

**USDA ORGANIC CROPPING RESEARCH FOR THE NORTHWEST
ORGANIC SEED TREATMENTS: 2006 PROGRESS REPORT**

Title: Organic Seed Treatments

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Cooperators: None

Date (period which report covers): 2006

Keywords: vegetable seed, seed treatments, organic, damping-off, seedling blight, *Fusarium*, *Pythium*, *Rhizoctonia*, seedborne, soilborne, diseases, fungi

Abstract: The new rules and standards of the USDA National Organic Program have increased the demand for organically produced seed, but have also raised concerns about losses due to both seedborne and soilborne pathogens because of the limited number of options available for organic seed treatment. This is compounded by a lack of unbiased scientific data on the efficacy of currently-available organic seed treatments on the spectrum of pathogens that can cause damping-off, seedling blight, or root rot problems. In spring and fall semesters of 2006, Jaime Cummings, the MS student working on this project, completed classes required for her MS degree in the WSU Department of Plant Pathology. Spinach was selected as a model vegetable crop to assess organic seed treatments for control of three damping-off and seedling blight pathogens that are common to many vegetables. Pathogenicity tests on spinach were completed for isolates of *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, and *Rhizoctonia solani*, to verify that the isolates are appropriate for this model crop system to evaluate seed treatments. A sand-oatmeal method of producing inoculum for application to soils and an organic potting medium was assessed for each isolate, and verified as a practical method of inoculation that could be used for all three pathogens. Inoculation rate trials were carried out in the greenhouse to determine appropriate rates at which to inoculate each pathogen in order to assess organic seed treatments.

Objectives:

1. Develop a list of potential organic seed treatment products that could be used by organic vegetable and vegetable seed growers.
2. Evaluate seed treatment products in greenhouse and field trials against common soilborne fungal pathogens that cause root rot and damping-off problems in vegetables, using spinach as a model system for small-seeded vegetables.

Procedures:

The MS student, Jaime Cummings, started an MS program in the WSU Dept. of Plant Pathology in the fall semester of 2005, and is currently completing a 3rd semester of classes for her MS

program. A literature search was completed to compile a tentative list of certified and potentially-certifiable organic seed treatments to evaluate for control of damping-off and seedling blight pathogens on spinach. Each of the products will be assessed in 2007 against an isolate of each of *Fusarium oxysporum* f. sp. *spinaciae* (an Ascomycete), *Pythium ultimum* (a Stramenopile), and *Rhizoctonia solani* AG4 HGII (a Basidiomycete), to determine the level and spectrum of activity of each product against the three types of seedling blight pathogens.

Isolates of *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, and *Rhizoctonia solani* were collected in 2005. Pathogenicity tests of these isolates on spinach were then completed in the greenhouse in 2005 and 2006. For the *Fusarium* seedling blight pathogenicity test, ~6 week-old seedlings of a proprietary female spinach inbred line that is tolerant of thrips damage (a persistent pest in the greenhouses at the WSU Mount Vernon NWREC), were inoculated with a spore suspension of *F. oxysporum* f. sp. *spinaciae* (produced in Kerr's broth on a shaker for 7 days). The root plug of each seedling was immersed in the spore suspension for 60 seconds, and the inoculated seedlings were transplanted into Sunshine Mix #1 in 4" diameter pots. Five replications of inoculated and non-inoculated (control treatment) seedlings were used. Seedlings were monitored for symptoms of wilt and dieback. Approximately 6 weeks after inoculation, the roots of the plants were washed, cut longitudinally, evaluated for rot and discoloration, and incubated in a moist chamber to monitor development of the pathogen. For the *R. solani* pathogenicity test, rye seed was soaked in water overnight, sterilized by autoclaving for 90 minutes in each of two consecutive days, cooled, and inoculated with the pathogen. After the rye was colonized by the fungus, it was air dried in a fume hood for 1 week, dried, and stored in a refrigerator. Seedlings were inoculated, at the time of transplanting into 4"-diameter pots, by placing 25 colonized rye seed around the plug in the potting medium. Non-colonized rye seed was used for the control plants. A randomized complete block design was used with 3 replications of each treatment, and one plant per experimental unit. The seedlings were monitored for wilt and dieback, and the roots assessed for discoloration as for the *Fusarium* wilt test. Pathogenicity of the *P. ultimum* isolate was assessed by placing around the root system of each plant about 10 pieces (~5 mm³) of potato dextrose agar colonized by the pathogen. A randomized complete block design was used with 3 replications of each treatment, and one plant per experimental unit.

