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

L.J. du Toit, E.W. Gatch, M.L. Derie, B.J. Holmes, and L.M. Brissey, Washington State University Mount Vernon NWREC, Mount Vernon, WA 98273-4768.

Effect of agricultural limestone amendment on Fusarium wilt and Verticillium wilt in a spinach seed crop, 2008.

Four rates of limestone (applied to main plots) and four proprietary female spinach inbred lines (A, B, C, and D planted in split plots, and ranging from highly susceptible to highly resistant to Fusarium wilt) were evaluated in a field trial on a Sumas silt loam soil in Skagit Co., WA. A randomized complete block design was used with five replications of the factorial combinations. The field had previously been planted to a spinach seed crop in 2003. Soil pH was 6.0 in Mar 08. The plots were plowed, chiseled, and mulched in Apr to prepare the seed bed. Agricultural limestone (TexLime General Purpose Limestone Flour, Oregon Lime Score = 96, CCE = 98.3%, with 97% $CaCO_3$ and 38.8% Ca) was applied at 0, 1, 2, and 4 t/A to appropriate main plots (each 36 ft x 36 ft) on 17 and 18 Apr using a 6 ft wide Gandy drop spreader, and incorporated 6 in. deep using a rototiller. On 1 May, RoNeet (pre-plant herbicide) was broadcast at 42 oz/A in 15 gal/A at 40 psi, and incorporated with a mulcher-packer. Spinach seeds were planted 0.5 in. deep 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 split plot, 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 (400 lb/A). Soil samples were collected from main plots (5 to 8 cores/plot) in three of five replications to a depth of 6 in. on 5 and 22 May, 16 Jun, 28 Jul, and 31 Aug for nutrient analyses. For the latter date, 10 g dried soil/plot was plated onto Komada's agar medium and 1 g onto NP-10 agar medium to quantify *F. oxysporum* and *Verticillium* spp., respectively. Soil samples were also collected from main plots in all five replications on 7 Jul for nutrient analyses. Plant stand and incidence of wilted seedlings were counted in two 10 ft sections of row/spinach line on 22 May, 13 Jun, 9 Jul, and 1 Aug. Isolations onto agar media were completed for a sample of 5 wilting seedlings/parent line in Jun. Plots were cultivated on 14 Jun, and hand-weeded as needed. Fertilizer was side-dressed (27-0-0 at 200 lb/A) on 20 Jun with a single-shank applicator. On 10 Jul and 4 Aug, whole plants were sampled from 4 ft row/parent line, dried at 95°F for 2 weeks, and weighed. Plants sampled on 10 Jul were subjected to nutrient analyses. Roots and crowns of the plants sampled on 4 Aug were cut lengthwise and rated for vascular discoloration typical of Fusarium wilt (prior to drying), except the male line and line C which had senesced, preventing vascular ratings. Plants in the middle 10 ft of the center 4 rows/plot were windrowed as each line matured (4, 22, and 24 Aug for females D, A and C, and B, respectively), and the seeds harvested manually. Seeds were cleaned, screened to marketable size (screen sizes 6 to 13), and weighed. A sample of 100 seeds/split 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 also completed for 100 seeds/split plot. For the latter, seeds were placed onto damp blotters in plastic petri plates (20 seeds/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 seeds were examined after 5, 9, and 14 d using a dissecting microscope (8 to 100X magnification). Mean temperature and total rainfall for Apr, May, Jun, Jul, and Aug were 45.4°F and 2.40 in., 53.7°F and 2.09 in., 55.6°F and 2.25 in., 59.7°F and 0.64 in., and 61.9°F and 2.23 in., respectively.

