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

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Effect of agricultural limestone and nitrogen fertilizers on Fusarium wilt and Verticillium wilt in a spinach seed crop, 2009.

Three rates of agricultural limestone application (in split-split plots); proprietary female spinach inbred lines moderately resistant (R), moderately susceptible (M), and highly susceptible (S) to Fusarium wilt (planted in split plots); and ammonium- vs. nitrate-based nitrogen (N) fertilizers (applied to main plots) were evaluated in a field trial in a Skagit silt loam soil near Mount Vernon, Skagit Co., WA. A split-split plot, randomized complete block design was used with four replications. The field had previously been planted to a spinach seed crop in 2005. Soil pH was 5.9 in Mar. Agricultural limestone (Imperial Ground limestone, Oregon Lime Score = 97, CCE = 97%, with 97% $CaCo_3$ and 38.8% Ca) was applied on 10 Apr at 0, 1, and 2 t/A with a 6 ft wide Gandy drop spreader, and rototilled 6 to 8 in. deep. On 22 Apr, RoNeet (42 oz/A) and Diazinon (2 qt/A) were broadcast in 15 gal/A at 40 psi and incorporated with a mulcher-packer for weed and insect control. Spinach seeds were planted on 22 Apr (0.5 in. deep) using a Monosem planter, with 22 in. spacing between rows and 2.5 in. spacing within rows. Six rows of the appropriate female line were planted in each split plot, with one row of a proprietary male line on each side of the six female rows. Fertilizer was applied in-furrow at planting: 11-52-0 monoammonium phosphate (270 lb/A) or 15-5-0 calcium nitrate (100 lb/A). Twelve soil cores (6 in. deep) were collected/split-split plot on 5 and 27 May, 18 Jun, 8 and 28 Jul, and 19 Aug for nutrient analyses. Plant stand and incidence of damped-off or wilted plants were counted in two 10 ft sections of row/spinach line on 15 May, 4 and 24 Jun, and 14 Jul. Isolations for plant pathogens were completed from roots and crowns of a sample of wilting seedlings/spinach line on 8 Jun. Plots were hand-weeded regularly. Fertilizer (27-0-0) was applied at 175 lb/A on 5 Jun with a single-shank applicator. On 29 Jun and 22 Jul, plants were collected from 3.3 ft of row/parent line. At the latter date, the root and crown of each plant were cut longitudinally and rated for dark vascular discoloration typical of Fusarium wilt. The plants were dried at 95°F and weighed. Plants sampled on 29 Jun were subjected to nutrient analyses. Seeds were harvested manually from 10 ft of the center 4 rows/female line, and dried at 78°F. Seeds were cleaned, screened to marketable size (screen sizes 7 to 13), and weighed. A sample of 100 seeds/plot was tested for germination using the blotter assay of the Association of Official Seed Analysts (AOSA). A freeze-blotter seed health assay for necrotrophic fungi was carried out on 100 seeds/plot. Seeds were placed on damp blotters in 4 in. x 4 in. acrylic boxes (32 to 34 seeds/box), imbibed in the dark for 25 h, and then incubated at -20°C for 25 h followed by 12 d at 24°C using a 12 h/12 h day/night cycle with near-UV and cool white fluorescent light by day. Seeds were examined 5, 9, and 14 d after plating using a dissecting microscope (8 to 100X magnification). Mean daily temperature and total rainfall for Apr, May, Jun, Jul, and Aug were 47.8°F and 1.69 in., 53.6°F and 2.63 in., 60.0°F and 0.16 in., 64.3°F and 0.51 in., and 61.7°F and 0.52 in., respectively. The trial was irrigated (1.3 in. water) on 18 Jun.