For the inoculation rate tests, a sand-oatmeal method of producing inoculum for application of the soilborne pathogens to soils and potting media was assessed for each of the pathogens used in the project. In summary for each pathogen, 1% ground rolled oats (Quaker Old Fashioned) was blended with soil (sandy-loam) that had been wet (10-15% water), placed in 1 quart Mason jars, sterilized twice for 45 minutes on consecutive days, and inoculated with the pathogen using mycelial plugs (or a spore suspension for *F. oxysporum* f. sp. *spinaciae*). The jars were stored at room temperature in the dark for 3 to 5 weeks to allow the pathogen to colonize the sand/oatmeal mix. The inoculum was then stored in a refrigerator for up to 6 months. The amount of inoculum of each pathogen in the jars was then quantified by plating a dilution series of the sand/oatmeal inoculum (suspended in 1% 'sloppy' agar) onto an agar medium that is semi-selective for the particular pathogen, i.e., Komada's medium for *F. oxysporum* f. sp. *spinaciae*, Rose Bengal agar for *P. ultimum*, and water agar for *R. solani*.

Inoculation rate trials were carried out in the greenhouse to determine an appropriate rate of inoculum to add to the organic potting medium for each pathogen. The spinach cultivar “Lazio” was selected for the project because it is a popular cultivar in the baby-leaf spinach market, and is very susceptible to damping-off. The objective was to achieve approximately 50% dieback of the seedlings with each pathogen in order to maximize the ability to differentiate efficacy of the seed treatments against each pathogen. In the initial inoculation rate trial for each pathogen, inoculum was added to an organic potting mix (Sunshine Professional Growing Mix) at rates of 0, 500, 1,000, 5,000, and 10,000 CFU (colony-forming units)/g potting medium. However, these inoculation rates proved too low for *F. oxysporum* f. sp. *spinaciae* and *R. solani*, so inoculation rates of 0, 5,000, 10,000, 50,000, and 100,000 CFU/g were then evaluated for each of *F. oxysporum* f. sp. *spinaciae* and *R. solani*. The inoculation rates were assessed as a separate trial for each pathogen, using a randomized complete block design with 5 replications of the 5 inoculation rates. In addition, the *P. ultimum* inoculation rate trial was carried out at 15°C using Percival growth chambers, and at ‘greenhouse’ temperature (approximately 25°C, as for the other two pathogens). Each experimental unit consisted of a 12” x 12” flat in which 36 seed of “Lazio” were planted (6 rows of 6 seed at a 2” spacing within rows). The number of emerged seedlings and the number of seedlings with post-emergence wilt were counted at 3 to 4-day intervals until 32 days after planting. Seedlings were fertilized with Alaska Fish Fertilizer (5:1:1, Lilly Miller Brands) weekly. Analyses of variance and Fisher’s protected least significant difference (LSDs) were carried out using SAS Version 9.1.

The Lazio seed lot used for the inoculation rate trials was subjected to a seed health assay to determine the incidence of seed infected with the following fungi: *Cladosporium variabile* and other *Cladosporium* spp., *Fusarium* spp., *Stemphylium botryosum*, *Verticillium* spp., and various other necrotrophic fungi that can be detected using the freeze-blotter seed health assay developed by du Toit et al. (2005. Plant Dis. 89:4-11).

The preliminary list of organic seed treatments (certified or potentially certifiable) to be evaluated in 2007 for control of damping-off and seedling blight pathogens includes:

1. Actinovate SP = *Streptomyces lucidus* (Natural Industries)
2. Kodiak vegetable biological fungicide = *Bacillus subtilis* (Bayer CropScience)
3. Mycostop = *Streptomyces griesoviridis* (Verdera Oy)
4. Natural II = an actinomycete (AgriCoat LLC)
5. Prestop Biofungicide Powder = *Gliocladium catenulatum* (Verdera Oy)
6. Rootshield = *Trichoderma harzianum* (Certis)
7. SoilGard-12G = *Gliocladium virens* (Certis)
8. Subtilex = *Bacillus subtilis* (Becker Underwood)
9. GTG I = a new organic treatment (Germain’s Technology Group, UK)
10. GTG II = a 2nd new organic treatment (Germain’s Technology Group, UK)

In addition, two control seed treatments will be included in each trial:

1. Non-treated seed, and
2. One of the following, as appropriate for the particular pathogen
 - a. Apron (mefenoxam = conventional seed treatment for *Pythium* spp.)
 - b. Maxim (fludioxonil = fungicide with some efficacy against *Fusarium* spp.)
 - c. PCNB (pentachloronitrobenzene = fungicide for *Rhizoctonia* spp.)

