Biocontrol Science and Technology (2000) 10, 677–686

Wood Chip-Polyacrylamide Medium for Biocontrol Bacteria Decreases *Verticillium dahliae* Infection on Potato

JAMES A. ENTRY,[1] CARL A. STRASBAUGH[2] AND R. E. SOJKA[3]

[1]*USDA Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 North, 3600 East, Kimberly, Idaho 83341, USA;* [2]*University of Idaho, Research and Extension Center, Kimberly, Idaho 83341, USA;* [3]*USDA Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 North, 3600 East, Kimberly, Idaho 83341, USA*

(*Received for publication 28 September 1999; revised manuscript accepted 1 May 2000*)

The lack of consistent success of biological control of soilborne plant pathogens may be due to the introduction of the organism into a foreign environment. We hypothesized that wood chip-polyacrylamide (PAM) cores surrounding host plant roots could alter the soil environment to favour growth of introduced biocontrol microorganisms, thereby reducing Verticillium dahliae *infection of potato (*Solanum tuberosum *L.) in a greenhouse. A 7 cm diameter × 15 cm deep hole (core) was drilled in the center of a 20 × 30 cm deep pot (1.9 kg) containing soil infested with* V. dahliae *inoculum. Cores were then filled with wood chip-PAM-biocontrol organism mixtures. Soils that had* Streptomyces lydicus *inoculated into wood chip-PAM cores had lower levels of* V. dahliae *symptoms (V_{vis}) and* V. dahliae *isolations (V_{iso}) than all other treatments in three soils.* V_{vis} *and* V_{iso} *on plants growing in soils amended with* S. lydicus *or* Pseudomona corrugata *inoculated into the soil itself (without wood chip-PAM cores) did not differ from soils that were unamended with these biocontrol organisms.* V. dahliae *biomass was lower in wood chip-PAM cores inoculated with* S. lydicus *than control or wood chip-PAM cores without biocontrol bacteria. Soils with wood chip-PAM cores inoculated with* S. lydicus *or* P. corrugata *generally had higher microbial biomass/*V. dahliae *biomass (MB/VB) ratios than control soils, or soils with* S. lydicus *or* P. corrugata *inoculated into the soil. Wood chip-PAM cores alone and wood chip-PAM cores inoculated with* S. lydicus *had higher MB/VB ratios than wood chip-PAM cores inoculated with* P. corrugata. V_{vis} *and* V_{iso} *were curvilinearly correlated with the MB/VB ratios in negative relationships, respectively* ($r^2 = 0.68$, $r^2 = 0.68$). *As the MB/VB ratio increased,* V_{vis} *and* V_{iso} *decreased. Although field studies and economic evaluations are necessary, amending soil with wood chips-PAM and a biocontrol bacterium may be a valuable method to increase the effectiveness of biocontrol organisms.*

Keywords: *biocontrol, polyacrylamide cores,* Verticillium dahliae, Streptomyces lydicus, Pseudomona corrugata

Correspondence to J. A. Entry. Tel: +1 208 423 6553; Fax: +1 208 423 6555; E-mail: jentry@kimberly.ars.pn.usbr.gov

ISSN 0958-3157 (print)/ISSN 1360-0478 (online)/00/050677-10

INTRODUCTION

Environmental concerns have prompted research into biocontrol of plant pathogens, but to date there are few practical alternatives to continued use of pesticides for many disease problems. *Verticillium* wilt of potato (*Solanum tuberosum* L.), caused by *Verticillium dahliae* (Kleb.), is a major factor limiting production. Treatment of soil with metam-sodium and long crop rotations are the only two practical approaches to reduce the density of *V. dahliae* inoculum in Idaho (Goth & Haynes, 2000; Guenther *et al.*, 1999). New potato varieties that are resistant to *V. dahliae* are currently being developed (Mohan *et al.*, 1992; Davis, 1994). Presently, proper nutrient regimes, crop rotation, irrigation management, and fumigation are being used to control the pathogen (Davis *et al.*, 1994a, b; James *et al.*, 1994). Since potato varieties respond to cultural management, research has focused on cultural methods to control *V. dahliae.*

Streptomyces lydicus (DeBoer) strain WYEC108 is used to control *Pythium ultimum* and *Rhizoctonia solani* infection in corn (*Zea mays* L.), cotton (*Gossypium mosseae* L.) and pea (*Lathyrus aphaca* L.) seed (Crawford *et al.*, 1993; Yuan & Crawford, 1995). The bacterium *Pseudomonas corrugata* has potential use as a biocontrol microorganism against fungal plant pathogens of several different crops including *Gaeumannomyces graminis* var *tritci* on wheat (*Triticum aestivum* L.), *Fusarium sambucinum, Helminthosporium solani, Phytophthora erythoseptica, P. infestans* and *Sclerotina sclerotiorum in vitro* (Ryder & Rovaria, 1993; Zhou & Paulitiz, 1993; Chun, 1996; Ristaino & Thomas, 1997).

