2011 FINAL REPORT TO THE CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

I. Abstract

Project Title: Assess the efficacy of seed treatments for eradicating *Verticillium dahliae* from spinach seed, preventing seed transmission of *V. dahliae*, and reducing the risk of infesting soils into which infected spinach seed is planted; and assess the influence of soil fumigation and soil pH on efficacy of these seed treatments.

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Cooperators: Mike L. Derie, Washington State University; **Jim C. Correll,** University of Arkansas, Fayetteville; and **KrishnaV. Subbarao**, University of California, Davis.

Summary: *Verticillium dahliae* is systemic and readily seed transmitted in spinach, raising concerns about introducing *V. dahliae* on spinach seed into fields subsequently planted to other susceptible crops. Based on prior research, a field trial was set up in western Washington in 2010 to evaluate the effects of seed treatments, soil fumigation (methyl-bromide:chloropicrin), and soil pH (modified with limestone) on seed transmission and soil infestation by *Verticillium* in a 'baby leaf' spinach-'head' lettuce crop rotation. Seeds of a spinach lot infected with *V. dahliae* at ~60% were planted on 7 May without fungicide seed treatment (= organic treatment) or treated with Apron + Thiram (standard seed treatment for conventional baby leaf spinach), Apron + Thiram + Topsin 4.5FL, or Farmore D300 + Mertect 340F (latter two were the most effective at preventing seed transmission in prior trials). A fifth treatment consisted of no spinach seed planted (no spinach preceding head lettuce). Soilborne *Verticillium* averaged 0.1 CFU/g soil in fumigated plots vs. 102 CFU/g soil in non-fumigated plots four weeks after fumigation. Seed treatments and soil fumigation significantly affected the rate of seed transmission of *V. dahliae* in spinach. Spinach plants assayed after harvest of leaves produced an average of 213 CFU *V. dahliae*/100 plants in plots planted with non-treated spinach seed. In comparison, Apron + Thiram + Topsin M 70WP reduced seed transmission to 5 CFU/100 plants (98% reduction compared to non-treated seed), and Farmore D300 + Mertect 340F reduced this to 15 CFU/100 plants (93% reduction). Apron + Thiram reduced seed transmission to 107 CFU/100 plants (50% reduction). The rate of transmission from non-treated seed was 706% greater in fumigated vs. non-fumigated plots (379 vs. 47 CFU/100 plants). This difference was much less in plots planted with fungicide-treated seed. When lettuce was planted, there were significant differences in the amount of *Verticillium* in fumigated vs. non-fumigated soils (21 vs. 162 CFU/g soil, respectively), with no significant effect of limestone (82 vs. 102 CFU/g soil in non-amended vs. amended plots, respectively). Planting infected spinach seed and incorporating spinach residues after harvest doubled the amount of *Verticillium* in soil compared to plots without spinach preceding lettuce (82 vs. 44 CFU/g soil, respectively). The amount of soilborne *Verticillium* detected after harvest and incorporation of lettuce residues was not affected by limestone or spinach seed treatments. However, soilborne *Verticillium* averaged 74 vs. 128 CFU/g soil in fumigated vs. non-fumigated plots, respectively = an increase in soilborne inoculum from the start of the trial by 19 CFU/g soil in non-fumigated plots vs. 74 CFU/g soil in fumigated plots = a 289% greater increase in soilborne *Verticillium* in fumigated vs. non-fumigated soils. The results demonstrate that soil fumigation can exacerbate seed transmission of *Verticillium* significantly when planting infected seed, but seed treatments can be highly effective at reducing seed transmission.

II. Main Body of Report

Project Title:
Assess the efficacy of seed treatments for eradicating *Verticillium dahliae* from spinach seed, preventing seed transmission of *V. dahliae*, and reducing the risk of infesting soils into which infected spinach seed is planted; and assess the influence of soil fumigation and soil pH on efficacy of these seed treatments.

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Objectives:
1. Assess the efficacy of conventional and organic fungicides for eradicating *Verticillium dahliae* from spinach seed, preventing seed transmission of *V. dahliae*, and reducing the risk of infesting soils into which infected spinach seed is planted.
2. Examine how soil pH might affect development of *Verticillium* in spinach and lettuce.

