

### Effect of Proline 480 SC on Fusarium wilt in a spinach seed crop in western Washington, 2012.

The maritime region of western Washington and Oregon is the only area of the USA with suitable conditions for spinach seed production. However, the acidic soils of this region are highly conducive to Fusarium wilt of spinach, necessitating rotations of 10 to 15 years between spinach seed crops to avoid major losses to this disease. The fungicide Proline was evaluated for managing spinach Fusarium wilt in a grower-cooperator's field in Skagit Co., WA. The field had been planted to a spinach seed crop in 2009, potato in 2010, and winter wheat in 2011-12. The soil was a Mount Vernon very fine sandy loam, with a pH of 5.9 in Mar 2012. Proprietary female spinach inbred lines differing in susceptibility to Fusarium wilt [moderately resistant (R), moderately susceptible (M), and highly susceptible (S)] were assigned to the main plots; and Proline or no Proline treatments were assigned to split plots in a split plot, randomized complete block design with four replications. On 23 Apr, RoNeet (42 oz/A) and Diazinon (2 qt/A) were broadcast in 15 gal/A at 40 psi and incorporated for weed and insect control, respectively. Agricultural limestone (Imperial Limestone, J. A. Jack & Sons, Seattle, WA, with 98.2 CaCO<sub>3</sub> and 1.2% MgCO<sub>3</sub>) was applied at 1 t/A on 11 Apr, and incorporated on 12 Apr. Spinach seeds were planted on 8 May (0.5 in. deep) using a Monosem planter, with a 22 in. inter-row spacing and a 2.5 in. intra-row spacing. Six rows of the appropriate female line were planted/plot plus a row of a proprietary male line on each side. Each split plot was 15 ft x 8 rows wide. Fertilizer was applied in-furrow at planting: 11-52-0-0.5B mono-ammonium phosphate + boron (333 lb/A). Proline 480 SC was applied on 10 May (5.7 fl oz/A) to appropriate plots using a CO<sub>2</sub>-pressurized backpack sprayer with a 2-nozzle boom (30 psi in 96.5 gpa), and again on 31 May and 21 Jun (5.7 fl oz/A + R-11 surfactant at the latter two applications). Plant stand and incidence of wilted plants were counted in two 10 ft sections of row/spinach line on 29 May, 19 Jun, and 9 and 31 Jul. For the latter two dates, wilt severity of each plant was also assessed on a 0-to-5 ordinal scale (0 = asymptomatic plant, 1 = 20% of aboveground foliage wilted, 2 = 40% wilted, 3 = 60% wilted, 4 = 80% wilted, and 5 = dead plant). Premature senescence of the male line precluded incidence and severity ratings on 31 Jul. On 29 May, isolations for plant pathogens were completed from roots and crowns of a sample of wilting seedlings of each spinach line. Plots were hand-weeded regularly. Fertilizer (27-0-0) was applied at 200 lb/A on 13 Jun with a single-shank applicator. On 21 Jun, a foliar application of chelated manganese (5%) + zinc (7%) was applied to plants (0.25 gal of each product/A, Foli-Gro Zn and Mn). On 11 Jul, plants were collected from 3.3 ft of row/parent line. The root and crown of each plant were cut longitudinally and rated for vascular discoloration typical of Fusarium wilt. The plants were dried at 95°F and weighed. On 2 Aug, pyraclostrobin + boscalid (Pristine WG at 0.7 lb/A) + Nu-Film 17 (1 pt/A) were applied to manage *Stemphylium* and *Cladosporium* leaf spots. Seeds were harvested manually from 10 ft of the center 4 rows/female line between 13 and 23 Aug, based on maturity of each 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 seed health assay for necrotrophic fungi was carried out on NP-10 agar medium with 100 seeds/plot. Seeds were placed on NP-10 agar medium in 4 in. x 4 in. acrylic boxes (32 to 34 seeds/box), and then incubated at 75°F 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 49.8°F and 4.3 in., 53.2°F and 2.4 in., 56.1°F and 3.1 in., 60.6°F and 1.3 in., and 62.8°F and 0 in., respectively.

