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Compost amendments in a wood- chip+ polyacrylamide medium decreased *Verticillium dahliae* infection on potato.

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Abstract

If the soil around the plant roots could be altered to favor the non-pathogenic indigenous soil microorganisms overrelative to the plant pathogens, the survival and proliferation of indigenous soil microorganisms, and thus effectiveness of biological control, may be increased. We used wood- chip+-polyacrylamide (PAM) cores to alter the soil environment in a greenhouse study to favor indigenous soil microorganisms in vegetable and manure compost to reduce *Verticillium dahliae* infection of potato (*Solanum tuberosum* L.) plants. Potato plants growing in soils amended with vegetable compost-wood chip-PAM cores had significantlyless disease because of 86 % lower visible (V_{vis}) and 66 % lower isolation (V_{iso}) V. dahliae infection rateings than control soils and soils with dairy or vegetable compost alone. Soils with wood chip-PAM cores and soils with wood chip-PAM-vegetable compost had greater 2.5 times greater microbial biomass/*Verticillium dahliae* biomass (MB/VB) ratios in soil than control soils or in soils amended with compost alone. MB/VB ratios in wood chip-PAM cores and wood chip-PAM-vegetable compost were2.5 to 3.2 imes greater than in wood chip-PAM-dairy compost cores. V_{vis} correlated in a quadratic relationship with the MB/VB ratio (r^2=0.76) indicating that as microbial biomass increased, *V. dahliae* biomass and symptoms decreased. As MB/VB ratio increased, Vvis decreased.

Introduction

Verticillium wilt caused by *Verticillium dahliae* is a major disease problem in potato (*Solanum tuberosum* L.) production. Indigenous microbes are effective contributors to disease suppression in cropping systems and mediate effects of numerous cultural practices to reduce pathogen populations and limit disease severity (Davis *et al.* 1996; Strausbaugh, 1993). A powerful biological control strategy in the soil environment is competition. Biocontrol of root disease is largely based upon competition for rhizosphere resources between the biocontrol organism and the pathogen, provided that their respective niches overlap. Competitive colonization of rhizosphere by the biocontrol organism and use of resources is thought to exclude many rhizosphere pathogens. Indigenous soil microorganisms will compete for colonization of soil organic material including host roots with facultatively obligate plant pathogens, until plant pathogenic microorganisms cannot find adequate resources to produce a population capable of infecting the host (Baker and Scher, 1987). It has been recognized that Ssuccessful control of plant diseases may be achieved ismay be affected by changing the environment in the rhizospere, so biocontrol agents can out compete plant pathogens for rhizosphere resources. behavior of pathogens in the rhizosphere . Biocontrol of root disease is largely based upon competition for rhizosphere resources between the biocontrol organism and the pathogen provided that their respective niches overlap. Competitive colonization of rhizosphere by the biocontrol organism and use of resources is thought to exclude many rhizosphere pathogens.

Polyacrylamides (PAMs) are polymers made up of many repeating subunits (monomers).

When PAM molecules are dissolved in water and they adhere to microorganisms or nutrients; some added PAM they may stay dissolved until the molecule adheres to an object. Polyacrylamide Superfloc® A836 was found to adhere indigenous microorganisms to roots. flocculate microorganisms along with suspended minerals, reducing their mobility in runoff (Sojka and Entry, 2000). In this experiment, the PAM Superfloc® A836 copolymer was used to help promote adherence of indigenous microorganisms in dairy waste to wood chips and potato roots. The objective of our greenhous study was to determine the efficacy of wood chips and PAM to manage soil microbes in order to alter the soil environment in a greenhouse study to favor indigenous microorganisms in vegetable and manure compost in order to andt reduce *Verticillium dahliae* infection of potato plants through competition.

Methods and Materials

The greenhouse experiment was arranged in a randomized block design with soil containing different concentrations of *V. dahliae* as blocks . Treatments were: 1) soil that did not receive wood chip-PAM treatments (control), 2) soil amended with a core containing a wood chip-PAM mixture without compost, 3) soil amended with a core containing a wood chip-PAM mixture with a dairy manure compost amendment, 4) soil amended with a core containing a wood chip-PAM mixture with a vegetable compost amendment, 5) soil that received dairy compost amendment without a wood chip-PAM treatment and 6) soil that received a vegetable compost amendment without a wood chip-PAM treatment. The experiment had 6 treatments x 3 soils with different concentrations of *V. dahliae* x 3 replications x 6 separate plants evaluated from each treatment x soil. A total of 324 samples for each parameter were taken during the experiment.

