Effect of seed treatments and soil fumigation on seed transmission and soil infestation by *Verticillium* in a baby leaf spinach-head lettuce rotation

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INTRODUCTION

Spinach (*Spinacia oleracea*) seed is produced in the few areas of the world with appropriate climatic conditions for high quality seed production: China, Denmark, France, Italy, Holland, New Zealand, and western Oregon and Washington in the USA (4,9). du Toit et al. (7) demonstrated the prevalence of *Verticillium dahliae* in spinach seed lots, seed transmission of the pathogen, the systemic nature of infection of spinach, and development of symptoms of Verticillium wilt only after initiation of bolting. The latter explains why Verticillium wilt is never observed in fresh market or processing spinach crops. Seedborne *V. dahliae* has impacted the international spinach seed industry because of regulations affecting movement of infested seed (1), and because of concerns about introduction of *V. dahliae* inoculum into soils as a result of planting infested spinach seed lots, with the potential for infection of subsequent crops susceptible to Verticillium wilt, e.g., lettuce (2,3).

van der Spek (10) demonstrated that soil microbial activity can influence the degree of seed transmission and soil infestation by *V. dahliae*, and subsequent development of wilt symptoms. Seed treatments can also reduce the incidence of spinach seed on which *V. dahliae* is detected, and reduce seed transmission rates (5,6,8). Of 20 conventional and organic fungicide seed treatments evaluated in greenhouse trials, 7 reduced the level of infection in a spinach seed lot from ~64% for non-treated seed to <10%, with a highly correlated reduction in rates of seed transmission (5,6). The most effective treatments were thiophanate-methyl, thiabendazole, triticonazole, a proprietary steam treatment, and a proprietary organic disinfectant. However, none of these treatments was registered for use on spinach seed in the USA at that time.

The objectives of this study were to evaluate the effects of spinach seed treatments and soil fumigation under field conditions on:
1) seed transmission of *Verticillium* from planting infested spinach seed, and
2) development of Verticillium wilt in a head lettuce crop following a 'baby leaf' spinach crop.

MATERIALS & METHODS

A field trial was set up in April 2010 at the Washington State University Mount Vernon NWREC on a silt loam soil in a split plot, randomized complete block design. Main plots were amended with 0 or 5 tons agricultural limestone/ha (97% calcium carbonate) on 11 April to assess the impact of soil pH (5.9 vs. 6.6 in non-amended vs. amended plots, respectively) on seed transmission and soil infestation by *Verticillium* (Fig. 1A). On 14 April, half the main plots were fumigated with methyl bromide-chloropicrin (57:34%) at ~3,500 liters/ha under plastic (Fig. 1B, 1C). Seeds of a commercial spinach seed lot naturally infested with *V. dahliae* at ~60% were planted on 7 May in split plots at ~3 to 5 million seeds/ha (Fig. 1D): 1) without fungicide seed treatment, 2) treated with mefenoxam + thiram (Apron + Thiram), 3) mefenoxam + thiram + thiophanate-methyl (Apron + Thiram + Topsin 4.5FL), 4) mefenoxam + azoxystrobin + fludioxonil + thiabendazole (Farmore D300 + Mertect 340F), or 5) no spinach seed preceding the lettuce crop.

A sample of leaves (200 g) cut from each split plot planted with non-treated seeds or Farmore D300 + Mertect 340F-treated seeds was tested for thiabendazole residues. After harvest of spinach leaves on 14 June (Fig. 1D), roots and crowns of 100 seedlings/split plot were assayed on NP-10 agar medium for *V. dahliae* to measure seed transmission rates. Remaining spinach residues were rototilled on 24 June. On 7 July, lettuce seeds of a Verticillium wilt-susceptible 'Salinas' cv. were planted in 2 rows/split plot (Fig. 1E). A sample of 400 lettuce seeds was tested for *Verticillium*. Lettuce heads (20/split plot) were harvested on 12 to 14 October, rated for Verticillium wilt, and a stem cross-section of each plant tested for *Verticillium.* Lettuce residues were then rototilled. Soil samples were collected from each split plot three times to quantify changes in soilborne *Verticillium* levels: 1) when spinach seed was planted, 2) when lettuce seed was planted, and 3) two weeks after lettuce residues were incorporated.

