50 a	92.25 ab	92.25 abc
Yield Shield Concentrate Biological Fungicide	49.50 cd	89.50 a	90.75 a	17.25 cde	81.50 abc	90.00 abcd	90.00 abcde
Apron XL LS	55.75 c	90.25 a	91.25 a	15.25 cde	73.75 d	84.25 f	84.25 g
Mertect 340F	42.50 cd	89.50 a	91.50 a	16.25 cde	82.25 abc	87.75 cdef	87.75 defg
LSD ($P < 0.05$)[z]	8.311	NS	NS	7.521	6.737	3.940	3.771

[x] The Association of Official Seed Analysts (AOSA) blotter germination assay (44) for four replications of 100 seeds/treatment. A different commercial seed lot of the hybrid Lazio was evaluated in each trial. The percent abnormally germinated seed, rotten seed, and nongerminated seed were recorded at 21 days, with no significant differences detected among treatments for these variables based on analyses of variance (*data not shown*).

[y] Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Apron XL LS (mefenoxam) and Mertect 340F (thiabendazole) were included as conventional fungicide seed treatments for control of *Pythium* spp. and *Fusarium* spp., respectively. Refer to Table 1 for details of each treatment.

[z] LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. NS = not significantly different at $P < 0.05$ (41).

of some treatments. However, the seed lots used in these trials were commercially available. The products also need to be evaluated under diverse field conditions to assess potential efficacy and consistency in performance. Of the 14 organic seed and drench treatments evaluated, GTG I and GTG II were the most consistent and effective at reducing damage to spinach from *P. ultimum* and *R. solani*, but not from *F. oxysporum* f. sp. *spinaciae*. None of the treatments provided complete protection against all three pathogens. Specific treatments may be more effective under certain conditions, as the consistency in performance of seed treatments can be affected by crop species, product formulation, seedborne or soilborne pathogens, and soil conditions (42). It is important to assess each treatment on specific crop species of interest under the diversity of production conditions in which the products might be used. Furthermore, the results highlight the need for potential users of such organic products to obtain accurate diagnoses of the specific damping-off pathogens present in fields in which the seed or drench treatments are to be used, in order to select the appropriate product(s) with highest efficacy against the particular pathogen(s) of concern. Research is also needed to evaluate combinations of seed and/or drench treatments against damping-off pathogens, individually and in combinations, in a diversity of field conditions.

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