Procedures:
A field trial was set up at the Washington State University Mount Vernon NWREC on a Sedro Wolley silt loam soil to examine the effects of soil fumigation, soil pH, and seed treatments on seed transmission of *Verticillium* from planting infected spinach seed, and on development of Verticillium wilt in a head lettuce crop following the 'baby leaf' spinach crop. A 2 x 2 x 5 factorial treatment design was used with five replications in a split plot, randomized complete block design: main plots (11 ft x 200 ft) were amended with 0 or 2 tons agricultural limestone/A on 11 Apr, and half of the main plots were not fumigated and the other half were fumigated with methyl bromide:chloropicrin (57:34%) at 350 gpa under plastic on 14 Apr.

Seeds of a spinach seed lot infected with *V. dahliae* at 60% were planted on 7 May in split plots without fungicide seed treatment (to mimic organic baby leaf spinach crops) or treated with Apron + Thiram (standard spinach seed treatment for conventional baby leaf spinach crops in CA), Apron + Thiram + Topsin 4.5FL (latter was the most effective at preventing seed transmission of *V. dahliae* in seed treatment trials we completed in 2009-10), or Farmore D300 + Mertect 340F (second most-effective seed treatment for reducing seed transmission of the pathogen in the 2009-10 trials). A fifth split plot treatment consisted of no spinach seed planted as a control treatment (no baby leaf spinach crop preceding the head lettuce crop). Fertilizer (0-0-60 + 0-0-21 + Mangro 31 + ZnSulfate 34) was broadcast at 325 lb/A, and incorporated by mulching on 10 Apr. Additional fertilizer (12-24-17 + 3 S + 1 Mg) was applied at 670 lb/A and incorporated on 29 Apr. Prior to planting spinach, Ro-Neet (pre-plant herbicide) was applied at 1.3 pt/A, and incorporated with a mulcher-packer. Spinach seeds were planted by hand using a Johnny's Select Seeds six-row precision planter (2.25 in. row spacing) in 15 ft x 3.5 ft split plots

with 15 ft alleys between adjacent split plots. Seeds were planted 1 in. deep with three passes of the planter/plot to achieve a seeding rate of approximately 1 to 2 million seeds/A.

A sample of 200 g of leaves was cut manually from each split plot planted with non-treated seeds or with Farmore D300 + Mertect 340F-treated seeds in non-fumigated, limestone-amended main plots of four replicate blocks. The leaves were frozen at -112°F and shipped to Syngenta Crop Protection for thiabendazole residue analysis. Spinach leaves were harvested on 14 Jun using a push lawn mower. The remaining roots and crowns of four sets of 25 seedlings were dug from each split plot (100 seedlings/split plot), the roots washed under tap water, and each set of 25 plants placed in an ELISA bag and stored overnight at 10°F. A 2 ml aliquot of 0.0125 M PO_4 buffer was added to each bag, the plants crushed, and 1 ml of sap from each bag/split plot pooled. From each pooled sample a 10-fold dilution series was plated onto NP-10 agar medium to quantify *V. dahliae* in the plants. Remaining spinach residues were tilled 6 in. deep with a roto-tiller on 24 Jun.

On 7 Jul, Kerb 50W (pre-plant herbicide) was applied to main plots at 3 lb/A, and incorporated with a mulcher. The same day, lettuce seeds of a Verticillium wilt-susceptible 'Salinas' cv. were planted in two rows/split plot spaced 22 in. apart with 2.75 in. spacing within rows, using a Monosem planter. A blend of 58% 12-24-17 and 42% 10-20-20 was applied at 325 lb/A in-furrow at planting. A sample of 400 seeds from this lettuce seed lot had 0% *Verticillium* when tested on NP-10 agar medium. Due to poor emergence, lettuce seeds were planted in each plot again on 16 Jul, and seed was hand-planted into missing sections of the rows on 26-27 Jul. Lettuce plants were thinned on 18 and 27 Aug to a spacing of 8 in. within rows. The plants were sprayed with Assail 70WP (1.4 oz/A) + Capture 2EC (4.25 fl oz/A) insecticides on 20 Aug to control loopers, aphids, and thrips; and cultivated and side-dressed with 15-5-0-0 calcium nitrate at 200 lb/A on 25 Aug. Tanos 50DF (10 oz/A) + Switch 62.5WDG (14 oz/A) were applied on 17 and 22 Sep to control downy mildew and grey mold, respectively, and Alliette WDG (5 lb/A) was applied on 29 Sep for downy mildew. Twenty lettuce heads were harvested from each split plot from 12 to 14 Oct, and total fresh weight measured. A cross-section of the base of the stem of each head was removed, surface-sterilized in 0.6% NaOCl for 60 sec, rinsed in tap water (60 sec), blotted dry, and sections of the stem tissue plated onto NP-10 agar medium and placed in moist chambers to isolate for *Verticillium*. Lettuce residues were rototilled into the plots on 14 Oct.