Fig. 1. Select stages of the 2010 'baby leaf' spinach- head lettuce trial.

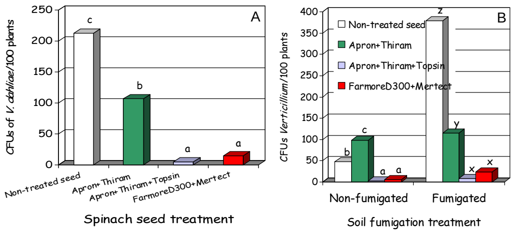

Fig. 2. Interaction of seed treatment (A) and soil fumigation effects (B) on seed transmission of *Verticillium dahliae* in 'baby leaf' spinach.

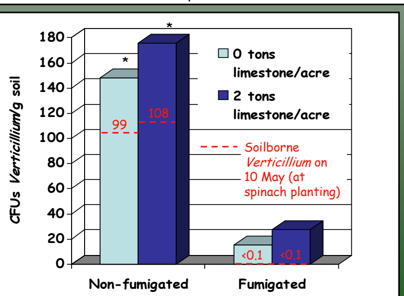

Fig. 3. Effect of soil fumigation and limestone amendment on soilborne *Verticillium* at lettuce planting (7 July 2010).

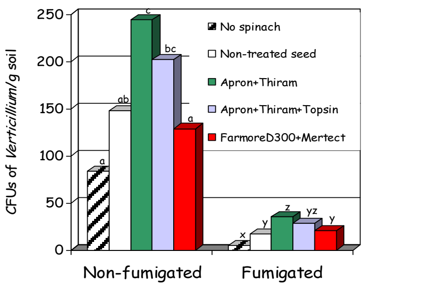

Fig. 4. Interaction of seed treatment and soil fumigation on soilborne *Verticillium* on 7 July 2010 (at lettuce planting).

RESULTS

Four weeks after fumigation, soilborne *Verticillium* averaged 0.1 vs. 102 CFU/g soil in fumigated vs. non-fumigated plots, respectively. Limestone had no significant effect on *Verticillium* soil counts.

Fungicide seed treatments and fumigation significantly affected seed transmission of *V. dahliae* in spinach (Fig. 2). Spinach plants assayed after harvest of the leaves averaged 213 CFU *V. dahliae*/100 plants in plots planted with non-treated seed (Fig. 2A). Apron + Thiram + Topsin 4.5FL reduced seed transmission by 98% (to 5 CFU/100 plants), Farmore D300 + Mertect 340F by 93% (to 15 CFU), and Apron + Thiram by 50% (to 107 CFU). Seed transmission of *V. dahliae* from non-treated seed was 706% greater in fumigated vs. non-fumigated plots (379 vs. 47 CFU/100 plants), but the difference was significantly less for treated seed (Fig. 2B).

At lettuce planting, fumigated vs. non-fumigated plots averaged 21 vs. 162 CFU *Verticillium*/g soil, respectively (Fig. 3). Planting infected spinach seed and incorporating spinach residues doubled the amount of *Verticillium* in soil compared to plots with no spinach (82 vs. 44 CFU/g soil, respectively, averaged across fumigated and non-fumigated plots) (Fig. 4). Spinach seed treatments significantly affected soilborne *Verticillium*, but opposite to the effects on seed transmission, i.e., the increase in soilborne *Verticillium* was greatest in plots planted with seed treated with Apron + Thiram or Apron + Thiram + Topsin 4.5FL, followed by non-treated seed and Farmore D300 + Mertect 340F-treated seed (Fig. 4).