The limited and inconsistent success of biocontrol organisms against soilborne plant pathogens may be due to the introduction of the biocontrol organism into an environment unsuitable to sustain large populations of the selected biocontrol organism (Powell & Faull, 1989; Renwich & Poole, 1989). The overriding difficulty seems to be the ability of the introduced microorganism to colonize and compete with established microflora in the soil. The biocontrol microbe is expected to survive and multiply, thereby out-competing or killing the pathogenic microorganism in a soil environment that was better suited to the pathogenic microorganism than the biocontrol microorganism (Powell & Faull, 1989; Renwick & Poole, 1989; Whipps, 1997). If the soil around the plant roots can be altered to favour the growth of biocontrol microorganisms relative to plant pathogens by amending the soil with non-toxic materials, the survival and proliferation of the biocontrol microorganism and thus effectiveness of biocontrol may be increased. In a greenhouse study, we sought to determine the efficacy of wood chip-polyacrylamide (PAM) cores to alter the soil environment around potato seed pieces to favour the biocontrol bacteria, *S. lydicus* WYEC108 and *P. corrugata*, thereby reducing *V. dahliae* infection of potato plants. A 7 cm diameter × 15 cm deep hole (core) was drilled in the center of the 20 × 30 cm deep pot (1.91 kg) containing soil *V. dahliae* inoculum. Cores were then filled with wood chip-PAM-biocontrol organism mixtures as described below. Potato seeds were then placed inside each core. Our hypothesis was that either the soil containing a high density of biocontrol microorganisms would protect roots from infection by *V. dahliae* by physically preventing growth of the pathogen, or it would alter the environment surrounding the roots making *V. dahliae* less competitive than the biocontrol bacteria.

MATERIALS AND METHODS

Experimental Design

The greenhouse experiment was arranged in a randomized block design using three different soil sources as main blocks (Kirk, 1982). Within each soil, treatments were: (1) soil unamended with wood chip-polyacrylamide (PAM) treatments (control); (2) soil amended with a core containing a wood chip-PAM mixture without biological control microorganisms; (3) soil amended with a wood chip-PAM core containing *S. lydicus* WYEC108; (4) soil amended with a wood chip-PAM core containing *P. corrugata*; (5) soil treated with *S. lydicus*

TABLE 1. Characteristics of soils used to determine effectiveness of biocontrol treatments for *V. dahliae* infection

Soil type	*V. dahliae* biomass (μg C g soil^{-1})	pH	Bulk density (g cm^{-3})	Water holding capacity (cm m^{-2})	Organic matter (g C kg soil^{-1})	Nutrients (g element kg soil^{-1})		
						NO_3	PO_4	K
Soil 1	0.43	8.2	1.08	20.0	13	14	12	148
Soil 2	0.36	8.0	1.17	18.0	9	23	5	62
Soil 3	0.30	7.8	1.02	24.0	16	16	10	120

All soils are classified as a Delco silt loam.

WYEC108 in talcum powder mixed into soil; (6) soil treated with *P. corrugata* in talcum powder mixed into soils. The experiment contained six treatments × three soils with different concentrations of *V. dahliae* colony forming units (cfu) g^{-1} soil × three replications. The entire experiment was repeated three times for a total of 54 experimental units.

Soil

The three soil sources, all with the same classification, used in this experiment were collected from the University of Idaho Research and Extension Center in Aberdeen, Idaho, USA, because they had extremely high concentrations of *V. dahliae* infection rates on potato in the last several growing seasons. All three soil sources are characterized as a Declo silt loam (Azad *et al.*, 1985). Detailed soil characteristics are summarized in Table 1. Prior to collection, the soils had been planted to a variety of crops for more than 70 years, including barley (*Horedum pusillum* Nutall.), wheat (*Triticum aesivum* L.) and potato; these soils have a long history of Verticillium wilt in potato crops (Davis *et al.*, 1996).