The three silt loam soils sources, all with the same classification, used in this experiment were collected from the University of Idaho Research and Extension Center at Aberdeen, Idaho. All three soil sources are characterized as a silt loam . Prior to collection, these soils had been planted to a variety of crops including barley (*Hordeum vulgare L.*), wheat (*Triticum aestivum* L.) and potatoes for more than 70 years. ;Potato crops grown in these soils had a long history of Verticillium wilt.V. dahliae infection in the potato crop (Davis *et al.* 1996).

The potatoes were grown in 3.0 L (11.3 gal) black plastic pots containing 3 kg (6.6 lb) soil. Control pots contained only soil (without PAM cores or amendments). The polyacrylamide copolymer used was a dry granular material having an approximate molecular weight of 12-15 Mg/mole (CYTEC Industries of Wayne, NJ and sold under the trade name Superfloc® A836 by CYTEC Industries of Wayne, NJ). A 7.0 cm diameter x 15 cm (2.57" diameter x 6.25" ") deep core was removed from the center of the soil in each pot receiving wood chip-PAM or wood chip- PAM-compost cores. Each hole was filled with a wood chip-PAM or wood chip-PAM compost core. All cores were comprised of a wood chip-PAM treatment which was a mixture of 48.9 g (1.7 oz) Ponderosa pine (*Pinus ponderosa* Dougl. Ex. Laws.) wood chips, 22.3g (0.78 oz) nutrient solution (Arnon and Hoagland, 1940) and 0.6 g PAM liter $^{-1}$ solution. Fresh wood chips were 5 x 5 x 1 cm (2 x 2 x 0.5 ") in size. Cores having the wood chip-PAM vegetable or dairy compost treatment contained the above mixture except with 143.2 g (0.32 lb) wood chips and 115.7 g (0.26 lb) vegetable or dairy compost. The three- month- old soil-dairy or soil-vegetable compost treatments without a core contained 2.7 kg (1.23 lb) soil mixed with 300 g (0.66 lb) dairy or vegetable compost. All soils were then watered to field capacity (QUANTIFY) with water.

Russet Burbank potato tubers were cut into 35 ± 5 g (1.23 oz) seed pieces and planted 4 cm deep in each pot. Potato plants were grown from May 24th to August 25th, 1997 in a greenhouse that was maintained at 26 ± 5°C. Plants were watered with well water to maintain field capacity and were fertilized with Arnon's nutrient solution (Arnon and Hoagland, 1940) each week. During that time, the plants were exposed to sunlight with was a photosynthetic active radiation of 400-700 $\mu mol\ m^{-2}\ S^{-1}$ and a 14-16 h photoperiod.

At harvest, plants were removed from the pots and separated into roots, shoots, and tubers. Roots and tubers were washed in water and then distilled deionized (RO?) water until all visible soil particles were removed. Verticillium symptoms, which were characterized by wilted and yellowish to brown leaves and stems, were separated from other wilt-producing symptoms in potato plants (drought stress, nutrient deficiency, senescence) by assaying the basal 3 cm of stem tissue for *V. dahliae* (Strausbaugh, 1993). *V. dahliae* was isolated from potato plants by slicing a 0.10 cm thick segment from the basal stem of each plant. Segments were surface-disinfected for 1 min in 0.5 % (v/v) NaOCl, rinsed in sterile distilled water and placed on bacto-agar . Colonies of *V. dahliae* with vertically branched conidiophores and conidia typical of *V. dahliae* formed in and around the vascular tissue in the segments of symptomatic plants (Strausbaugh, 1993)(SENTENCE FRAGMENT). Verticillium symptoms were evaluated at termination of the experiment and data were expressed as 1) a percentage of stems with Verticillium symptoms (V_{vis}) and 2), a percentage of plants from which V. *dahliae* was isolated (V_{iso}). All root, shoot, and tuber tissue was then dried at 80°C for 48 h and weighed.