Verticillium wilt was not observed in the lettuce crop, and *Verticillium* was isolated from only 2 of 2,000 lettuce plants. By the end of the trial, the amount of soilborne *Verticillium* was not affected significantly by spinach seed treatments (Fig. 5), but averaged 74 vs. 128 CFU/g in fumigated vs. non-fumigated plots, respectively, which equated to an increase in soilborne inoculum from the start to the end of the trial of 19 CFU/g in non-fumigated plots vs. 74 CFU/g in fumigated plots (= 289% greater increase in fumigated plots).

Residue analyses of spinach leaves harvested from plots planted with Farmore D300 + Mertect 340F-treated seed had <10 ppb thiabendazole.

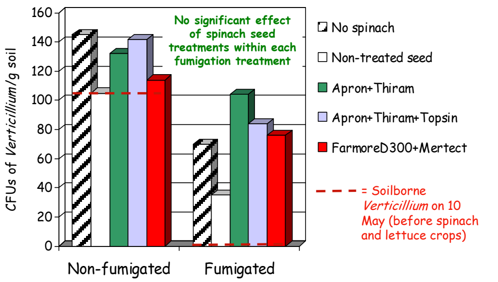

Fig. 5. Effect of spinach seed treatment and soil fumigation on soilborne *Verticillium* on 28 October 2010 (after incorporation of lettuce residues).

CONCLUSIONS

- Soil fumigation increased seed transmission of *V. dahliae* in spinach by >700%.
- Seed treatments effectively reduced seed transmission in spinach.
- Under the conditions of this trial, planting infested spinach seed did not cause Verticillium wilt in the subsequent lettuce crop, and had no significant effect on soilborne *Verticillium* levels after the lettuce crop.
- The trial is being repeated in 2011 at the WSU Mount Vernon NWREC, WA (acid soil) and at a grower-cooperator farm in Salinas, CA (alkaline soil).

SELECTED LITERATURE CITED

1. Anonymous. 2003. International Phytosanitary Certificate No. 4051. Phytosanitary Federal Law of the Mexican United States, Phytosanitary Regulation and Inspection Department, Mexico.
2. Atallah, Z.K., Hayes, R.J., and Subbarao, K.V. 2011. Fifteen years of Verticillium wilt of lettuce in America's salad bowl: A tale of immigration, subjugation, and abatement. Plant Dis. 95:784-792.
3. Atallah, Z.K., Maruthachalam, K., du Toit, L.J., Koike, S.T., Davis, R.M., Klosterman, S.J., Hayes, R.J., and Subbarao, K.V. 2010. Population analyses of the vascular plant pathogen *Verticillium dahliae* detect recombination and transcontinental gene flow. Fung. Gen. Biol. 47:416-422.
4. Deleuran, L.C., and Boelt, B. 2006. Spinach seed production in Denmark. Pages 13-15, in: Proc. 2006 Int. Spinach Conf., 13-14 July 2006, La Conner, WA.
5. du Toit, L.J., Derie, M.L., Brissey, L.M., and Holmes, B.J. 2010. Evaluation of seed treatments for management of seed borne *Verticillium* and *Stemphylium* in spinach, 2009. Plant Dis. Manag. Rep. 4:ST038.
6. du Toit, L.J., Derie, M.L., Brissey, L.M., Holmes, B., and Gatch, E.W. 2009. Evaluation of seed treatments for management of seed borne *Verticillium* in spinach, 2008. Plant Dis. Manag. Rep. 3:ST020.
7. du Toit, L.J., Derie, M.L., and Hernandez-Perez, P. 2005. Verticillium wilt in spinach seed production. Plant Dis. 89:4-11.
8. du Toit, L.J., and Hernandez-Perez, P. 2005. Efficacy of hot water and chlorine for eradication of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from spinach seed. Plant Dis. 89:1305-1312.
9. Foss, C.R., and Jones, L.J. 2005. Crop Profile for Spinach Seed in Washington. USDA National Pest Management Centers.
10. van der Spek, J. 1973. Seed transmission of *Verticillium dahliae*. Med. Facult. Landb. Rijks. Gent 38:1427-1434.

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