Rainfall (0.75 in.) 10 d after planting caused leaching of nitrate in plots fertilized in-furrow with nitrate-N. Spinach in these plots remained nitrogen-deficient (pale green) throughout the trial. The incidence of damping-off did not differ significantly among treatments on 15 May. By 4 Jun, the R female had significantly more damping-off than the M female, and all three female lines had significantly more damping-off than the male line. There was also a significant interaction between parent line and nitrogen fertilizer; the M line had significantly more damping-off in plots fertilized with ammonium-N vs. nitrate-N. The incidence of wilt increased through the season. By 24 Jun, wilt incidence was significantly affected by rates of limestone, nitrogen fertilizer, and the interaction of these factors. Plots with 2 t limestone/A had fewer wilted plants (5.3%) than plots with 1 (12.9%) or 0 t/A (33.6%). In plots with 0 and 1 t/A, nitrate-N fertilizer significantly increased wilt compared to ammonium-N, but the fertilizers had no effect in plots with 2 t/A. By 14 Jul, the S and M lines had significantly more wilt (>90% incidence) than the R line (80.4%), and the early-maturing male line had fully senesced. The R line had less wilt in plots fertilized with ammonium-N vs. nitrate-N. On 29 Jun, the male and S female lines had significantly greater biomass than the M line, which had greater biomass than the R line, reflecting the inherent smaller stature of the R female line. Spinach biomass on 29 Jun in plots with 2 t limestone/A was >200% that of plots with no limestone, and 31% more than in plots with 1 t/A. By 22 Jul, plots with 1 or 2 t limestone/A had 56 to 85% more spinach biomass than plots with no limestone. Spinach biomass on 29 Jun was greater in plots fertilized with ammonium-N vs. nitrate-N for each parent except the M female. Spinach biomass was not affected by N-fertilizer in plots with 2 t limestone/A, but in plots with 0 or 1 t/A biomass was greater in plots fertilized with ammonium-N vs. nitrate-N. The M and R lines had 62 and 50% more marketable seed yield, respectively, than the S line. Application of 2 t limestone/A increased seed yield to 494 lb/A compared to 184 lb/A in plots with no limestone. Ammonium-N fertilized plots out-yielded nitrate-N plots by 83%. *F. oxysporum* was isolated from wilted seedlings of all parent lines in May. The S line had 100% vascular discoloration typical of Fusarium wilt on 22 Jul, which was greater than that of the M and R lines (93 to 94%). Incidence of vascular discoloration was significantly less in plots with 2 t limestone/A vs. 0 or 1 t/acre. The M line had more vascular discoloration in plots fertilized with ammonium-N vs. nitrate-N, but the fertilizers did not affect vascular discoloration of the other two female lines. Limestone increased soil pH significantly within three weeks of application, from 5.9 in control plots to 6.4 and 6.6 in plots with 1 and 2 t/A, respectively. Although soil pH declined gradually through the season, significant differences in pH among limestone treatments were detected through harvest, when pH was 5.2, 5.8, and 6.3 for plots with 0, 1, and 2 t/A, respectively. On 27 May, plots fertilized with nitrate-N had significantly higher pH (6.3) than the ammonium-N plots (6.1), but this effect disappeared by 19 Aug. On 27 May, ammonium-N fertilized plots had significantly more ammonium-N than nitrate-N plots, but soil nitrate-N did not differ among the fertilizer treatments because nitrate was leached by severe rainfall 10 d after planting. Available soil calcium (Ca) increased significantly with increasing rate of limestone. In contrast, soil zinc (Zn) and manganese (Mn) were significantly greater in plots with 0 vs. 1 and 2 t limestone/A. Soil iron (Fe) was not affected by limestone treatments, but was lower in plots fertilized with nitrate-N vs. ammonium-N. Plant Ca, phosphorus (P), and sulfur (S) were lower and magnesium (Mg), Mn, and Zn were higher in plots with 0 t limestone/A compared to 2 t limestone/A. S female plants had significantly less boron (B), Ca, Mg, S, and Zn than the other two females. Plant N, P, and S levels were greater in plots fertilized with ammonium-N vs. nitrate-N, but B and Zn were the opposite. Germination of harvested seeds was significantly less from nitrate-N vs. ammonium-N fertilized plots. The incidence of harvested seeds infected with *Fusarium* spp. was greater for the S line than the M and R lines. Soil sampled in the rows after harvest from plots with S and M lines had significantly more *F. oxysporum* than soil from plots with the R line. Soil from nitrate-N plots had 89% more *V. dahliae* compared to ammonium-N plots. The results demonstrate the potential for limestone to render acidic soils of western Washington less conducive to Fusarium wilt, and the influence of spinach line susceptibility to Fusarium wilt on this response.

