SPINACH (*Spinacia oleracea* 'Lazio')
Damping-off; *Pythium ultimum*
Fusarium wilt; *Fusarium oxysporum* f. sp. *spinaciae*

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Evaluation of seed and drench treatments for organic management of soilborne diseases of spinach in western WA, 2007.

A field trial was conducted at two locations each certified for organic production in western Washington to evaluate selected seed and drench treatments for organic spinach production. One trial was planted at the Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU Mount Vernon NWREC) in Mount Vernon, WA on 18 May, and the second trial at the WSU Vancouver Research and Extension Unit (WSU Vancouver REU) in Vancouver, WA on 5 Jun. The Mount Vernon site had a Puget silt loam soil and the Vancouver site had a Hillsboro silt loam soil. Soil pH prior to planting was 6.42 ± 0.10 and 6.29 ± 0.17 at the Mount Vernon and Vancouver sites, respectively. Average daily temperature at the Mount Vernon site was 12.7 ± 1.9°C, and ranged from 4.2 to 26.1°C for the duration of the trial. Average daily temperature at the Vancouver site was 19.2 ± 8.1°C, and ranged from 6.2 to 40.6°C for the duration of the trial. A total of 47.0 and 65.5 mm rainfall and irrigation were recorded over the duration of the trials at Mount Vernon and Vancouver, respectively. Each trial was set up as a split plot, randomized complete block design with five replications of a 3 x 12 factorial treatment design. The three main plot treatments included: inoculation of the soil with ***P. ultimum*** or ***F. oxysporum*** f. sp. ***spinaciae***, and a non-inoculated control treatment. Split plot treatments consisted of 12 seed and/or drench treatments, which included two proprietary experimental seed treatments (Experimental #1 and Experimental #2), and the experimental treatment Natural X (Agricoat LLC, Soledad, CA). Each split plot was 3.00 m long x 0.76 m wide with five rows of spinach (15 cm between rows and 250 seed/3 m row). Natural soilborne populations of *Fusarium* and *Pythium* were determined for each site from soil cores (30 to 50 cores of 25 mm diameter) collected to a 15 cm depth prior to inoculation and planting. Three 10 g subsamples of each soil sample were used to prepare a dilution series spread onto Petri plates containing appropriate semi-selective agar media (Mircetich and Kraft, and Komada). Inoculum for ***F. oxysporum*** f. sp. ***spinaciae*** was produced on sterilized organic rye seed inoculated with colonized potato dextrose agar (PDA) plugs of the pathogen, incubated for 4 to 5 wk, and then dried. The colonized, dried rye seed was ground and sieved to a particle size of 1.0 to 1.8 mm. ***P. ultimum*** inoculum was produced by adding colonized PDA plugs of the pathogen to a soil/oatmeal mixture (1% ground oatmeal by weight) with 15% water (w/w) in Mason jars. The jars were stored in the dark at room temperature for 4 to 5 wk, and then at 4°C until needed. Inoculum for each pathogen was quantified by dilution plating onto semi-selective media. The ***F. oxysporum*** f. sp. ***spinaciae*** inoculum contained $6.3 \times 10^6 \pm 1.6 \times 10^6$ cfu/g, and the ***P. ultimum*** inoculum contained $4.5 \times 10^5 \pm 1.5 \times 10^5$ cfu/g. At each site, 150 g ***F. oxysporum*** f. sp. ***spinaciae*** inoculum and 75 g ***P. ultimum*** inoculum were applied over each appropriate split plot using a soil sieve, and incorporated 7 to 10 cm deep using a Honda FR800 rototiller. Mount Vernon plots were fertilized 2 wk after planting with Alaska fish fertilizer (5-1-1) (Lilly Miller Brands, Clackamas, OR) at 7.68 ml/liter water and Acadian Seaplants Seaweed Extract fertilizer (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) at 2.23 g/liter water. Fertilizers were applied in 7.6 liters water/split plot. PAR4 granular fertilizer (9-3-7) (Boyer Valley Organic Proteins, Arion, IA) was incorporated at the Vancouver field site at 4,347 kg/ha the day prior to planting. The number of emerged seedlings and the incidence of wilted plants were counted in 1.22 m of each of the middle three rows/split plot every 7 d for 5 or 6 wk after planting at Vancouver and Mount Vernon, respectively. Biomass was measured as above-ground dry weight of seedlings cut at the soil line in 1.22 m of each of the middle three rows/split plot. Data were subjected to analyses of variance (ANOVA) and means comparisons using Fisher's protected least significant difference (LSD).