Soil samples (6-8 cores/split plot) were collected on 10 May, 7 Jul, and 28 Oct from each plot to test soil pH and quantify *Verticillium*. At each sampling, soil cores were pooled for each split plot, dried, crushed, sieved to 1 mm particle size, and stored at 50°F. A 1g sub-sample of each sample was plated onto NP-10 agar medium to quantify *Verticillium* spp. Soil pH was measured for a 40 g subsample of each plot not planted with spinach seed for each fumigation-limestone treatment combination.

Data were subjected to analyses of variance, and means compared using Fisher's protected least significant differences (LSD, at $P < 0.05$). Mean temperature and total rainfall for May, Jun, Jul, Aug, Sep, and Oct were 52.1°F and 4.57 in., 56.8°F and 1.66 in., 60.7°F and 0.05 in., 60.8°F and 1.17 in., 58.8°F and 2.87 in., 51.9° F and 1.76 in., respectively. Plots were irrigated with a total

of 2.02 in. water split over 11 May, 8 and 27 Jul, and 19 Aug.

Results and Discussion:
Soil pH was significantly greater in plots amended in Apr with 2 tons limestone/A compared to non-amended plots: pH of 5.9 vs. 6.6 by 10 May when spinach seed was planted, 5.7 vs. 6.8 by 21 Jul when lettuce seed was planted, and 5.6 vs. 6.8 on 28 Oct, two weeks after incorporation of lettuce residues, respectively. Soilborne *Verticillium* averaged 0.1 CFU/g soil in fumigated plots compared to 102 CFU/g soil in non-fumigated plots sampled on 10 May, four weeks after fumigation. Within non-fumigated plots, there was no significant difference in *Verticillium* soil counts between limestone-amended vs. non-amended plots (103 vs. 113 CFU/g soil, respectively), i.e., the change in soil pH resulting from the spring limestone amendment did not have a significant effect on the amount of *Verticillium* detected at planting (background level of *Verticillium* in the field site), as expected.

Fungicide seed treatments and soil fumigation treatments significantly ($P < 0.05$) affected the rate of seed transmission of *V. dahliae* in spinach, detected by plant sap assays after harvest of leaves. There was a significant interaction between seed treatments and soil fumigation treatments (Table 1). Spinach plants assayed after harvest of the leaves produced an average of 213 CFU *V. dahliae*/100 plants in plots planted with non-treated seed. In comparison, Apron + Thiram + Topsin M 70WP seed treatment reduced seed transmission to 5 CFU/100 plants (98% reduction in seed transmission compared to non-treated seed), and Farmore D300 + Mertect 340F reduced seed transmission to 15 CFU/100 plants (93% reduction), with no significant difference between the latter two seed treatments. Apron + Thiram, the 'conventional' fungicide seed treatment for baby leaf spinach in the USA, reduced seed transmission to 107 CFU/100 plants (50% reduction in seed transmission). The rate of seed transmission of *V. dahliae* from non-treated spinach seed was 706% greater in fumigated plots than in non-fumigated plots (379 vs. 47 CFU/100 plants). This difference in seed transmission rates in fumigated vs. non-fumigated soils was much less in plots planted with fungicide-treated seed.