In winter/spring 2007, when Jaime returns to the WSU Mount Vernon NWREC full-time, the seed treatments will be evaluated separately for control of each of the three soilborne pathogens. The treatments will be assessed using a randomized complete block design with 5 replications of the 12 treatments (10 organic treatments and 2 control treatments). Additional organic seed treatments could be added, if appropriate. The greenhouse trials will be carried out using the same inoculation protocol as for the inoculation rate trials, and will be repeated to assess reproducibility of the results. Those products that demonstrate efficacy against one or more of the three pathogens will be assessed in field trials located: 1) on the certified organic land at the WSU Mount Vernon NWREC, 2) on the certified organic land at the WSU Vancouver REU, and 3) on a commercial organic farm (probably Nash Huber's Organic Produce in Sequim, WA). At each site except the commercial organic farm, the field will be inoculated with the pathogens using the same inoculation protocol as for the greenhouse trials. "Lazio" seed with each of the seed treatments will be planted in the field. A randomized complete block design will be used with 5 replications of each of the seed treatments. Each plot will consist of two 10'-long rows. Irrigation may be needed if conditions are dry, because damping-off diseases are favored by moist soils. Plots will be monitored for stand count, post-emergence wilt, and biomass. The commercial organic farm site will not be inoculated, but the trial will be irrigated as needed to create favorable conditions for damping-off. Isolations will be carried out from wilted and healthy seedlings at each field site to confirm the cause of damping-off.

The organic seed treatments that prove efficacious in the greenhouse and field trials will be assessed further as combinations of seed treatments in soils inoculated with combinations of the three pathogens. The combination seed treatment and inoculation trials will be carried out in the greenhouse, using the same protocols as described above. Based on results of the greenhouse trials, the combination trials will be duplicated in at least two of the three field sites.

Progress towards objectives:

Isolates of *F. oxysporum* f. sp. *spinaciae*, *P. ultimum*, and *R. solani* have been collected and verified as pathogenic on spinach. An efficient method of inoculum production using a sand-oatmeal mix was evaluated for each pathogen and proved effective in the greenhouse inoculation rate trials for all three pathogens. The first replication of inoculation rate trials was carried out for each of the pathogens in the summer of 2006, to identify a rate of inoculation to assess efficacy of organic seed treatments, i.e., a rate of inoculation that results in about 50% dieback of the seedlings. Based on the greenhouse trials, an inoculation rate of ~5,000 CFU/g soil or potting medium will be used for *P. ultimum*, and ~50,000 CFU/g will be used for each of *F. oxysporum* f. sp. *spinaciae* and *R. solani*. For *P. ultimum*, the inoculation rate trial in the greenhouse (~25°C) proved to be more reliable and efficient than the trial carried out at 15°C in the Percival growth chambers, because of the limitation of space and size of the flats that can be accommodated in the growth chambers. Therefore, seed treatment efficacy trials for all three pathogens will be carried out in the greenhouse in the winter/spring of 2007.

Field and greenhouse trials evaluating efficacy of the organic seed treatments (individually and in various combinations) are expected to be completed by mid- to late summer of 2007. Jaime's MS thesis will be written up in the fall/winter of 2007-08.

Results of the freeze-blotter seed health assay of the “Lazio” seed lot used for the inoculation rate trials and to be used for the greenhouse and field seed treatment efficacy trials are shown below (completed in May 2006):

Seed treatment	Mean ± standard deviation (%) of 400 “Lazio” spinach seed infested/infected						
	<i>Stemphylium botyrosom</i>	<i>Cladosporium variabile</i>	Other <i>Cladosporium spp.</i>	<i>Colletotrichum</i>	<i>Fusarium spp.</i>	<i>Verticillium spp.</i>	<i>Alternaria spp.</i>
Non-sterilized	35.0 ± 2.4	2.5 ± 1.0	33.5 ± 6.0	0	4.8 ± 1.7	42.0 ± 4.3	36.5 ± 4.8
Sterilized (60 s)	37.0 ± 6.4	0.3 ± 0.5	7.0 ± 2.4	0	0	31.0 ± 6.2	24.8 ± 9.6

Outputs: See “Progress towards objectives”.

Impact: None to date.

Institution: Washington State University

State: Washington

Funding source(s) (matching): None

Funding amount(s): \$32,500 from the USDA CSANR has been assigned to continue this project through 2007 (and possibly spring 2008, if needed for Jaime to complete her MS thesis). WSU account #11D-3461-4644.

Organic research land (# of acres):

Station 0 non-organic 0 transitional 2 certified (WSU Mount Vernon NWREC & WSU Vancouver REU)

On-farm 0 non-organic 0 transitional 1 certified (Nash Huber’s farm near Sequim, WA)

Farmer cooperators: The field trial may be duplicated at Nash’s Organic Produce near Sequim, WA, where organic spinach is produced commercially. Spinach damping-off problems have been documented in several fields on that farm by Lindsey du Toit and Jaime Cummings (isolations were carried out in spring 2006 from damped-off spinach plants).