Polyacrylamide Application

The polyacrylamide (PAM) copolymer used was a dry granular material having an approximate molecular weight of 12–15 g mole^{-1} (CYTEC Industries of Wayne, NJ and marketed under the trade name Superfloc A 836). This PAM formulation had a negative charge density of approximately 18%, achieved by substitution of a sodium formate group for one of every five amide groups, with the negative charge resulting from disassociation of sodium when the PAM was dissolved in water. This commercial PAM product also has approximately 15% urea by weight linked to the molecule. PAM is a nontoxic polymer (Shanker *et al.*, 1990; Barvenik, 1994) that will degrade at approximately 10%/year when mixed into soil (Abdelmagid & Tabatabai, 1982).

S. lydicus and *P. corrugata* Preparation

The wood chip-PAM-*S. lydicus* cores were made with strain WYEC108, obtained from Dr Don Crawford, University of Idaho. Cultures were maintained on 1% (w/v) potato-dextrose agar (Yuan & Crawford, 1995). A 0.1% (w/v) potato-dextrose broth was inoculated with 7-day old cultures of *S. lydicus*. Cultures were stirred and maintained at 23°C, and contained 2×10^5 cells ml^{-1} after 6 days as determined by direct counting, and staining with iodonitrotetrazolium (INT) as described below.

The wood chip-PAM-*P. corrugata* cores were made with *P. corrugata*, obtained from Dr Wesley W. C. Chun, University of Idaho. Cultures were maintained on a medium containing triphenyltetrazolium chloride (Kelman, 1988). Four-day old cultures were inoculated to a minimal medium containing 40 mM-K_2HPO_4, 14 mM-KH_2PO_4, 0.4 mM-$MgSO_4 \cdot 7H_2O$, 7.6 mM-$(NH_4)_2SO_4$ and 1.0% dextrose l^{-1}. Cultures were stirred and maintained at 23°C and contained 1.4×10^6 cells ml^{-1} after 6 days. *S. lydicus* strain WYEC108 in talc powder (lot E9706018) was obtained from Innovative Biosystems Inc. (Moscow, Idaho, USA),

contained 1×10^8 cells g^{-1} as determined by direct counting and staining with INT as described below. *P. corrugata* in talc powder, obtained from Dr Wesley Chun, contained 1×10^6 cells g^{-1}.

Treatments

Soil (3 kg) was placed in 1.9 l black plastic pots and saturated to field capacity with well water. Pots receiving wood chip-PAM or wood chip-PAM with or without biocontrol microorganisms had a 7 cm diameter × 15 cm deep (350 ml) hole (core) drilled in the center of the soil. Cores were then filled with wood chip-PAM-biocontrol organism mixtures as described below. The wood chip-PAM-treatment without a biocontrol organism consisted of cores made of a mixture of 7 g of Ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws.) wood chips, 1.0 ml of Arnon's nutrient solution (Arnon & Hoagland, 1940) and 0.55 g of PAM. Cores consisting of the wood chip-PAM-*S. lydicus* or *P. corrugata* treatment, contained the above mixture with 10 ml of the stock solution (described above) of *S. lydicus* or *P. corrugata*. For the soil-*S. lydicus* in talc powder treatment, 3 kg of soil was mixed with 1 g of *S. lydicus* in talc. For the soil-*P. corrugata* in talc powder treatment, 2.9 kg of soil was mixed with 100 g of *P. corrugata* in talc. All pots were then watered with well water to field capacity. The bulk density of wood chip-PAM cores averaged 0.11 g cm^{-3}.

Growing Conditions

Russet Burbank tubers were cut into 35 ± 5 g seed pieces and planted 4 cm deep in each pot. In soils with a wood chip-PAM or wood chip-PAM-biocontrol microorganism core, the potato seed piece was planted in the center of the core. Potato plants were grown for 3 months in a greenhouse that was maintained at 25 ± 3°C. Plants were watered to field capacity with Arnon's nutrient solution (Arnon & Hoagland, 1940) each week over the course of the experiment. During that time, the seedlings were exposed to light having a photosynthetic active radiation of 400–700 μmol m^{-2} S^{-1} and a 14–16 h photoperiod.

Harvesting and Disease Assessment

At harvest, plants were removed from the pots and separated into roots, shoots, and tubers. Roots and tubers were washed in well water and then distilled deionized water until all visible soil particles were removed. Verticillium symptoms, which were 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* using methods described in Strausbaugh (1993). *V. dahliae* was isolated from potato plants by slicing a 10 mm 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 (Difco Laboratories, Detroit, Michigan, USA). Colonies of *V. dahliae* with vertically branched condiophores and condia typical of *V. dahliae* formed in and around the vascular tissue in the segments of symptomatic plants (Strausbaugh, 1993). 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}) (plants were given a score of 1 if *V. dahliae* was isolated from the stem and 0 if *V. dahliae* was not isolated). All root, shoot, and tuber tissue was then dried at 80°C for 48 h and weighed.

