SPINACH SEED CROP (*Spinacia oleracea*)
Fusarium wilt; *Fusarium oxysporum* f. sp. *spinaciae*

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Evaluation of limestone amendments for control of Fusarium wilt in a spinach seed crop, 2006.

A limestone amendment field trial was set up on a Puget clay loam soil in Skagit Co., WA. A randomized complete block design was used with five replications of a factorial combination of six rates of limestone amendment (applied to main plots) and two proprietary female spinach inbred lines, one highly susceptible to Fusarium wilt and one moderately susceptible to Fusarium wilt (planted in subplots). The field had previously been planted to a spinach seed crop in 2001, and the soil pH was 5.5 in Mar 06. Agricultural limestone (TexLime General Purpose Limestone Flour, Oregon Lime Score = 96, CCE = 98.3%, 97% $CaCO_3$, and 38.8% Ca) was applied manually using a drop spreader at 0, 1.4, 2.1, 2.8, 3.5, and 4.2 tons/acre onto the appropriate main plots (each measuring 35 ft x 35 ft) on 4 and 5 Apr, and incorporated to a depth of 6 in. on 5 Apr using a rototiller. The plots were mulched mechanically on 28 Apr. On 2 May, RoNeet (herbicide) was broadcast at 42 oz/acre in 15 gpa and at 40 psi, and incorporated with a mulcher-packer for weed control. Spinach seed were planted at a depth of 0.5 in. using a Monosem planter, with 22 in. spacing between rows and 2 in. spacing within rows. Six rows of the appropriate female line were planted in each subplot, along with one row of a proprietary male line on each side of the six female rows. Fertilizer (11-52-0) was applied in-furrow at 350 lb/acre at planting. Soil samples were collected from each main plot (8 cores/plot) to a depth of 6 in. on 11 May, dried, and tested for pH. Plant stand and incidence of wilted seedlings were counted in 10 ft of row for each spinach line on 30 May, 21 Jun, and 11 Jul. Isolations were carried out from a sample of wilting seedlings from all three spinach lines on 31 May. Spin-Aid was broadcast at 46 oz/acre in 11 gpa and at 35 psi on 6 Jun for weed control. Plots were also weeded manually. Fertilizer (27-0-0) was applied at 130 lb/acre on 12 Jun with a single-shank applicator. On 26 Jun, plants were sampled from 3.3 ft of row of each parent line, dried for 2 weeks, and weighed. Soil cores (20/main plot) were collected on 26 Jun, dried for 5 days, and soil nutrient analyses completed. Plant biomass samples were collected again on 11 Aug, dried, and weighed. Plants in the middle 10 ft of each of the center 4 rows of each female line were windrowed on 16 to 18 Aug, dried for a week in the field, and hand-threshed. An additional 20 soil cores/subplot were collected on 22 Aug from within rows of the female spinach plants, dried, and soil pH determined. The harvested seed were cleaned and screened to marketable size (6 to 13). A sample of 100 seed/subplot/replication was then tested for germination using the blotter assay of the Association of Official Seed Analysts (AOSA). In addition, a freeze-blotter seed health assay for necrotrophic fungi was carried out on 100 seed/subplot/replication. The seed were placed onto damp blotters in plastic petri plates (20 seed/plate), imbibed in the dark for 25 h, and then incubated at –20ºC for 25 h followed by 12 d at 24ºC under a 12 h/12 h day/night cycle with near-UV and cool white fluorescent light by day. The seed were examined 5, 9, and 14 to 17 d after plating, using a dissecting microscope (8 to 100X magnification). Mean temperature and total rainfall for Apr, May, Jun, Jul, and Aug were 46.8°F and 3.16 in., 45.0°F and 1.49 in., 58.6°F and 1.10 in., 61.1°F and 0.82 in., and 59.2°F and 0.23 in., respectively.