Soil dilution-plating revealed a ***F. oxysporum*** population of $4.5 \times 10^4 \pm 0.4 \times 10^4$ cfu/g and a *Pythium* population of $3.0 \times 10^2 \pm 1.5 \times 10^2$ cfu/g prior to inoculation at the Mount Vernon site, compared to $6.1 \times 10^3 \pm 1.9 \times 10^3$ cfu/g for ***F. oxysporum*** and $1.5 \times 10^2 \pm 7.1 \times 10^1$ cfu/g for *Pythium* spp. at the Vancouver site. Dilution-plating of soil samples collected from inoculated plots two weeks post-inoculation revealed a ***F. oxysporum*** population of $1.3 \times 10^4 \pm 0.2 \times 10^4$ cfu/g and a *Pythium* population of $1.0 \times 10^3 \pm 0.1 \times 10^3$ cfu/g at the Mount Vernon site, compared to $3.2 \times 10^4 \pm 1.0 \times 10^4$ cfu/g for ***F. oxysporum*** and $9.1 \times 10^2 \pm 1.4 \times 10^2$ cfu/g for *Pythium* spp. at the Vancouver site. Based on the ANOVAs, there was no significant interaction between the main plot factor (pathogen inoculation) and split plot factor (seed and drench treatments) for any dependent variable at any of the weekly ratings at either site. However, main plot and split plot factors had a significant effect on emergence each week at Mount Vernon. Seed and drench treatments also had a significant effect on emergence each week at Vancouver, but the inoculations did not. The opposite was true for post-emergence disease between the two sites. At Mount Vernon, neither the inoculations nor the seed/drench treatments had a significant effect on post-emergence disease. At Vancouver, plots inoculated with ***F. oxysporum*** f. sp. ***spinaciae*** had significantly higher post-emergence disease compared to plots inoculated with ***P. ultimum***, which had higher post-emergence disease than non-inoculated plots. At Mount Vernon, plots inoculated with ***F. oxysporum*** f. sp. ***spinaciae*** also had significantly lower emergence and final biomass than plots inoculated with ***P. ultimum***, which had significantly lower emergence and final biomass than the non-inoculated plots. Seed treatment with Experimental #1 and Experimental #2 resulted in significantly higher emergence at 7 d in each trial compared to both the non-treated seed and the conventional fungicide treatment. In contrast, plots drenched with compost tea or SoilGard 12G Microbial Fungicide, planted with seed treated with Experimental #1, or with Micro 108 Seed Inoculant + Actinovate AG drench had significantly lower final emergence (35 or 42 d) compared to the two control treatments at each site. Additionally, at Mount Vernon, seed treatment with Experimental #2, Kodiak Concentrate Biological Fungicide, and Yield Shield Concentrate Biological Fungicide resulted in significantly lower final emergence. At Mount Vernon, none of the treatments affected post-emergence disease significantly compared to the control treatments. At Vancouver, only plots with Experimental #2 seed treatment had significantly higher disease compared to non-treated seed. At Mount Vernon, only plots with Experimental #2 had significantly lower biomass than that of plots with non-treated seed, but all treatments resulted in significantly lower biomass compared to that of the conventional fungicide treatment. At Vancouver, only the compost tea drench resulted in significantly lower biomass than that of the non-treated seed. Experimental #1 or Kodiak Concentrate Biological Fungicide resulted in significantly higher biomass than that of the conventional fungicide treatment. The results indicate that two of the seed treatments consistently improved early germination of seedlings (i.e., Experimental #1 and Experimental #2). The ability to evaluate the efficacy of the treatments for damping-off of spinach was limited by 1) the predominance of Fusarium wilt at the Mount Vernon site in all main plots, regardless of inoculation treatment, which primarily developed late in the trial (>28d), and 2) the minimal effectiveness of the ***P. ultimum*** inoculum at both sites.