Spinach stand counts from May to Aug differed significantly among parent lines, with the greatest stands for female B, followed by female A, and then the other three lines (*data not shown*). The effects of limestone treatments on stand counts increased through the season, with significantly more plants in plots that received limestone vs. control plots (*data not shown*). The incidence of wilted plants did not differ significantly among spinach lines or rates of limestone on 22 May (*data not shown*), but thereafter significantly more wilt was observed in the male line, followed by females A and C, with the least wilt for females B and D at each rating (Table 1). On 13 Jun, incidence of wilt decreased significantly at rates of limestone amendment >1 t/A. By Jul, the beneficial effect of limestone at suppressing Fusarium wilt was no longer significant for the most susceptible lines, but was still significant for lines B and D (Table 1). Dark vascular discoloration typical of Fusarium wilt was most prevalent in females A and C (96 to 100%), followed by female B. The opposite was true for the light vascular discoloration typical of Verticillium wilt (0 and 4% for females A and C, respectively, vs. 75% for female B). Rates of limestone amendment did not significantly affect incidence of vascular discoloration. On 10 Jul, dry plant weight was greatest for female D, followed by females B, C, and A, with male plants the smallest (Table 1); dry plant weights by 4 Aug were affected similarly (*data not shown*). On 10 Jul, plant weight was significantly greater in plots treated with limestone vs. the control plots, and by 4 Aug there was a significant increase in plant weight with increasing rate of limestone amendment (*data not shown*). Similarly, seed yield was greatest for female D, followed closely by female B, and then female A (which yielded approximately 50% less seed than D and B). Female A (highly susceptible to Fusarium wilt) had very poor seed yield (17% of the yield of female C, and 8% of the yield of D). For each female line, seed yield increased significantly with increasing rate of limestone amendment (no significant interaction term). *F. oxysporum* was isolated from wilting seedlings of each spinach line, but it was difficult to find wilting seedlings of females D and B. Limestone had increased soil pH significantly three weeks after application, from 6.2 for non-limed plots to 6.5, 6.7, and 6.9 for plots with 1, 2, and 4 t/A, respectively (*data not shown*). Soil pH differed similarly among limestone treatments by 7 Jul (Table 2), and through the season (*data not shown*). The soil concentration of available calcium (Ca) increased significantly with increasing rate of limestone (Table 2), whereas available nitrate (NH_4-N) and manganese (Mn) decreased. Similarly, plant nutrient analyses showed a significant increase in Ca and decrease in zinc (Zn) in plants with increasing rate of limestone amendment. There were also significant differences in plant nutrient concentrations among spinach lines, with the lowest boron (B), sulfur (S), and Zn concentrations in the parent lines more susceptible to Fusarium wilt (male and female A) (Table 2). Except for soil Ca measured on 7 Jul, there was no significant interaction in the soil or plant assays between rate of limestone amendment and spinach parent line in the analyses of variance (ANOVA) for any dependent variable; similarly for the seed germination and health assays (Table 2). Soil Ca levels increased with increasing rate of limestone amendment for all spinach lines, but the degree of increase differed among lines (*data not shown*). Germination of harvested seed was significantly greater in plots with 4 t limestone/A vs. seed harvested from control plots (Table 2), and seed germination was poorer for female A than the other three females. The opposite was true for the incidence of rotten seed. The incidence of harvested seed infected with *V. dahliae* increased with increasing rate of limestone amendment, but this was not true for the incidence of seed with *Fusarium* spp. (Table 2). Seeds harvested from female A plants had a greater incidence of *Fusarium* spp. than harvested seeds of females B, C, or D. The earliest maturing female line, D, had the smallest incidence of seeds infected with necrotrophic fungi like *S. botryosum* and *Alternaria* spp. Overall, these results demonstrate the potential value of limestone amendment for suppressing Fusarium wilt in spinach seed crops, and the potential influence of the susceptibility of spinach parent lines to Fusarium wilt on the degree of suppression.

Table 1.

