Project Title

Investigation of the possible role of winter cover crops in Verticillium wilt epidemiology

Project Investigators

Steven Koike and Richard Smith
University of California Cooperative Extension
1432 Abbott Street
Salinas, CA 93901
831-759-7350
stkoike@ucdavis.edu/rifsmith@ucdavis.edu

Tom Gordon and Margaret Lloyd
Department of Plant Pathology
University of California
Davis, California 95616

Abstract
Verticillium wilt has become one of the most threatening diseases of lettuce in coastal California, and the isolates that infect lettuce also cause disease in strawberry. Because lettuce and strawberry growers often rotate ground between them, the development of Verticillium wilt in one crop could likely have serious implications for the other crop. Work conducted at Davis indicates that legume cover crops potentially could increase *V. dahliae* inoculum. Because of these findings, we explored the interaction of winter-planted cover crops placed into a *V. dahliae* infested field in coastal California. 12 cover crops and two spinach cultivars were planted on December 29, 2011 in a Watsonville field that was previously cropped to strawberries that had significant Verticillium wilt. A sample of each seed lot was tested and determined to be free of seedborne *V. dahliae*. Mature plants were randomly sampled on April 18, 2012, and roots and stems were tested for *V. dahliae*. The pathogen was recovered from plants in only four cases: two stems of purple vetch, one root sample of cayuse oat, one root sample of Merced rye. None of the plants showed symptoms of disease. Soil inoculum levels were approximately 50% lower in the post-evaluation than in the pre-planting assay; this differential likely reflects a combination of an altered distribution and dilution of propagules due to soil mixing in preparation for planting, and natural attrition during the season. Overall, these data show low susceptibility of cover crops to colonization by *V. dahliae* at the relatively low inoculum levels present in soil and during this winter period. Recovery from purple vetch stems indicates that inoculum build-up could be a concern with this species, whereas recovery only from roots, as was the case for oats and rye, is less cause for concern.

Introduction
Verticillium wilt has become one of the most threatening diseases of lettuce in coastal California. In an unfortunate coincidence, the isolates of *Verticillium dahliae* that infect lettuce also cause Verticillium wilt of strawberry. The loss of methyl bromide and other pre-plant soil fumigants, as

well as changes in regulations governing soil fumigation, creates a situation in which strawberry growers may in future seasons see a resurgence of Verticillium wilt. Because lettuce and strawberry growers often rotate ground between them, the development of Verticillium wilt in one crop could likely have serious implications for the other crop.

Questions persist as to the possible impact of other plants grown in rotation with lettuce or strawberry. In particular, what impact might cover crops have regarding soil populations of *V. dahliae*? Work by Lloyd and Gordon indicates that legume cover crops potentially could increase *V. dahliae* inoculum. In a series of experiments, ten different legume cover crops were inoculated (by either conidial root dips or solid mixtures of microsclerotia) and grown in either a greenhouse or the field. These experiments were conducted in Davis during both spring and winter seasons. Lloyd and Gordon found that all ten species became systemically colonized to some degree and that microsclerotia were present in colonized tissues. However, the cover crop species remained symptomless. Common vetch, black-eyed pea, sesbania, and sunn hemp (Crotolaria) had the highest infection percentage. Field pea, lana vetch, fava bean, bell bean, purple vetch, and hairy vetch were infected at a significantly lower percentage. Fava bean has been previously reported as a *V. dahliae* host by other researchers.

Because of these findings, we were interested in exploring the interaction of winter-planted cover crops planted into a *V. dahliae* infested field in coastal California.

Objective
Investigate the role of cover crops in Verticillium wilt epidemiology

Procedures
Commonly grown cover crops were planted on December 29, 2011 in a field east of Watsonville that was rotated out of strawberries in the 2011 production season. The prior strawberry crop was severely damaged by Verticillium wilt, caused by *Verticillium dahliae*. Nine cover crops were planted to assess their susceptibility to colonization by *V. dahliae* (Table 1). All cover crops were tested for seedborne *V. dahliae* by imbedding seed (200 per species) into petri plates containing the semi-selective NP-10 medium and incubating the plates in the dark for two weeks. Two spinach cultivars, seeds of which were also tested for *V. dahliae*, were included to determine whether they could become infected from field inoculum during this winter period. The plot was established in a section where previously planted strawberry plants had been affected by Verticillium wilt. Each plot was two 40-inch beds wide by 20-feet long, with cover crops planted on the top of each bed. Each plot was replicated four times in a randomized complete block design.