On 7 Jul, when lettuce was planted, there were significant differences in amount of *Verticillium* detected in fumigated vs. non-fumigated soils (21 vs. 162 CFU/g soil, respectively), with no significant effect of limestone treatments (82 vs. 102 CFU/g soil in non-amended vs. amended plots, respectively) (Table 1). Planting infected spinach seed and incorporating spinach residues after harvest of the leaves doubled the amount of *Verticillium* detected in soil compared to plots without spinach preceding the lettuce crop (82 vs. 44 CFU/g soil, respectively). Spinach seed treatments had a significant effect on soilborne *Verticillium* detected on 7 Jul but, surprisingly, opposite to the effects on seed transmission, i.e., the increase in *Verticillium* soil counts was greatest in plot planted with seed that had been treated with either Apron + Thiram or Apron + Thiram + Topsin M 70 WP, followed by non-treated seed and Farmore D300 + Mertect 340F-treated seed. Seed treatments may have reduced colonization of spinach by other soilborne fungi, enabling *Verticillium* to colonize residues rapidly after harvest.

Verticillium wilt was not observed in the head lettuce crop following the baby leaf spinach crop, and *V. dahliae* was only isolated from 2 of the 2,000 lettuce stem sections evaluated at harvest. The amount of soilborne *Verticillium* detected at the end of the trial after harvest of the lettuce

and incorporation of lettuce residues (28 Oct) was not affected significantly by limestone treatment or spinach seed treatments, but was affected significantly by soil fumigation. Soilborne *Verticillium* averaged 74 vs. 128 CFU/g soil in fumigated vs. non-fumigated plots, respectively (Table 1). This translated to an increase in soilborne inoculum from the start of the trial by 19 CFU/g soil in non-fumigated plots vs. 74 CFU/g soil in fumigated plots, which translated to a 289% greater increase in soilborne *Verticillium* in fumigated soil vs. non-fumigated soil.

Table 1. Amount of *Verticillium* detected in the roots and crowns of spinach plants (14 Jun), and amount detected in the soil at lettuce planting (7 Jul) and following harvest of lettuce heads and incorporation of the lettuce residues (28 Oct), when a *Verticillium*-infected spinach seed lot with different fungicide seed treatments was planted as a baby leaf crop preceding a Verticillium wilt-susceptible head lettuce crop in Skagit Co., Washington.

Date of *Verticillium* assay, spinach seed treatment	Averaged across fumigation and limestone treatments	Averaged across limestone treatments	
		Non-fumigated plots	Fumigated plots
	CFU *Verticillium*/g soil		
10 May (at spinach planting)	54	108	0.06
	CFU *V. dahliae*/100 spinach plants after harvest of leaves		
14 Jun (at spinach harvest)			
Non-treated	213 a*	47	379
Apron + Thiram	107 b	99	115
Apron + Thiram + Topsin M 70 WP	5 c	3	8
Farmore D300 + Mertect 340F	15 c	5	24
	CFU *Verticillium*/g soil (change from CFUs detected on 10 May)		
7 Jul (at lettuce planting)			
No spinach planted ('fallow')	44 (-10) d	84 (-24)	5 (5)
Non-treated	82 (28) bc	148 (40)	17 (17)
Apron + Thiram	140 (86) a	244 (136)	35 (35)
Apron + Thiram + Topsin M 70 WP	115 (61) ab	202 (94)	28 (28)
Farmore D300 + Mertect 340F	75 (21) d	129 (21)	21 (21)
	CFU *Verticillium*/g soil (change from CFUs detected on 10 May)		
28 Oct (after lettuce residue incorporation)			
No spinach planted ('fallow')	107 (53) a	145 (37)	70 (70)
Non-treated	70 (16) a	105 (-3)	35 (35)
Apron + Thiram	118 (64) a	132 (24)	104 (104)
Apron + Thiram + Topsin M 70 WP	113 (59) a	142 (34)	84 (84)
Farmore D300 + Mertect 340F	95 (41) a	114 (6)	76 (76)

*At each date, means within a column followed by the same letter are not significantly different based on Fisher's protected LSD.

Overall, the results demonstrate that soil fumigation can exacerbate seed transmission rates of *Verticillium* significantly when planting infected spinach seed, but seed treatments can be highly effective at reducing seed transmission rates of *Verticillium*. Therefore, there may also be a significant benefit from using an effective seed treatment on lettuce to manage Verticillium wilt when infected lettuce seed is a source of inoculum.

Residue analyses of spinach leaves harvested from plots planted with Farmore D300 + Mertect 340F-treated spinach seed had <10 ppb thiabendazole. These results are being utilized by Syngenta Crop Protection to pursue a federal seed treatment registration for Mertect 340F on spinach.