Stand counts on 30 May, 21 Jun, and 11 Jul were not affected significantly by limestone applications (Table 1). Similarly, the incidence of wilted plants on each of 30 May and 21 Jun was not affected significantly by limestone applications. However, by 11 Jul, limestone applications >1.4 tons/A significantly suppressed Fusarium wilt. Plant biomass on 26 Jun was significantly greater in all plots treated with limestone compared to the control plots, with the highest biomass from plots amended at 4.2 tons/A. By 11 Aug, limestone applications ≥2.1 tons/A had significantly larger plants than plots treated with 1.4 tons/A or no lime. The suppressive effect of lime on Fusarium wilt was significant for the susceptible female line, with 45% reduction in incidence of wilt, and 318% increase in seed yield on plots amended at 3.5 or 4.2 tons/A compared to control plots. For the moderately susceptible female, limestone applications did not decrease the incidence of wilt significantly, but seed yield was greatest (2,468 lb seed/A) on plots amended with limestone at 2.8 tons/A, and decreased at higher and lower rates of amendment. Stand counts were significantly higher, incidence of wilt lower, plant biomass greater, and seed yields almost eight-fold greater for the moderately susceptible female line compared to the highly susceptible female line. The male line was the most susceptible of the three lines to Fusarium wilt. *F. oxysporum* f. sp. *spinaciae* was the primary organism isolated from wilting seedlings of the susceptible female line and the male line on 31 May, but *Pythium* spp. and *Rhizoctonia* spp. were primarily isolated from a few wilting plants of the moderately susceptible female (*data not shown*). Limestone amendments increased soil pH significantly on 15 May, 26 Jun, and 18 Aug, with the highest pH measured on 26 Jun, approximately 2 months after application (Table 2). The highest rate increased soil pH from means of 5.4, 5.7, and 5.5 in the control plots to 6.2, 6.9, and 6.7 on 15 May, 26 Jun, and 18 Aug, respectively. Even 4.2 tons/A did not render this soil alkaline. Both soil and plant calcium (Ca) concentrations increased significantly with increasing rates of limestone application. Applications of >1.4 tons/A significantly reduced the concentration of available zinc (Zn) and manganese (Mn) in the soil and plants. The moderately susceptible female line had significantly lower concentrations of potassium (K), magnesium (Mg), sulfur (S), boron (B), zinc, and copper (Cu) than the highly susceptible female line, reflecting stunted growth of the latter compared to the former. Conversely, calcium concentrations were significantly higher in the moderately susceptible female than the highly susceptible female. Calcium plays an important role in host resistance, while zinc and manganese are thought to promote aggressiveness of some Fusarium wilt pathogens. Other micro- or macronutrients, or other properties measured (NO_3-N, NH_4-N, P, K, Na, Fe, Cl, organic matter, and electrical conductivity) were not affected significantly by rate of limestone or parent line (*data not shown*). There was no significant interaction between rate of limestone amendment and parent line in the analyses of variance for any variable in the soil and plant analyses, and in the seed germination and health assays. Limestone amendments did not affect germination of the harvested seed, but germination was significantly greater for the moderately susceptible female than the highly susceptible female (62.0 vs. 21.3%, respectively) (Table 2). The incidence of rotten seed was significantly lower for the moderately susceptible female than the highly susceptible female. The incidence of harvested seed infected with *Fusarium* spp. was very low (<1%) for both female lines and all rates of limestone amendment (Table 2), despite significant differences in incidence of Fusarium wilt in the field in Jul. In contrast, the incidence of seed infected with *Verticillium* spp. increased significantly with increasing rate of limestone amendment for both female lines, from a mean of 11.2% for non-limed plots to 35.7% for plots limed at 3.5 tons/acre. The moderately susceptible female had significantly more seed infected with *Verticillium* spp. (38.7%) than the highly susceptible female (8.6%). The incidences of other seedborne necrotrophic fungi were not affected by parent line or rate of limestone amendment. The results demonstrate that limestone amendments may reduce losses to Fusarium wilt in spinach seed crops on acid soils of western Washington.

Table 1.