At harvest, soil, cores and plant roots from each pot were separated. Soil and wood chip-PAM or wood chip - PAM - compost amended cores from each pot were collected at harvest and analyzed separately for *V. dahliae,* and other active fungal and bacterial biomass. *V. dahliae* colony forming units (cfus) in soil and in cores were estimated using procedures described by Butterfield and DeVay (1977). *V. dahliae* cfu were converted to *V. dahliae* biomass to be able to compare the amount of *V. dahliae* inoculum to the amount of total fungi and bacteria in the soils and cores. In the conversion from *V. dahliae* cfus to *V. dahliae* biomass, it was assumed that each cfu arose from a piece of *V. dahliae* hyphae or spore able to cause an infection in potato. Active bacterial and fungal biomass for each soil and wood chip core in each pot at harvest were estimated using direct counting methods as described by Ingham and Klein, (1984).

Results

Soil amended with wood chip PAM cores and wood chip- PAM-vegetable compost cores had lower V_{vis} rateings than all other treatments (Table 1). Soil amended with wood chip-PAM-dairy cores and compost soils amended with vegetable compost only had lower V_{vis} and V_{iso} rates than the control soil or soil amended with dairy compost. No significant differences in plant and tuber weight were evident, although a pattern with regard to soil source was evident. Active fungal and bacterial biomass did not differ consistently with treatment or soil source. Soil 1 in the control treatment had higher *V. dahliae* biomass than all other treatments, except soil 1 in the dairy compost treatment. Wood chip cores in soil amended with dairy compost and vegetable compost had higher *V. dahliae* biomass in soils 1 and 2 than wood chips+PAM alone. The control, vegetable compost and dairy compost treatments had lower MB/VB ratios thant the other three treatments. The MB/VB ratios in the vegetable and dairy compost treatments were higher than the control treatment. V_{vis} correlated curveinlinearly with the MB/VB ratio in a negative relationship (r^2=0.76). As the MB/VB ratio increased, visible infection of *V. dahliae*

decreased.

Discussion

The major challenge facing commercial production of biological control of plant pathogens is to obtain effective and reproducible disease control. The lack of disease control by organic treatments and biological control products for plant pathogens is often ascribed to environmental factors, which are often difficult to define. In field conditions, fluctuations in moisture, temperature and nutrient and carbon availability can play a role in limiting effectiveness of biological control treatments. Various communities of indigenous microorganisms in any one soil have adapted to the specific environmental conditions. One method of controlling plant pathogens in a soil may be to change the soil environment to favor indigenous microorganisms that will out compete plant pathogens. In our greenhouse study, we found that Vvis and Viso infection rates were Verticillium wilt was substantially reduced by adding wood chips amended with PAM, with or without dairy or vegetable compost to soils containing high concentrations of *V. dahliae* biomass. Since as the MB/VB ratio increased as V_{vis} and V_{iso} rateings decreased, competition from indigenous microorganisms may be responsible.

In this experiment the PAM Superfloc® A836 copolymer was used to loosely adhere indigenous microorganisms to wood chips and potato roots. Changing the soil environment by adding green manure crops and nutrient relationships (Davis et al. 1996) has been shown to limit Verticillium wiltearly dying in potato. We found that soil amended with vegetable compost, but not with dairy compost, increased the MB/VB ratios and reduced *V. dahliae* infection rates. Simply adding vegetable compost or proper nutrient management may be able to reduce *V. dahliae* infection oin potato. However, amending the soil with wood chip-PAM or wood chip-PAM-vegetable compost mixture may further reduced *V. dahliae* infection.