Estimation of *V. dahliae* in Soil

At harvest, soil, cores and plant roots from each pot were separated. Soil and wood chip-PAM or wood chip-PAM-biocontrol bacteria cores from each pot were collected at harvest and analyzed separately for *V. dahliae*, and other active fungal and bacterial biomass. Soil and core material was collected, stored in air-tight and moisture-tight plastic freezer bags at 4°C and at moisture conditions at harvest. Soil was prepared for estimation of *V. dahliae* and microbial biomass within 24 h of collection to minimize the effects of storage on

microbial activity (West *et al.*, 1986). *V. dahliae* colony forming units (cfu) 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* cfu 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. Previous studies (Jenkinson & Ladd, 1981; Lodge & Ingham, 1991) have found that a carbon to volume conversion factor of 120 μg carbon mm^{-3}, a 1.1 g cm^{-3} wet density, 20% dry matter content, and a 41% carbon content in fungi is appropriate for hyphal length to fungal biomass. These estimations were used to convert *V. dahliae* colonies to *V. dahliae* biomass.

Microbial Biomass Measurements

Active bacterial biomass and fungal biomass for each soil and wood chip core at harvest were estimated using methods described by Ingham and Klein (1984). Active fungi were estimated by taking a 1 ml water sample which was diluted in 9 ml of a phosphate buffer (pH 6.0) and shaken at approximately 120 rpm for 5 min. A 1 ml sample was removed and stained with 1 ml of a 20 μg ml^{-1} fluorescein diacetate (FDA) solution in a 0.2 M-phosphate buffer for 3 min. One ml of 1.5% agar in a 0.1 M-phosphate buffer (pH 9.5) was added to the FDA suspension. The sample was mixed and a sample placed on a microscope slide containing a cavity of known volume (Ingham & Klein, 1984). Immediately after preparation, slides were examined for FDA-stained hyphal length by epifluorescent microscopy. Total fungal biomass was estimated by measuring the length and diameter of hyphae in 3 to 60 fields with phase-contrast microscopy. Three slides were evaluated from each sample and 10 fields/slide were evaluated with phase contrast microscopy at X1600 magnification for total hyphal length, and three transects were evaluated for FDA-stained (active) hyphal length.

Iodonitrotetrazolium (INT) stain was used for counting active bacteria (Stamatiadis *et al.*, 1990). A 1 ml sample of initial soil suspension was diluted to a final dilution in a 0.2 mg soil in 4 ml buffer. The suspension was incubated with 4 ml of filtered INT buffer for 60 min in the dark at 20°C. Total bacteria ml^{-1} of water were estimated from the mean number of bacteria (fluorescent and non-fluorescent bacteria), their average diameter and length/field. Three slides were evaluated for each sample and 10 fields/slide were evaluated using epifluorescent oil-immersion microscopy to determine numbers, and size of fluorescent and total bacteria (Lodge & Ingham, 1991).

Bacterial biomass was computed from the numbers of active and total bacteria, and active and total fungal biomass were determined from hyphal length. Bacterial biomass was computed from the number of soil bacteria g^{-1} of soil by considering that the bacterial spheres were 1 μm in diameter (Jenkinson & Ladd, 1981). Active and total fungal biomass were computed by considering average hyphal diameter to be 1 μm in diameter, and then multiplying by the length of observed hyphae (Jenkinson & Ladd, 1981). A carbon to volume conversion factor of 120 μg^{-1} C mm^{-3} was used for both bacteria and fungi, assuming 1.1 g cm^{-3} wet density, 20% dry matter content, and a 0.41 carbon content in the bacterium or fungus (Jenkinson & Ladd, 1981). Microbial biomass is the sum of active fungal and bacterial biomass. Microbial biomass/*V. dahliae* biomass (MB/VB) was calculated by dividing microbial biomass by *V. dahliae* biomass.

Statistical Analysis

All dependent variables were tested for normality with univariate procedures. Data were then analyzed by means of two way analysis of variance (ANOVA) procedures for a randomized block design with Statistical Analysis Systems (SAS, 1996). Residuals were equally distributed with constant variances. Differences were judged significant at $P = 0.05$, as determined by the Least Squares Means test. Correlations were analyzed with *V. dahliae*

infection rating and presence or absence of *V. dahliae* in tissue as dependent (x) variables and *V. dahliae* biomass, microbial biomass or MB/VB as independent (y) variables.