The severity of spinach wilt increased from 0.02% on 29 May to 3.20% on 31 Jul, by which time every plant had some degree of wilt. The main effect of spinach parent line susceptibility to Fusarium wilt was highly significant for wilt severity on 19 Jun, and 9 and 31 Jul, as well as vascular discoloration, seed yield, harvested seed germination, and incidence of harvested seed infested with *Fusarium* spp. The main effect of Proline was significant for wilt severity on 31 Jul. The M and R female spinach lines had the least severe wilt as early as 19 Jun; and by 9 and 31 Jul, wilt was the least severe for the R female, followed by the M and S lines. The Proline 480 SC treatment reduced wilt severity from an average of 3.30 to 3.09 by 31 Jul, with all female lines showing a similar trend. The S female line and male line had greater vascular discoloration (98.2 and 96.5%, respectively) than the M and R female lines (70.2 and 75.8%, respectively). Proline did not affect vascular ratings. Plant dry weight did not differ among spinach parent lines or Proline treatments. Marketable seed yield was 62 and 27% greater in plots of the R female than the S and M female plots, respectively. The Proline main effect was significant at  $P = 0.0872$ , i.e., there was a 91.3% probability that the Proline treatments increased seed yield significantly. Plots of the S female treated with Proline had 18.4% more marketable seed than non-treated plots; likewise, Proline increased seed yield of the M female line by 11.4%, and of the R female line by 6.9%. Germination assays revealed significantly less germination of seed harvested from the S female plants, and an increase in the incidence of rotten seed of that parent line compared to the M and R female lines. An average 6.4% of seed from the S female plots was infested with *Fusarium* spp., which was far greater than 0.9% infested seed for the M female and 0.1% for the R female. The Proline treatment did not affect seed health or seed germination significantly. This study demonstrated the potential for Proline 480 SC to reduce severity of spinach Fusarium wilt, and the influence of spinach parent line susceptibility to Fusarium wilt on this response.

Factor	Severity of wilt/20 ft row (1-5)				Incidence of vascular discoloration on 11 Jul (%)	Dry plant weight on 11 Jul (lb/3.3 ft row)	Marketable seed yield (lb/A) <sup>x</sup>
	29 May	19 Jun	9 Jul	31 Jul			
Spinach parent line and susceptibility to Fusarium wilt							
♀ Susceptible .....	0.02 a <sup>z</sup>	0.23 a	2.59 a	3.79 a	98.2 a	199 a	462 c
♀ Moderate .....	0.02 a	0.08 b	1.61 b	3.16 b	70.2 b	210 a	892 b
♀ Resistant .....	0.04 a	0.09 b	1.03 c	2.65 c	75.8 b	178 a	1,218 a
♂ .....	0.03 a	0.26 a	2.84 a	-	96.5 a	209 a	-
LSD .....	NS	0.09	0.32	0.20	Arcsine <sup>y</sup>	NS	138
Proline treatment							
None .....	0.03 a	0.16 a	2.10 a	3.30 a	85.8 a	200 a	815 a
Proline .....	0.02 a	0.16 a	1.93 a	3.09 b	84.6 a	198 a	900 a
LSD .....	NS	NS	NS	0.09	NS	NS	104
Interaction of female spinach parent line and Proline treatment							
♀ Susc.-None .....	-	-	-	3.89 a	-	-	423 c
-Proline .....	-	-	-	3.69 a	-	-	501 c
♀ Mod.-None .....	-	-	-	3.29 b	-	-	844 b
-Proline .....	-	-	-	3.02 b	-	-	940 b
♀ Res.-None .....	-	-	-	2.73 c	-	-	1,178 a
-Proline .....	-	-	-	2.56 c	-	-	1,259 a
♂-None .....	-	-	-	-	-	-	- <sup>x</sup>
-Proline .....	-	-	-	-	-	-	-
LSD .....	-	-	-	0.20	-	-	138
Assays of harvested seed <sup>x</sup>							
Factor	AOSA germination assay (% of seed)		Freeze-blotter seed health assay (% of seed)				
	Germinated	Rotten	<i>Fusarium</i> spp.	<i>Verticillium dahliae</i>	<i>Stemphylium botryosum</i>	<i>Alternaria</i> spp.	
Spinach parent line and susceptibility to Fusarium wilt							
♀ Susceptible .....	0.51 b	30.70 a	6.40 a	4.50 a	72.40 ab	29.40 b	
♀ Moderate .....	0.62 a	18.80 b	0.90 b	9.90 a	76.90 a	48.40 a	
♀ Resistant .....	0.67 a	7.60 c	0.10 c	5.80 a	65.10 b	41.10 a	
♂ .....	-	-	-	-	-	-	
LSD .....	0.08	6.79	Rank	NS	8.72	9.80	
Proline treatment							
None .....	0.58 a	20.07 a	2.67 a	6.13 a	72.73 a	39.40 a	
Proline .....	0.62 a	18.00 a	2.27 a	7.33 a	70.20 a	39.87 a	
LSD .....	NS	NS	NS	NS	NS	NS	

<sup>z</sup> Data were subjected to analyses of variance (ANOVAs) with parent lines and Proline treatments as fixed effects, using SAS Version 9.3. For main plot (parent line) and split plot (Proline treatment) factors, each mean is the average of four replications and all levels of the other factors. 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  $P = 0.05$ .

<sup>y</sup> 'Arcsine' and 'Rank' = original means are presented but means separation is based on arcsine 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, or data are not shown if the interaction term between spinach parent lines and Proline treatments in the analysis of variance was not significant.

<sup>x</sup> Seeds were not harvested from the male spinach line.