A wood chip-PAM core was practical for a greenhouse study. When growing potatoes on a commercial basis, adding a wood chip-PAM core when planting would be impractical. A more practical method is might be to make a shallow trench adding the wood chip- PAM mixture in the same pass. Although field studies and economic evaluations are necessary, amending soil with wood chips+-PAM or wood chips+-PAM+-vegetable compost mixtures might be valuable methods to control some soilborne diseases. The use of wood chip+-PAM+-vegetable compost treatments may be a possible method to control soilborne diseases in high value crops. **This approach may also offer an alternative soil treatment regime to the use of methyl bromide fumigation which is being banned from commercial agriculture and which is primarily used in high value crops, for which our alternative may be economically practical. Some of these approaches are also likely compatible with organic production strategies, or could be modified to meet the criteria.**

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Table 1. Infection ratings, potato biomass, yield, active microbial and *Verticillium dahliae* biomass and the microbial biomass/*Verticillum dahliae* biomass ratio in soil and wood chip and polyacrylamide cores with vegetable and compost treatments.

				–Potato	Yieild--	Soil Microbial Biomass			*Verticillum dahliaedahaliae* biomass			
		Infection Rating		plant	tuber	Fungi	Bacteria	Total	Soil	Chips	MB/VB	
Treatment	Soil	V_{vis}	V_{iso}	$(g)g^{-1}$	$(g)g^{-1}$	----------μg C g^{-1} Soil----------					Soil	Core
Control	1	27.1b	0.89a	11.6ab	4.2b	1.6a	3.6b	5.2bc	0.169a	–	30.7c	–
	2	38.1ab	1.0a	13.9ab	5.0b	1.7a	3.4ab	4.1bc	0.085b	–	48.2c	–
	3	19.8bc	1.0a	14.1ab	6.9b	0.9a	1.1c	2.0c	0.076b	–	26.3c	–
Wood Chips + PAM	1	4.7e	0.25c	4.5b	1.0b	4.3a	6.3a	10.6a	0.055b	0.008c	192.7a	132.5a
	2	0.0e	0.57b	6.1b	1.1b	6.0a	3.7b	9.6ab	0.048b	0.005c	200.0a	192.0a
	3	0.0e	0.0c	4.4b	0.6b	5.3a	5.5a	10.8a	0.045b	0.057b	240.0a	189.0a
Wood Chips + Vegetable Compost + PAM	1	2.5e	0.16c	8.3b	4.3b	5.4a	4.7ab	10.1a	0.067b	0.060b	150.7a	168.3a
	2	2.7e	0.11c	10.0b	5.4b	5.2a	4.7c	9.9ab	0.053b	0.090a	186.8a	110.0ab
	3	0.0e	0.13c	12.5ab	8.3ab	3.4a	4.6c	8.0ab	0.053b	0.076b	150.9a	105.2b
Wood Chips Dairy Compost + PAM	1	17.2c	0.56b	11.0ab	6.2b	3.6a	4.8ab	8.4ab	0.080b	0.048b	105.0ab	175.0a
	2	15.6c	0.49b	15.8ab	10.0ab	2.9a	4.6ab	7.5b	0.072b	0.072ab	104.2ab	104.2b
	3	9.5d	0.44b	17.3a	11.9a	2.6a	3.7bc	7.3b	0.069b	0.068ab	105.7ab	107.3b
Vegetable Compost	1	9.4d	0.24c	7.3ab	2.8b	3.1a	3.4b	6.6bc	0.085b	–	77.7b	–
	2	15.2c	0.22c	9.8b	4.0b	2.5a	1.5c	3.7c	0.074b	–	50.0c	–
	3	8.6d	0.00d	10.6b	8.7ab	0.6a	1.4c	2.0c	0.047b	–	70.0b	–
Dairy Compost	1	24.4b	0.67a	7.9ab	0.5b	3.0a	4.8ab	7.8b	0.101b	–	77.2b	–
	2	60.3a	1.0a	8.3ab	1.7b	2.2a	2.6bc	4.8c	0.067b	–	71.6b	–
	3	53.4a	1.0a	12.0ab	4.1b	0.9a	1.7c	2.5c	0.050b	–	50.0c	–

a V_{vis} = visible Verticillium dahliae infection; percent of the plant with visible symptoms of *V. dahliae* infection
b V_{iso} = *Verticillium dahliae* infection as measured by isolation; graded as no isolation = 0, *V. dahliae* isolated = 1.
c MB/VB = microbial biomass in soil or core/*Verticillium dahliae* biomass in soil or core.
d In each column, values followed by the same letter are not significantly different as determined by the Least Square Means Test ($P \leq 0.05$) n=54.

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