Mature plants were randomly sampled from each plot on April 18, 2012, and roots and stems were tested for the presence of *V. dahliae*. Soil from each plot was collected before planting and after cover crop evaluation and then assayed to determine the density of infective propagules. Soils were dried for two weeks, ground into a fine powder, and dispersed (using an Anderson sampler) onto plates containing NP-10. Plates were incubated in the dark for 3 weeks and then examined for *V. dahliae*.

Table 1. Winter cover crops evaluated for susceptibility to *Verticillium dahliae*

	Cover crop	Variety/Type
1	Vetch	Lana Woolly Pod
2	Vetch	Common
3	Vetch	Purple
4	Pea	Dundale
5	Fava	Bell
6	Oat	Cayuse
7	Rye	AGS 104
8	Rye	Merced
9	Barley	UC 603
10	Triticale	Juan
11	Mustard	Kodiak
12	Mustard	Ida Gold
13	Spinach	Amazon
14	Spinach	PV 0290

Results and Discussion

All cover crop and spinach seeds tested for seedborne inoculum were negative for *V. dahliae*. From plants, *V. dahliae* was recovered in only four cases. Two isolations were made from stem tissue of purple vetch, and one isolation each was made from the roots of cayuse oats and Merced rye. The infected purple vetch plants came from a plot that had one of the highest densities of pathogen inoculum (28 microsclerotia/gram of soil). None of the plants showed Verticillium wilt symptoms. These preliminary results indicate that *Verticillium* can colonize purple vetch during the winter period if relatively high inoculum levels are present. A similar trend was observed for rye, in which infection by *Verticillium* occurred in the rye plot with a high inoculum count. For spinach plants, we recovered *V. tricorpus* from a number of plants; however, no *V. dahliae* colonies were found.

Soil inoculum levels were approximately 50% lower in the post-evaluation than in the pre-planting assay (Table 2). This differential likely reflects a combination of an altered distribution and dilution of propagules due to soil mixing in preparation for planting, and natural attrition during the season.

Table 2. Pre-plant and post-evaluation counts of microsclerotia of *Verticillium dahliae* in cover crop plots

	Mean microsclerotia/gm soil	
	Pre-plant	Post-eval.
Lana woolly pod vetch	4	3
Common vetch	16	6
Purple vetch	14	9
Dundale pea	18	8
Fava bean	11	7
Cayuse oat	19	3
AGS 104 rye	25	5
Merced rye	18	8
UC 603 barley	5	7
Juan triticale	10	10
Kodiak mustard	13	7
Ida Gold mustard	10	9
Spinach	10	8
Spinach	12	8

Overall, these data show low susceptibility of cover crops to colonization by *V. dahliae* at the relatively low inoculum levels present in soil and during winter conditions at this coastal location. The mean range of *V. dahliae* microsclerotia (ms) for all plots pre-plant was 4 to 25 ms/gram of soil, and the mean range for all plots at cover crop maturity was 3 to 10 ms/gram of soil. Recovery from the stem of purple vetch indicates that inoculum build-up could be a concern with this species, whereas recovery only from roots, as was the case for oats and rye, is less cause for concern. Further study will be needed to accurately gauge the magnitude of the risk posed by each cover crop and to identify those that are most suitable for rotations with susceptible crops such as lettuce and strawberry.

Acknowledgments

We thank our grower cooperators for hosting this trial, and California Leafy Greens Research Board and California Strawberry Commission for jointly supporting the research. We thank the following for assistance with the study: Patty Ayala, Adam Coleman, Aaron Heinrich, Kat Kammeijer, Laura Murphy, and Tom Hearne and LA Hearne Seed Company (for help with the cover crops).