RESULTS

Since ANOVA indicated that treatment × soil interactions for *V. dahliae* (V_{vis}) and isolation (V_{iso}), plant weight, active bacterial and fungal biomass, total microbial biomass, *V. dahliae* biomass and microbial biomass/*V. dahliae* biomass (MB/VB) ratios were significant, results are described with regard to treatments in each soil. Soils with wood chip-PAM cores inoculated with *S. lydicus* had lower V_{vis} and V_{iso} ratings than all other treatments in the three soil sources (Table 2). Soils with wood chip-PAM without a biocontrol microorganism had lower V_{vis} ratings in soil 3 than in soils 1 and 2. V_{vis} and V_{iso} ratings in soils with wood chip-PAM cores inoculated with *P. corrugata*, and soils with *S. lydicus* or *P. corrugata* inoculated into the soil (without wood chip cores) did not differ from the control treatment. Although there were statistically significant differences, plant biomass and yield showed to patterns with regard to treatment or soil source with the control tending to be lower than the other treatments, especially the wood chip-PAM cores inoculated with *S. lydicus*. Active fungal biomass did not differ with treatment or soil source. Although there were statistically significant differences, active bacterial and total microbial biomass showed no patterns with regard to treatment or soil source. *V. dahliae* biomass did not significantly differ with treatments or soils. *V. dahliae* biomass was higher in wood chip-PAM cores inoculated with *S. lydicus* than control, wood chip-PAM cores inoculated with *P. corrugata* and wood chip-PAM cores without biocontrol microorganisms. Soils with wood chip-PAM cores generally had higher MB/VB ratios in soil than control soils, or soils inoculated with *S. lydicus* or *P. corrugata*. Wood chip-PAM cores inoculated with *P. corrugata* had lower MB/VB ratios than the other PAM treatments. V_{vis} and V_{iso} correlated with the MB/VB ratios in quadratic negative relationships ($r^2 = 0.68$, $r^2 = 0.68$), respectively. As the MB/VB ratio increased V_{vis} and V_{iso} decreased.

DISCUSSION

The major challenge facing commercial production of biocontrol agents of plant pathogens is to obtain effective, reproducible, and economically and environmentally acceptable disease control. The lack of disease control by organic treatments and biocontrol microorganisms is often ascribed to environmental factors, which are often difficult to define (Renwick & Poole, 1989). In field conditions, fluctuations in moisture, temperature and nutrient and carbon availability can play a role in limiting the effectiveness of the biocontrol treatment. Various communities of indigenous microorganisms in any particular soil have adapted to the specific environmental conditions to which the soil has been subjected. One method of controlling plant pathogens in a soil environment may be to change a soil in and around the root zone to favour indigenous microorganisms that out-compete plant pathogens. If potatoes are planted into a wood chip-PAM-*S. lydicus* core, *V. dahliae* infection may be substantially reduced. As the MB/VB ratio increased infection of *V. dahliae* (as measured by visible symptoms and isolations from the stem) decreased, the competition from indigenous microorganisms may be responsible.

Polyacrylamides are polymers made up of many repeating subunits (monomers). As with all polymers, the properties of PAMs are very dependent on the size of the polymer. Several studies found that PAM degradation in soil is fairly rapid (Lande *et al.*, 1979; Shanker *et al.*, 1990; Kay-Shoemake *et al.*, 1998a). Enrichment cultures showed that soil microorganisms are capable of utilizing PAM as a sole source of nitrogen, but not carbon (Kay-Shoemake *et al.*, 1998b). The polyacrylamide Superfloc A836 copolymer is a nontoxic polymer (Barvenik, 1994; Seybold, 1994) that will degrade at approximately 10%/year (Abdelmadgid & Tabatabai, 1982). Microbial degradation of PAM on a particular site likely depends on

TABLE 2 Infection ratings, potato biomass yield, active microbial biomass, *V. dahliae* biomass and the microbial biomass/*V. dahliae* biomass ratio in soil and wood chip cores with the biocontrol bacteria *S. lydicus* and *P. corrugata*.