Factor	Stand count (no. of plants/10 ft of row)			Incidence of wilted plants (%/10 ft of row)			Dry plant weight (lb/3.3 ft of row)		Marketable seed yield (lb/A)
	30 May	21 Jun	11 Jul	30 May	21 Jun	11 Jul	26 Jun	11 Aug	
Spinach parent line and susceptibility to Fusarium wilt									
♀ Moderate ……..	111 a*	109 a	101 a	0.6 a	0.5 a	2.2 a	0.19 a	1.03 a	1,660 a
♀ Susceptible …..	104 b	91 b	86 b	4.4 b	49.7 b	65.1 b	0.11 b	0.37 b	211 b
♂ ……..	93 c	89 c	84 b	7.0 c	68.8 c	90.6 c	-	0.20 c	-
LSD …….	Log	3.9	4.7	Arcsin	4.95	6.05	0.014	Sq rt	Sq rt
Rate of limestone amendment (tons/A)									
0 ……...	104 a	93 a	87 a	4.8 a	48.4 a	62.4 a	0.12 c	0.42 c	706 a
1.4 ……	101 a	94 a	89 a	4.4 a	41.8 a	62.1 a	0.14 b	0.44 c	754 a
2.1 ……	103 a	96 a	91 a	3.8 a	38.8 a	52.2 b	0.15 b	0.52 b	935 a
2.8 ……	106 a	98 a	93 a	4.0 a	35.8 a	49.1 b	0.14 b	0.65 a	1,340 a
3.5 ……	103 a	99 a	93 a	2.9 a	35.1 a	44.9 b	0.16 b	0.58 ab	911 a
4.2 ……	101 a	96 a	89 a	4.1 a	38.1 a	45.0 b	0.19 a	0.58 ab	881 a
LSD ……..	NS	NS	NS	NS	NS	8.55	0.024	Sq rt	NS
Interaction of spinach parent line and rate of limestone amendment (tons/A)									
♀ Moderate – 0 …	109 a	106 a	96 a	0.2 a	1.6 a	1.7 d	72.8 a	404.8 a	1,334 b
1.4 ..	107 a	104 a	98 a	0.4 a	0.2 a	8.4 d	89.3 a	442.8 a	1,430 b
2.1 ..	112 a	106 a	102 a	0.7 a	0.4 a	1.7 d	81.0 a	456.6 a	1,611 ab
2.8 ..	114 a	112 a	106 a	0.8 a	0.2 a	0.0 d	87.1 a	565.8 a	2,468 a
3.5 ..	118 a	112 a	104 a	0.3 a	0.6 a	0.6 d	93.1 a	451.8 a	1,491 b
4.2 ..	107 a	111 a	99 a	1.1 a	0.0 a	0.6 d	97.7 a	483.2 a	1,587 b
♀ Susceptible – 0	107 a	90 a	87 a	4.8 a	64.5 a	86.9 a	32.7 a	121.0 a	78 d
1.4	103 a	88 a	83 a	5.0 a	51.9 a	81.3 ab	38.2 a	102.4 a	72 cd
2.1	106 a	94 a	88 a	5.1 a	47.3 a	64.6 bc	53.8 a	150.4 a	253 cd
2.8	106 a	92 a	85 a	5.6 a	47.0 a	63.0 bc	40.0 a	196.0 a	205 cd
3.5	98 a	91 a	86 a	2.4 a	43.0 a	47.2 c	49.0 a	216.4 a	326 c
4.2	104 a	88 a	83 a	3.4 a	44.7 a	47.7 c	71.5 a	206.8 a	326 cd
♂ - 0 …….	95 a	83 a	79 a	9.5 a	79.3 a	98.6 a	-	42.1 a	-
1.4 ……..	93 a	90 a	85 a	7.9 a	73.3 a	96.5 a	-	50.1 a	-
2.1 ……..	90 a	87 a	82 a	5.5 a	68.8 a	90.4 a	-	100.7 a	-
2.8 ……..	96 a	90 a	86 a	5.6 a	60.3 a	84.3 a	-	123.6 a	-
3.5 ……..	94 a	94 a	90 a	5.9 a	61.7 a	86.7 a	-	127.8 a	-
4.2 ……..	90 a	89 a	85 a	7.9 a	69.6 a	86.8 a	-	101.2 a	-
LSD …….	NS	NS	NS	NS	NS	19.41	NS	NS	Sq rt

* Each mean is the average of 5 replications. For the two main factors (spinach parent line and liming rate), each mean is averaged over 5 replications and all levels of the other main factor. For each main factor or interaction, means followed by the same letter within a column are not significantly different based on Fisher's protected least significant difference (LSD) at $P < 0.05$. Interaction means are only presented for those variables for which the interaction term was significant in the analysis of variance. Arcsin, Log, and Sq rt = original means presented, but means separation is based on arcsin, logarithmic, and square root transformations, respectively, to meet assumptions for parametric analyses. NS = not significantly different at $P < 0.05$.

Table 2.

Factor	Soil nutrient analyses[z]: pH 15 May	pH 26 Jun	pH 18 Aug	Nutrient concentration (on 26 Jun) Ca (meq/100 g)	Zn (mg/kg)	Mn (mg/kg)
Spinach parent line and susceptibility to Fusarium wilt						
♀ Moderate	-[y]	-	6.3 a	-	-	-
♀ Susceptible ..	-	-	6.1 b	-	-	-
♂ …….	-	-	-	-	-	-
LSD …….	-	-	0.07	-	-	-
Rate of limestone amendment (tons/A)						
0 ……..	5.4 c[x]	5.7 d	5.5 e	8.9 c	1.48 a	3.0 a
1.4 ……...	5.7 b	6.1 d	5.9 d	11.0 b	1.24 ab	1.7 ab
2.1 ……...	5.9 ab	6.4 c	6.2 c	11.9 b	1.06 bc	1.3 bc
2.8 ……...	6.0 a	6.7 bc	6.5 b	13.9 a	1.06 bc	1.4 bc
3.5 ……...	6.1 a	6.8 ab	6.6 b	13.9 a	0.92 bc	1.2 c
4.2 ……...	6.2 a	6.9 a	6.7 a	15.0 a	0.80 c	1.1 c
LSD …….	0.28	Rank	0.12	1.43	0.385	Rank