Factor	Incidence (%) of damping-off seedlings/20 ft of row		Incidence (%) of wilted plants/20 ft of row		Incidence (%) of vascular discoloration on 22 Jul	Dry plant weight (lb/3.3 ft of row)		Marketable seed yield (lb/A)
	15 May	4 Jun	24 Jun	14 Jul		29 Jun	22 Jul	
Form of N-fertilizer								
Ammonium (NH_4)	2.2[z]	4.6	8.3 b	88.8	96.7	0.25	0.32	452 a
Nitrate (NO_3)	1.9	3.4	26.3 a	92.9	94.2	0.16	0.22	246 b
LSD	NS[y]	NS	Rank	NS	NS	NS	NS	109.7
Spinach parent line and susceptibility to Fusarium wilt								
♀ Susceptible	2.4	4.7 ab	24.4	99.5 a	100.0 a	0.21 a	0.23	269 b
♀ Moderate	2.2	3.6 b	18.2	92.6 b	94.0 b	0.20 b	0.27	409 a
♀ Resistant	1.6	7.0 a	9.3	80.4 c	92.5 b	0.16 c	0.28	378 a
♂	2.0	0.6 c	-	-	-	0.25 a	0.29	-
LSD	NS	Rank	NS	Rank	Rank	Rank	NS	108.4
Rate of limestone amendment (t/A)								
0	1.9	14.9	33.6 a	92.0	98.3 a	0.13 c	0.18 b	184 b
1	2.4	11.5	12.9 ab	91.4	97.1 a	0.21 b	0.29 a	368 ab
2	1.8	11.0	5.3 b	89.1	91.0 b	0.27 a	0.34 a	494 a
LSD	NS	NS	Rank	NS	Rank	Rank	Rank	212.3
Interaction of spinach parent line and N-fertilizer								
♀ Susceptible - NH_4	2.9	14.0 ab	4.1	99.0 a	100.0 a	0.27 ab	0.32	401
- NO_3	1.8	13.0 ab	44.8	100.0 a	100.0 a	0.16 d	0.15	118
♀ Moderate - NH_4	2.5	16.0 a	13.5	95.1 b	99.2 ab	0.23 bc	0.31	465
- NO_3	1.9	8.9 bc	22.8	90.0 b	88.9 c	0.17 cd	0.24	353
♀ Resistant - NH_4	1.0	18.4 a	7.2	72.3 c	91.1 bc	0.18 bc	0.32	489
- NO_3	2.2	16.9 a	11.4	88.5 b	93.8 c	0.13 d	0.24	268
♂ - NH_4	2.3	6.0 c	-	-	-	0.31 a	0.32	-
- NO_3	1.7	6.8 c	-	-	-	0.19 cd	0.26	-
LSD	NS	Rank	NS	Rank	Rank	0.06	NS	NS
Interaction of limestone and form of N-fertilizer								
0 - NH_4	2.7	5.5	13.4 bc	87.8	100.0	0.22 bc	0.27	316
- NO_3	1.0	5.3	53.8 a	96.3	96.7	0.05 d	0.10	52
1 - NH_4	2.5	4.3	6.4 c	90.0	100.0	0.25 ab	0.32	443
- NO_3	2.4	2.6	19.5 b	88.2	94.2	0.17 c	0.25	294
2 - NH_4	1.3	4.0	4.9 c	88.8	90.2	0.28 a	0.36	596
- NO_3	2.3	2.2	5.7 c	94.0	91.8	0.27 ab	0.31	393
LSD	NS	NS	Rank	NS	NS	Rank	NS	NS

	Soil pH[x]						Soil nutrient analyses (27 May)[x]					
	5 May	27 May	18 Jun	8 Jul	28 Jul	19 Aug	Ca (meq/100g)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	NO_3 (mg/kg)	NH_4 (mg/kg)
N-fertilizer												
NH_4	-	6.1 b	-	-	-	5.76	4.8	2.3	2.3	67.3 b	33.5	9.1 a
NO_3	-	6.3 a	-	-	-	5.79	4.8	2.0	2.1	81.7 a	32.3	2.2 b
LSD	-	0.16	-	-	-	NS	NS	NS	NS	8.63	NS	Rank
Rate of limestone amendment (t/A)												
0	5.9 c	5.7 c	5.7 c	5.7 c	5.4 b	5.2 c	3.5 c	2.6 a	3.2 a	80.0	29.7	9.9
1	6.4 b	6.2 b	6.1 b	6.2 b	6.3 a	5.8 b	5.0 b	2.1 b	1.9 b	72.9	30.2	3.9
2	6.6 a	6.6 a	6.5 a	6.6 a	6.6 a	6.3 a	6.0 a	1.7 b	1.6 b	70.6	38.7	3.2
LSD	0.16	0.19	0.26	0.13	0.42	0.14	0.55	0.50	0.44	NS	NS	NS