Field site, pathogen inoculation, and seed and/or drench treatment (rate/100 kg seed or rate/100 liters water)[z]	Emergence/3.6 m row[y]		AUPC[x]		Dry biomass (g/3.6 m row)[w]
	7 d	35 or 42 d	Emergence	Disease	
Mount Vernon, WA					
Pathogen inoculation					
Non-inoculated	10.2 a[v]	92.3 a	3018 a	163 a	79.5 a
P. ultimum	8.4 a	87.6 a	2778 b	162 a	71.5 b
F. oxysporum f. sp. *spinaciae*	6.0 b	70.9 b	2375 c	212 a	55.5 c
LSD	Log	5.01	168.8	NS	6.48
Seed and/or drench treatment					
Non-treated seed	6.1 b	97.7 ab	3179 ab	239 a	71.3 b
Combination conventional fungicides[u]	4.5 b	103.7 a	3301 a	127 a	88.9 a
Compost tea 50 liter	1.3 c	63.0 d	2059 d	105 a	60.2 bc
Experimental #1	29.0 a	65.5 d	2248 d	130 a	62.0 bc
Experimental #2	28.4 a	59.4 d	2150 d	144 a	53.2 c
Kodiak Concentrate Biological Fungicide 31.2 g	3.7 b	86.4 c	2786 c	157 a	68.0 b
Micro 108 Seed Inoculant 1.76 kg + Actinovate AG 2.58 g	3.3 bc	84.9 c	2780 c	219 a	71.7 b
Natural II 750.7 g	4.4 b	90.0 bc	2936 bc	218 a	70.7 b
Natural X 750.7 g	3.9 b	90.9 bc	2884 bc	221 a	72.7 b
SoilGard 12G Microbial Fungicide 239.7 g	5.2 b	86.9 c	2771 c	175 a	70.8 b
Subtilex Biological Fungicide 15.6 g	4.2 b	88.8 bc	2833 c	211 a	68.3 b
Yield Shield Concentrate Biological Fungicide 6.26 g	4.3 b	85.7 c	2755 c	204 a	68.3 b
LSD	Log	10.03	337.6	NS	12.96
Vancouver, WA					
Pathogen inoculation					
Non-inoculated	1.2 a	57.5 a	1426 a	35 c	79.5 a
P. ultimum	1.6 a	56.5 a	1401 a	44 b	80.6 a
F. oxysporum f. sp. *spinaciae*	1.8 a	57.5 a	1464 a	62 a	74.4 a
LSD	Rank	4.11	96.7	9.0	6.90
Seed and/or drench treatment					
Non-treated seed	0.9 cd	62.2 a	1543 ab	46 bc	80.4 ab
Combination conventional fungicides[u]	0.5 d	61.1 a	1502 ab	54 ab	73.8 bc
Compost tea 50 liter	2.2 bcd	44.5 c	1081 c	39 bc	61.5 c
Experimental #1	5.5 a	51.2 bc	1403 b	40 bc	92.0 a
Experimental #2	2.9 ab	60.3 a	1535 ab	69 a	80.1 ab
Kodiak Concentrate Biological Fungicide 31.2 g	0.4 d	64.8 a	1606 a	47 bc	92.1 a
Micro 108 Seed Inoculant 1.76 kg + Actinovate AG 2.58 g	1.3 bcd	45.7 c	1159 c	42 bc	69.4 bc
Natural II 750.7 g	1.1 cd	61.1 a	1516 ab	43 bc	80.9 ab
Natural X 750.7 g	0.5 d	61.5 a	1479 ab	35 c	74.4 bc
SoilGard 12G Microbial Fungicide 239.7 g	2.9 abc	48.7 c	1198 c	47 bc	71.1 bc
Subtilex Biological Fungicide 15.6 g	0.3 d	65.5 a	1661 a	53 ab	81.7 ab
Yield Shield Concentrate Biological Fungicide 6.26 g	0.3 d	58.9 ab	1473 ab	52 abc	79.9 ab
LSD	Rank	8.22	193.4	17.9	13.80

[z] Each treatment applied as a seed treatment at the rate shown/100 kg seed, except for the compost tea, Actinovate AG, and SoilGard 12G Microbial Fungicide treatments which were each applied as a soil drench at the rate shown/100 liters water. Ingredients of the compost tea included vermicompost (5 liters), seaweed powder (100 ml), liquid humic acids (200 ml), and Azomite rock dust (300 g), which were aerated in 95 liters water for 24 h prior to application (Scheuerell and Mahaffee, 2004). The compost tea was applied as 3.79 liters tea in 7.57 liters water per split-plot. Actinovate AG was applied as 0.2 g product dissolved in 7.57 liters water per split plot. SoilGard 12G Microbial Fungicide was applied as 70.94 g product suspended in 23.65 liters water per split plot.

[y] The Mount Vernon and Vancouver field trials were carried out for 42 and 35 d, respectively.

[x] Area under progress curves (AUPCs) for emergence and post-emergence disease (damping-off or wilt). AUPC is a cumulative measure of emergence or disease ratings over time: $[(\Sigma(y_i + y_{i+1}/2)(t_i - t_{i+1})]$, where y_i = the number of emerged or diseased seedlings at the i^{th} rating, y_{i+1} = the number of emerged or diseased seedlings at the (i+1) rating, t_i = the number of days at the i^{th} rating, and t_{i+1} = the number of days at the (i+1) rating.

[w] Above-ground dry weight of plants sampled at the final rating (35 or 42 d).

[v] For main plot pathogen inoculations, and for split plot seed and/or drench treatments, each mean is averaged over five replications and all levels of the other factor. For each dependent variable at each location, main plot means followed by the same letter within a column are not significantly different based on Fisher's protected least significant difference (LSD) at $P < 0.05$; similarly for split plot means. Means are not presented for the interaction of pathogens with seed or drench treatments because the interaction term in the analysis of variance was not significant for any dependent variable. Log = original means presented but means separation is based on logarithmic transformation to meet requirements for parametric statistical analyses. Rank = original means presented, but means separation is based on Friedman's non-parametric rank test because assumptions for parametric analyses could not be met using transformations.

[u] A combination conventional fungicide treatment consisting of seed treatment with Apron XL LS (20.8 ml/100 kg seed) and Mertect 340F (122.4 ml/100 kg seed), and a drench with Terraclor 75% WP (59.9 or 30.0 g/100 liters water at Mount Vernon and Vancouver, respectively) for control of *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., respectively.