Factor	Plant nutrient analyses (26 Jun)[z]: K (%)	Ca (%)	Mg (%)	S (%)	B (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
Spinach parent line and susceptibility to Fusarium wilt								
♀ Moderate ….	5.12 b	1.49 a	0.55 b	0.29 b	26.3 b	33.3 b	42.6 a	7.9 b
♀ Susceptible ..	6.40 a	1.41 b	0.63 a	0.35 a	36.4 a	43.5 a	51.9 a	10.4 a
♂ …….	-	-	-	-	-	-	-	-
LSD[z] ……...	0.277	0.075	0.30	0.015	1.48	2.13	NS	0.42
Rate of limestone amendment (tons/A)								
0 ……..	5.49 a	1.15 d	0.60 a	0.31 a	31.9 a	43.6 a	72.5 a	8.3 a
1.4 ……...	5.53 a	1.39 c	0.57 a	0.31 a	32.0 a	40.7 ab	50.4 b	9.1 a
2.1 ……...	5.97 a	1.48 bc	0.62 a	0.34 a	32.2 a	39.7 bc	46.2 bc	9.7 a
2.8 ……...	5.92 a	1.43 bc	0.59 a	0.31 a	29.9 a	35.8 d	38.3 d	9.2 a
3.5 ……...	5.72 a	1.68 a	0.59 a	0.31 a	30.9 a	36.1 cd	39.7 cd	9.3 a
4.2 ……...	5.93 a	1.53 b	0.57 a	0.32 a	31.0 a	34.4 d	36.5 d	9.3 a
LSD …….	NS	0.13	NS	NS	NS	3.69	Log	NS

Factor	AOSA seed germination assay (% of seed)[w]: Germinated	Rotten	Freeze-blotter seed health assay (% of seed)[w]: *Fusarium* spp.	*Verticillium* spp.	*Stemphylium botryosum*	*Cladosporium variabile*	All other fungi
Spinach parent line and susceptibility to Fusarium wilt							
♀ Moderate ….	62.0 a	26.4 b	0.6 a	38.7 a	13.0 a	0.6 a	17.0 a
♀ Susceptible ..	21.3 b	66.9 a	0.1 a	8.6 b	17.5 a	1.3 b	20.0 a
♂ …….	-	-	-	-	-	-	-
LSD …….	4.54	4.85	NS	Log	NS	0.76	NS
Rate of limestone amendment (tons/A)							
0 ……..	43.2 a	45.6 a	0.3 a	11.2 c	15.8 a	0.8 a	20.6 a
1.4 ……...	36.1 a	49.9 a	0.1 a	19.6 bc	14.6 a	0.7 a	20.8 a
2.1 ……...	39.2 a	49.0 a	0.5 a	20.0 bc	17.6 a	1.5 a	18.9 a
2.8 ……...	47.1 a	42.5 a	0.4 a	29.1 ab	17.7 a	0.6 a	19.5 a
3.5 ……...	42.2 a	45.8 a	0.6 a	35.7 a	12.9 a	1.6 a	15.9 a
4.2 ……...	44.3 a	45.0 a	0.2 a	26.4 ab	12.7 a	0.6 a	15.3 a
LSD …….	NS	NS	NS	Log	NS	NS	NS

[z] Soil and plant analyses were done by Soiltest Farm Consultants (Moses Lake, WA), except for soil pH tests on samples from 15 May and 18 Aug, which were completed at the WSU Mount Vernon NWREC.

[y] For each main factor (spinach parent line and rate of limestone amendment), each mean is averaged over 5 replications and all levels of the other main factor.

[x] Within each main factor, means followed by the same letter in a column are not significantly different based on Fisher's protected least significant difference (LSD) at $P < 0.05$. Interaction means are not presented because the interaction term in the analysis of variance was not significant for any of the variables measured. 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. NS = not significantly different at $P < 0.05$.

[w] Seed was not harvested from the male parent line.