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Factor	Plant nutrient analyses (29 Jun)[x] N (%)	P (%)	Ca (%)	Mg (%)	S (%)	B (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
N-fertilizer									
NH_4	3.18 a	0.54 a	1.19	0.54 b	0.24 a	23.5 b	93.2 b	155.1	3,728
NO_3	2.81 b	0.41 b	1.29	0.70 a	0.16 b	30.8 a	120.8 a	148.2	5,064
LSD	0.279	Sq root	NS	Rank	Arcsin	Rank	Log	NS	NS
Spinach parent line and susceptibility to Fusarium wilt									
♀ Susceptible	2.57 c	0.47 b	1.07 c	0.56 b	0.17 b	23.7 b	80.3 c	147.9 bc	5,068 a
♀ Moderate	3.14 b	0.52 a	1.22 b	0.65 a	0.22 a	28.8 a	122.8 a	166.5 a	5,063 a
♀ Resistant	3.73 a	0.47 b	1.37 a	0.71 a	0.23 a	30.6 a	98.8 b	153.8 b	4,086 a
♂	2.53 d	0.43 c	1.28 b	0.59 b	0.17 b	25.6 b	126.3 a	138.4 c	3,367 b
LSD	0.12	Sq root	0.087	Rank	Arcsin	Rank	Log	Rank	Rank
Rate of limestone amendment (t/A)									
0	2.96	0.43 b	1.07 b	0.75 a	0.17 c	29.3	117.2 a	178.2 a	6,021
1	3.09	0.48 a	1.27 a	0.62 a	0.20 b	27.6	106.8 ab	143.0 b	3,699
2	2.94	0.52 a	1.37 a	0.51 b	0.22 a	24.5	97.1 b	133.8 b	3,468
LSD	NS	Sq root	0.127	Rank	Arcsin	NS	Log	Rank	NS
Interaction of spinach parent line and form of N-fertilizer									
♀ Susceptible - NH_4	2.95 c	0.54 b	1.07 d	0.49 d	0.21 b	21.6	73.5	144.9	3,624
- NO_3	2.20 e	0.39 d	1.07 d	0.62 c	0.13 d	25.7	87.0	150.9	6,511
♀ Moderate - NH_4	3.33 b	0.60 a	1.16 d	0.58 c	0.27 a	25.1	108.3	171.6	4,743
- NO_3	2.96 c	0.44 c	1.28 bc	0.72 ab	0.18 c	32.6	137.2	161.3	5,384
♀ Resistant - NH_4	3.93 a	0.54 b	1.34 ab	0.62 bc	0.29 a	26.4	84.5	158.7	3,616
- NO_3	3.54 b	0.41 d	1.40 a	0.79 a	0.17 c	34.7	113.1	149.0	4,556
♂ - NH_4	2.51 d	0.47 c	1.17 cd	0.49 d	0.18 bc	21.1	106.6	145.3	2,929
- NO_3	2.55 d	0.39 d	1.40 a	0.68 abc	0.16 cd	30.2	146.1	131.5	3,806
LSD	0.226	0.032	0.112	Rank	Arcsin	NS	NS	NS	NS

	Assays of harvested seeds[w]						Soil dilution assays (cfus/g soil)	
	AOSA seed germination assay (% seed)		Freeze-blotter seed health assay (% of seed)					
Factor	Germinated	Rotten	*Fusarium* spp.	*Verticillium dahliae*	*Stemphylium botryosum*	*Alternaria* spp.	*Fusarium* spp.	*V. dahliae*
Form of N-fertilizer								
Ammonium (NH_4)	83.2 a	7.4 a	0.9	3.3	4.9	20.8	5,328	25.3 a
Nitrate (NO_3)	58.9 b	16.6 b	2.9	9.1	4.4	21.3	4,016	47.9 b
LSD	Rank	Log	NS	NS	NS	NS	NS	21.8
Spinach parent line and susceptibility to Fusarium wilt								
♀ Susceptible	64.8	20.0 a	4.2 a	1.8	3.2 b	17.2	4,874 a	31.0
♀ Moderate	75.3	8.3 b	0.9 b	7.9	7.4 a	23.6	5,339 a	34.5
♀ Resistant	75.1	7.4 b	0.7 b	7.9	3.3 b	21.7	3,802 b	44.3
♂	-	-	-	-	-	-	-	-
LSD	NS	Log	Rank	NS	Arcsin	NS	Rank	NS
Rate of limestone amendment (t/A)								
0	69.8	13.1	3.4	2.2	5.5	20.4	4,721	34.5
1	68.6	13.7	1.7	6.3	4.6	20.0	4,875	29.7
2	77.0	8.6	0.6	9.1	3.9	22.6	4,420	45.7
LSD	NS	NS	NS	NS	NS	NS	NS	NS

[z] For main plot (N-fertilizer treatment), split plot (parent line), and split-split plot (limestone treatment) factors, each mean is the average of four replications and all levels of the other factors, except soil nutrient analyses on dates when samples were only collected for limestone and/or N-fertilizer treatments. 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 $P = 0.05$.

[y] 'Log', 'Arcsin', 'Sq root', and 'Rank' = original means are presented but means separation is based on logarithmic, arcsin square root, square root, or rank transformations, respectively, to meet requirements for parametric analyses of variance. 'NS' = not significantly different at $P = 0.05$. '-' = data not collected for a given factor/variable combination.

[x] Soil and plant nutrient analyses were done by Soiltest Farm Consultants, Moses Lake, WA.

[w] Seeds were not harvested from the male spinach line.