Treatment	Soil	Infection rating		Plant		Soil microbial biomass (μg C g^{-1} soil)			Verticillium biomass (μg C g^{-1} soil)		MB/VB[c]	
		V_{vis}[a]	V_{iso}[b]	Biomass	Yield	Fungal	Bacterial	Total	Soil	Chips	Soil	Chips
Control	1	39a[d]	1.0a	10.0ab	2.6c	0.7c	1.1c	1.9c	0.032a	—[e]	59c	—
	2	32a	0.9a	9.8b	3.1c	0.4c	2.0c	2.4c	0.031a	—	77c	—
	3	31a	0.9a	9.0b	3.7c	0.4c	2.9bc	3.3c	0.041a	—	80c	
Wood chips + PAM	1	29a	0.4a	7.5b	2.9c	3.8a	1.9c	5.6b	0.021a	0.031a	181b	382a
	2	34a	0.3b	11.0ab	5.2bc	2.2ab	1.9c	4.0bc	0.028a	0.067a	143bc	128b
	3	26a	0.3b	11.2ab	6.0b	4.9a	5.3a	10.2a	0.046a	0.031a	222b	495a
Wood chips + PAM *S. lydicus*	1	0c	0.1c	11.4ab	5.5bc	3.8a	3.6b	7.4a	0.041a	0.08b	180b	306a
	2	1c	0.0c	18.9a	12.6a	3.5a	3.1b	6.6b	0.043a	0.11b	153ab	139b
	3	1c	0.0c	12.7ab	8.1b	2.7ab	3.2b	5.9b	0.141a	0.08b	144ab	215ab
Wood chips + PAM *P. corrugata*	1	27a	0.7a	11.0ab	5.4bc	3.4a	6.0a	9.4a	0.030a	0.37a	313a	20c
	2	29a	0.7a	15.6a	9.1ab	1.0b	1.6c	2.6c	0.024a	0.15b	108c	27c
	3	21ab	0.7a	17.9a	10.9a	1.8b	4.9a	6.7ab	0.041a	0.19b	148bc	30c
S. lydicus	1	23ab	0.8a	8.2b	3.4c	0.8bc	3.4b	4.2bc	0.041a	—	102c	—
	2	21ab	0.8a	11.0ab	4.1c	0.5c	3.8b	4.3bc	0.041a	—	93c	—
	3	22ab	0.8a	10.2ab	4.0bc	2.7ab	3.1b	2.9c	0.027a	—	74c	—
P. corrugata	1	28a	0.9a	8.2b	2.8bc	0.7bc	3.2b	3.9c	0.040a	—	98c	—
	2	29a	0.6ab	13.3a	7.2b	2.2ab	2.7bc	4.9bc	0.042a	—	116bc	—
	3	26a	0.9a	7.6b	1.9c	0.8bc	2.2c	3.0c	0.036a	—	83c	—

[a] V_{vis} = visible *V. dahliae* infection; per cent of the plant with visible symptoms of *V. dahliae* infection.
[b] V_{iso} = *V. dahliae* infection as measured by isolation; graded as no isolation = 0, *V. dahliae* isolated = 1.
[c] MB/VB = microbial biomass in soil or core/*V. 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 ($P \leq 0.05$) $n = 54$.
[e] Indicates that no cores were present. No cores were present in the control, *S. lydicus* without wood chip PAM treatments and *P. corrugata* without wood chip PAM treatments.

soil moisture, temperature, carbon and nutrient status as well as the amount and type of PAM application.

Changing the soil environment by simply adding *S. lydicus* or *P. corrugata* had no significant effect on the MB/VB ratio and/or V_{vis} and V_{iso} ratings. Planting potato seed pieces in a wood chip-PAM alone core significantly reduced V_{vis} rating in soil 3 and the V_{iso} rating in soils 2 and 3. The wood chip-PAM alone core reduced *V. dahliae* infection by providing a medium that was less favourable for growth of the pathogen. This additional measure of control may allow growers to grow potatoes with reduced amount of rotations with other crops. Although adding a wood chip-PAM core was practical for a greenhouse study it might not be practical when growing crops on a commercial basis. A cost effective method may be to add the wood chip-PAM mixture during planting. PAM is currently used for erosion control in irrigated crop land and, at a cost of US$7.50–12.50 per ha, it is extremely economical. Although field studies and economic evaluations are necessary, amending soil with wood chips-PAM or wood chips-PAM mixture inoculated with a biocontrol microorganism may be a valuable method to control soilborne diseases.