Factor	Incidence of wilted plants (%/20 ft row)		Incidence (%) of plants on 4 Aug with vascular discoloration typical of		Dry plant weight on 10 Jul (lb/4 ft row)	Colony forming units/g soil sampled on 31 Aug		Marketable seed yield (lb/A)
	13 Jun	9 Jul	Fusarium wilt	Verticillium wilt		*F. oxysporum*	*Verticillium* spp.	
Spinach parent line								
♂ ……...	77.7 a[z]	100.0 a	-[y]	-	0.03 e	-	-	-
♀A ……...	39.3 b	92.3 b	100.0 a	0.0 b	0.11 d	11,550 a	108 c	186 d
♀B …….	0.1 d	2.9 d	15.7 b	74.8 a	0.39 b	10,600 a	356 a	2,050 b
♀C …….	11.3 c	62.1 c	95.8 a	4.2 b	0.30 c	12,900 a	184 bc	1,054 c
♀D …….	0.2 d	4.2 d	-	-	0.63 a	9,850 a	264 ab	2,307 a
LSD…….	Rank[y]	Rank	Rank	Rank	Square rt	Log	Log	Arcsin
Rate of limestone amendment (t/A)								
0……..	41.9 a	63.1 a	75.8 a	23.2 a	0.27 b	11,683 a	145 c	1,268 c
1……..	37.0 a	57.8 ab	68.5 a	27.1 a	0.29 a	12,283 a	219 bc	1,344 bc
2……..	31.4 b	61.9 ab	68.1 a	29.1 a	0.31 a	10,250 a	200 b	1,447 ab
4……..	27.3 b	58.3 b	69.5 a	26.0 a	0.30 a	10,683 a	348 a	1,540 a
LSD……	Rank	Rank	Rank	Rank	Square rt	Log	Log	Arcsin
Interaction of spinach parent line and rate of limestone amendment (t/A)								
♂ - 0……	-[x]	100.0 a	-	-	-	-	-	-
1……	-	100.0 a	-	-	-	-	-	-
2……	-	100.0 a	-	-	-	-	-	-
4……	-	100.0 a	-	-	-	-	-	-
♀A – 0…	-	95.8 bc	-	-	-	-	83 gh	-
1…	-	77.8 c	-	-	-	-	59 h	-
2…	-	99.0 ab	-	-	-	-	121 efgh	-
4…	-	96.6 c	-	-	-	-	169 efgh	-
♀B – 0…	-	6.7 efg	-	-	-	-	162 efgh	-
1…	-	2.4 efg	-	-	-	-	477 a	-
2…	-	1.8 gh	-	-	-	-	312 bcd	-
4…	-	2.7 h	-	-	-	-	472 a	-
♀C – 0…	-	70.7 d	-	-	-	-	125 efgh	-
1…	-	60.6 d	-	-	-	-	107 fgh	-
2…	-	67.5 d	-	-	-	-	122 efgh	-
4…	-	49.7 d	-	-	-	-	382 ab	-
♀D – 0…	-	5.2 e	-	-	-	-	210 defg	-
1…	-	6.0 e	-	-	-	-	233 cdef	-
2…	-	3.2 fgh	-	-	-	-	245 cde	-
4…	-	2.4 gh	-	-	-	-	368 abc	-
LSD……...	-	Rank	-	-	-	-	Log	-

[z] Each split plot mean is averaged over five replications. For the two main factors (spinach parent line and rate of limestone amendment), each mean is averaged over five 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 $P = 0.05$.

[y] '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. 'Arcsin', 'Log', and 'Square rt' = original means are presented, but means separation is based on arcsin square root, log, and square root transformations, respectively, to meet assumptions for parametric analyses.

[x] For the main effect of spinach parent line, '-' indicates the dependent variable was not measured for that parent because the plants had already senesced by 4 Aug.

[w] For the interaction term between parent line and limestone treatment in the ANOVA, '-' indicates the interaction means are not presented because the interaction term was not significant. CFUs *Verticillium* spp./g soil were not measured for the plot borders planted to the male spinach line.

Table 2.

	Soil nutrient analyses[z]										
	Nutrient concentration (7 Jul)										
	pH	Ca	Mg	K	NH_4-N	Zn	Mn	Cu	Fe	S	B
Factor	7 Jul	meq/100 g			mg/kg						
Spinach parent line											
♂…….	-	-	-	-	-	-	-	-	-	-	-
♀A.....	6.6 a[y]	8.54 a	1.23 a	2 a	2.9 a	1.63 a	2.7 a	3.45 a	124 a	15.1 a	0.926 a
♀B.....	6.5 a	8.29 a	1.10 a	199 a	5.1 a	1.51 a	3.0 a	3.48 a	111 a	13.4 a	0.804 a
♀C.....	6.6 a	8.82 a	1.20 a	417 a	3.7 a	1.62 a	2.4 a	3.49 a	117 a	14.5 a	0.933 a
♀D.....	6.8 a	8.59 a	1.09 a	567 a	5.8 a	1.58 a	3.1 a	3.95 a	130 a	11.8 a	0.898 a
LSD[x]..	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rate of limestone amendment (t/A)											
0…….	6.2 c	6.62 d	1.12 a	306 a	7.7 a	1.86 a	4.2 a	3.72 a	137 a	13.7 a	0.952 a
1…….	6.6 b	8.07 c	1.16 a	284 a	4.3 b	1.63 a	2.9 b	3.53 a	117 a	13.1 a	0.848 a
2…….	6.7 ab	8.97 b	1.17 a	296 a	3.7 bc	1.44 a	2.2 c	3.45 a	112 a	14.5 a	0.820 a
4…….	7.0 a	10.57 a	1.16 a	309 a	1.8 c	1.38 a	1.9 c	3.59 a	110 a	13.7 a	0.924 a
LSD[x]..	0.29	Log	NS	NS	Rank	NS	0.62	NS	NS	NS	NS

