

Evaluation of yield loss caused by leaf spot fungi in spinach seed crops, 2004.

A hybrid spinach seed crop field trial was established on a Puget silt loam soil at the Washington State University - NWREC to quantify yield losses caused by *C. variabile* and *S. botryosum*. The field site was fumigated on 2 Oct 03 with Tri-Con 57/43 (methyl bromide/chloropicrin) at 350 lb/A to a depth of 8 in. and sealed with plastic tarp, for control of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *spinaciae*. The tarp was removed on 6 Nov 03. Male and female stock seed of a proprietary spinach hybrid were planted on 23 Apr 04 at a ratio of two male rows for every six female rows, with 22 in.-spacing between rows and 2 in.-spacing within rows. Fertilizer (18-46-0) was applied in-furrow (375 lb/A) at planting. The trial was tilled between rows on 1 Jun and side-dressed with 34-0-0 (150 lb/A) on 2 Jun. Plots, 20 ft-long and consisting of six female rows and a male row on each side, were established in the field on 17 Jun with 15 ft alleys separating plots at each end. The experiment was arranged in a randomized complete block design with five replications of six treatments. Treatments consisted of plots sprayed 0, 1, 2, 3, 4, or 5 times with Cabrio EG at 0.7 lb/A. The first fungicide application was initiated at the start of anthesis, on 9 Jul. Due to windy and rainy conditions, the control plots were sprayed (water) on 9 Jul, and Cabrio EG was applied on 11 and 12 Jul. Cabrio EG was applied again on 22 Jul, and 8, 19, and 26 Aug. All applications were made in 44.5 gal/A at 40 psi using a CO₂-pressurized tractor-mounted sprayer fitted with a 7 nozzle boom (8003 flat fan tips at 20-in. spacing). R-11 surfactant was used with all applications at 1.0 pt/100 gal. Each plot was inoculated with approx. 0.18 gal of a mixed spore suspension of *C. variabile* and *S. botryosum* in 0.01% Tween 80 on 13 Jul (1.7 x 10⁵ and 1.7 x 10⁴ spores/ml, respectively), 23 Jul (2.1 x 10⁴ and 1.1 x 10⁴ spores/ml, respectively), 9 Aug (2.1 x 10⁴ and 1.5 x 10⁴ spores/ml, respectively), 20 Aug (1.9 x 10³ and 9.0 x 10³ spores/ml, respectively), and 27 Aug (6.1 x 10³ and 2.1 x 10⁴ spores/ml, respectively). All inoculations were made at 15 psi using the sprayer described above. Male rows were tilled under on 30 Jul. Severity of leaf spot was assessed on 17 and 31 Aug. The middle 10 ft of the center four female rows were cut by hand from each plot on 7 Sep. Because of persistent rains, harvested plants were placed onto 10 ft x 12 ft sections of Remay and transported to covered storage to dry. Plants from the driest plots were threshed on 16 Sep and the remaining plots were threshed on 23 Sep. The seed was cleaned and sized according to commercial specifications for the proprietary hybrid. In Oct, a freeze-blotter assay was used to determine the incidence of *Stemphylium*, *Cladosporium*, and other fungi in 100 seed/plot. The seed was surface-sterilized for 1 min in 1.2% NaOCl, triple-rinsed in sterile deionized water, and placed onto damp blotters in plastic Petri plates (20 seed/plate). The seed imbibed on the blotters in the dark for 24 h, and was then incubated at -4°F for 24 h followed by 12 d at 75°F under a 12 h/12 h day/night cycle with near-UV and cool white fluorescent light by day. The seed was examined using a dissecting microscope (8 to 100X magnification) 5, 8, and 11 days after plating. Germination of 100 seeds/plot was assessed using the AOSA (Association of Official Seed Analysts) protocol. Mean temperature and total rainfall for Apr, May, Jun, Jul, Aug, and Sep were 52.3°F and 0.28 in., 56.2°F and 3.68 in., 61.2°F and 1.8 in., 64.5°F and 0.51 in., 65.3°F and 2.6 in., 57.6°F and 3.4 in., respectively.

Control plots averaged 80 and 89% severity of leaf spot on 17 and 31 Aug, respectively. Each additional application of Cabrio EG reduced severity of leaf spot, although severity of leaf spot was not significantly different for plots receiving 4 or 5 applications of Cabrio EG. Seed yield did not differ significantly among plots receiving 1, 2, 3, 4, or 5 applications of Cabrio EG. Seed germination and incidence of *C. variabile* on the harvested seed did not differ significantly among treatments. However, the incidence of *S. botryosum* on the harvested seed differed significantly among treatments, ranging from <10% for plots receiving 4 or 5 applications of Cabrio EG to 64% for control plots. Severity of leaf spot on 17 and 31 Aug was significantly correlated with incidence of *S. botryosum* on the seed ($r = 0.92$ and 0.87 , respectively at $P < 0.0001$). Regression calculations showed the percentage of *S. botryosum* on the harvested seed (Y) = $64.4 - 24.3X + 2.6 X^2$, where X = number of applications of Cabrio EG ($R^2 = 0.92$, $CV = 22.47$). Similarly, $Y = 28.65 - 1.15Z + 0.02Z^2$, where Z = severity of leaf spot one week prior to harvest ($R^2 = 0.85$, $CV = 30.18\%$). The predominant leaf spot symptoms were of *Stemphylium* leaf spot, as reflected in the higher incidence of seedborne *S. botryosum* compared to *C. variabile*. Applications of Cabrio EG during conditions conducive for *Stemphylium* leaf spot did not have much effect on total seed yield but increased seed quality significantly as measured by the incidence of seedborne *S. botryosum*.

Number of applications of Cabrio EG ^z	Leaf spot severity (%) ^y		Seed yield (lb/40 ft) ^x	% Seed infected ^w	
	Aug 17	Aug 31		<i>Stemphylium botryosum</i>	<i>Cladosporium variabile</i>
0	80 a ^v	89 a	0.56 a	63.8 a	1.8 a
1	49 b	82 a	0.66 ab	43.8 b	0.2 a
2	12 c	65 b	0.92 b	26.8 c	0.2 a
3	10 cd	58 bc	0.75 ab	15.0 d	0.8 a
4	7 de	47 cd	0.79 ab	9.8 d	0.2 a
5	6 e	39 d	0.77 ab	9.0 d	0.4 a

^z All except the control (0) plots received the first application of Cabrio EG. An additional set of plots was dropped for each subsequent application of Cabrio EG.

^y Severity of leaf spot rated as % foliage with symptoms of *Cladosporium* leaf spot and *Stemphylium* leaf spot per plot.

^x Seed yield = lb of marketable seed (between screen sizes 6 and 11) per 40 ft of row (10 ft of the center four rows per plot).

^w Mean % of 100 seed infected per plot. Seed assayed using a freeze-blotter assay described above.

^v Means followed by the same letter within a column are not significantly different based on Fisher's Protected LSD ($P \leq 0.05$).