The use of wood chip-PAM-biocontrol cores may be one way to control soilborne plant pathogens in high value crops. Methyl bromide is a widely used fumigant in the commercial production of strawberries (*Fragaria chiloensis* D.), tomatoes (*Lycopersicon esculentum* L.), peppers (*Capsicum* spp.), melons (*Cucumis melo* L.), cucumbers (*Cucurbita sativus* and *Cucurbita anguria* L.), and various ornamentals (Ragsdale & Wheeler, 1995; Ristaino & Thomas, 1997). Evidence has accumulated that methyl bromide is implicated in the destruction of stratospheric ozone (WMO, 1994), and the compound is scheduled to be phased out of production and use in the near future (USEPA, 1993). Other currently available fumigants such as methyl iodide, Vorlex (1,3-dichloropropene, and methyl isocyanate), Vapam (metam sodium), Telone ([1,3]-dichloropropene) and Basmid (dazomet) have an increased cost, reduced efficacy, and higher toxicity compared to methyl bromide (Ragsdale & Wheeler, 1995; Ristaniao & Thomas, 1997). The National Pesticide Impact Assessment Program estimated that the ban on methyl bromide will result in economic losses to US producers from US$800 to 900 million, the greatest losses occurring in tomato and strawberry production (USDA, 1993). This study provides evidence that changing the soil environment to favour biocontrol of soil organisms to control soil pathogens may give producers an alternative to soil fumigants.

REFERENCES

ABDELEMAGID, H.M. & TABATABAI, M.A. (1982) Decomposition of acrylamide in soils. *Journal of Environmental Quality* **11,** 701–704.

ARNON, D.I. & HOAGLAND, D.R. (1940) Crop production in artificial culture solutions, and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. *Soil Science* **5,** 463–485.

AZAD, H.R., DAVIS, J.R., SCHNATHOST, W.C. & KADO, C.I. (1985) Relationships between rhizoplane and rhizosphere bacteria and verticillium wilt resistance in potato. *Archives of Microbiology* **140,** 347–351.

BARVENIK, F.W. (1994) Polyacrylamide characteristics related to soil applications. *Soil Science* **158,** 235–243.

BUTTERFIELD, E.J. & DEVAY, J.E. (1977) Reassessment of soil assays for *Verticillium dahliae. Phytopathology* **67,** 1073–1078.

CHUN, W. (1996) The use of phytopathogenic bacteria as biological control agents. *Recent Reviews and Development in Plant Pathology* **1,** 113–121.

CRAWFORD, D.L., LYNCH, J.M., WHIPPS, J.M. & OUSLEY, M.A. (1993) Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Applied and Environmental Microbiology* **59,** 3899–3905.

DAVIS, J.R. (1994) *Verticillium Wilt of Potato.* University of Idaho, College of Agriculture Current Information Series Bulletin 977.

DAVIS, J.R., HUSIMAN, O.C., WESTERMAN, D.T., EVERSON, D.O., SORENSEN, L.H., SCHNIDER, A.T. & STARK, J.C. (1994a) The influence of cover crops on the suppression of Verticillium wilt of potato, in *Advances*

in Potato Pest Biology and Management (ZEHENDER, G.W., POWLSEN, M.L., JANNSON, R.J. & RAMAN, K.V., Eds), American Phytopathological Society Press, St Paul, MN, USA, pp. 332–341.

DAVIS, J.R., PAVIK, J.J., CORSINI, D.L., SORENSEN, L.H., SCHNIDER, A.T., EVERSON, D.O., WESTERMAN, D.T. & HUSIMAN, O.C. (1994b) Influence of continuous cropping of several potato clones on the epidemiology of verticillium wilt of potato. *Phytopathology* **73,** 207–214.

DAVIS, J.R., HUSIMAN, O.C., WESTERMAN, D.T., EVERSON, D.O., SORENSEN, L.H. & SCHNIDER, A.T. (1996) Effects of green manures on verticillium wilt of potato. *Phytopathology* **86,** 444–453.

GOTH, R.W. & HAYNES, K.G. (2000) Evaluation of potato clones for severity of Verticillium wilt, yield and specific gravity in Maine. *American Journal of Potato Research* **77**, 89–94.

GUENTHER, J.F., WIESE, M.V., PAVLISTA, A.D., SIECZKA, J.B. & WYMAN, J. (1999) Assessment of pesticide use in the US potato industry. *American Journal of Potato Research* **76**, 25–29.

INGHAM, E.R. & KLEIN, D.A. (1984) Soil fungi relationships between hyphal activity and staining with fluorescein diacetate. *Soil Biology and Biochemistry* **16,** 273–278.

JAMES, D.W., HURST, R.L., WESTERMAN, D.T. & TINDALL, T.A. (1994) Nitrogen and potassium fertilization of potatoes: Evaluating nutrient interactions in petioles with response surfaces. *American Potato Journal* **71,** 249–265.

JENKINSON, D.S. & LADD, J.M. (1981) Microbial biomass in soil: Measurement and turnover, in *Soil Biochemistry, Vol. 5* (PAUL, E.A. & LADD, J.N., Eds), Marcel Dekker, New York, USA, pp. 415–471.