	Plant nutrient analyses (10 Jul)[z]										
	N	P	K	Ca	Mg	S	B	Zn	Mn	Cu	Fe
	%						mg/kg				
Spinach parent line											
♂…….	2.1 e	0.35 d	2.5 d	1.27 a	0.47 a	0.17 d	7.3 d	48 b	147 a	11.8 a	7,348 a
♀A.....	3.0 c	0.38 d	4.5 c	1.28 a	0.43 b	0.24 c	4.9 d	32 c	87 b	8.4 c	2,282 b
♀B.....	4.0 a	0.58 a	8.0 a	1.31 a	0.50 a	0.40 a	17.8 a	65 a	90 b	12.1 a	1,167 bc
♀C.....	3.8 b	0.53 b	7.5 a	1.27 a	0.50 a	0.40 a	12.8 b	64 a	95 b	11.7 a	1,600 bc
♀D.....	2.7 d	0.47 c	5.8 b	1.26 a	0.38 b	0.36 b	9.9 c	69 a	78 b	9.8 b	696 c
LSD[x]...	Rank	0.035	0.51	Rank	Rank	Rank	Rank	Log	Log	Rank	Log
Rate of limestone amendment (t/A)											
0……..	3.1 a	0.45 a	5.5 a	1.10 c	0.47 a	0.30 a	47.4 a	61 a	114 a	10.7 a	2,914 a
1……..	3.1 a	0.46 a	5.6 a	1.20 b	0.45 a	0.31 a	47.4 a	56 a	99 ab	10.5 a	2,453 a
2……..	3.2 a	0.46 a	5.8 a	1.29 b	0.46 a	0.32 a	46.2 a	53 a	96 b	10.8 a	2,605 a
4……..	3.2 a	0.47 a	5.8 a	1.51 a	0.45 a	0.32 a	48.6 a	52 a	89 b	11.1 a	2,503 a
LSD[x]..	Rank	NS	0.33	Rank	Rank	Rank	Rank	Log	Log	Rank	Log

	AOSA seed germination assay (% of seed)[w]		Freeze-blotter seed health assay (% of seed)[w]				
	Germinated	Rotten	*Fusarium* spp.	*Verticillium dahliae*	*Stemphylium botryosum*	*Cladosporium variabile*	*Alternaria* spp.
Spinach parent line							
♂…….	-	-	-	-	-	-	-
♀A.....	81.5 b	11.3 a	6.8 a	6.0 c	72.0 a	0.65 a	64.2 b
♀B.....	91.1 a	2.6 c	1.4 b	33.6 b	62.2 b	0.05 a	75.4 a
♀C.....	90.7 a	5.4 b	0.7 bc	29.6 b	52.0 c	0.20 a	51.0 c
♀D.....	90.5 a	6.3 b	0.5 c	45.5 a	29.2 d	0.35 a	31.1 d
LSD[x]...	Arcsin	Rank	Arcsin	Arcsin	Log	NS	9.35
Rate of limestone amendment (t/A)							
0…….	87.7 b	7.3 a	2.4 a	20.5 d	54.0 ab	0.45 a	57.5 a
1…….	87.1 b	6.8 a	2.3 a	25.0 c	57.8 a	0.35 a	57.3 a
2…….	88.2 ab	6.8 a	3.0 a	32.2 b	52.3 b	0.30 a	54.8 ab
4…….	90.8 a	4.7 b	1.6 a	37.0 a	51.3 b	0.15 a	52.2 b
LSD[x]...	Arcsin	Rank	Arcsin	Arcsin	Log	NS	2.85

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

[y] For each main factor (spinach parent line and rate of limestone amendment), each mean is averaged over five replications and all levels of the other main factor, except for soil nutrient analyses which were only completed for the limestone treatments.

[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 these variables. 'Log' and 'Arcsin' = original means presented but means separation is based on logarithmic or arcsin square root transformations, respectively, 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] Seeds were not harvested from plants of the male spinach line.