KAY-SCHOEMAKE, J.L., WATWOOD, M.E., LENTZ, R.D. & SOJKA, R.E. (1998a) Polyacrylamide as an organic nitrogen source for soil microorganisms with potential effects on inorganic soil nitrogen in agricultural soil. *Soil Biology and Biochemistry* **30,** 1045–1052.

KAY-SHOEMAKE, J.L., WATWOOD, M.E., LENTZ, R.D. & SOJKA, R.E. (1998b) Polyacrylamide as a substrate for microbial amidase in culture and in soil. *Soil Biology and Biochemistry* **30,** 1647–1654.

KELMAN, A. (1988) The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. *Phytopathology* **44,** 693–695.

KIRK, R.E. (1982) *Experimental Design: Procedures for the Behavioral Sciences*, 2nd edn, Brooks Cole, Belmont, CA, USA, 546 pp.

LANDE, S.S., BOSCH, S.J. & HOWARD, P.H. (1979) Degradation and leaching of acrylamide in soil. *Journal of Environmental Quality* **8,** 133–137.

LODGE, D.J. & INGHAM, E.R. (1991) A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. *Agriculture, Ecosystems and Environment* **5,** 31–37.

MOHAN, S.K., DAVIS, J.R., SORENSEN, J.R. & SCHNIDER, A.T. (1992) Infection of aerial parts of potato plants by *Colletotrichum coccodes* and its effects on premature death and yield. *American Potato Journal* **69,** 547–559.

POWELL, K.A. & FAULL, J.L. (1989) Commercial approaches to the use of biological control agents, in *Biotechnology of Fungi for Improving Plant Growth* (WHIPPS, J.M. & LUMSDEN, R.D., Eds), Cambridge University Press, New York, USA, pp. 259–275.

RAGSDALE, N.N. & WHEELER, W.B. (1995) Methyl bromide: risks, benefits and current status in pest control. *Reviews of Pesticide Toxicology* **3,** 21–44.

RENWICK, A. & POOLE, N. (1989) The environmental challenge to biological control of plant pathogens, in *Biotechnology of Fungi for Improving Plant Growth* (WHIPPS, J.M. & LUMSDEN, R.D., Eds), Cambridge University Press, New York, USA, pp. 277–291.

RISTANIO, J.B. & THOMAS, W. (1997) Agriculture, methyl bromide and the ozone hole. Can we fill the gaps. *Plant Disease* **81,** 964–977.

RYDER, M.H. & ROVARIA, A.D. (1993) Biological control of take-all glass grown wheat using strains of *Pseudomonas corrugata* isolated from field soil. *Soil Biology and Biochemistry* **25,** 311–320.

SAS INSTITUTE INC. (1996) *SAS User's Guide to Statistics*, SAS Institute Inc., Cary, NC, USA.

SEYBOLD, C.A. (1994) Polyacrylamide review: soil conditioning and environmental fate. *Communications in Soil Science and Plant Analysis* **25,** 2171–2185.

SHANKER, R., RAMAKRISNA, C. & SETH, P.K. (1990) Microbial degradation of acrylamide monomer. *Archives of Microbiology* **154,** 192–198.

STAMATIADIS, S., DORAN, J.W. & INGHAM, E.R. (1990) Use of staining inhibitors to separate fungal and bacterial activity in soil. *Soil Biology and Biochemistry* **22,** 81–88.

STRAUSBAUGH, C.A. (1993) Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Phytopathology* **83,** 1253–1258.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (1993) 40 CFR, part 82. *Protection of Stratospheric Ozone: Final Rule.* Federal Register, v **58,** no. 236 Dec 10, 1993. 65018–65062.

UNITED STATES DEPARTMENT OF AGRICULTURE (1993) *The Biologic and Economic Assessment of Methyl Bromide*, National Agricultural Pesticide Assessment Program, US Department of Agriculture Report, 1993.

WORLD METEOROLOGICAL ORGANIZATION (1994) *Scientific Assessment of Ozone Depletion: 1994.* Global ozone research and monitoring. Project No. 37. Geneva, Switzerland.

WEST, A.W., ROSS, D.J. & COWLING, J.C. (1986) Changes in microbial C, N, P, and ATP contents, numbers and respiration on storage of soil. *Soil Biology and Biochemistry* **18,** 141–148.

WHIPPS, J.M. (1997) Ecological considerations involved in commercial development of biological control agents for soil-borne diseases, in *Modern Soil Microbiology* (VAN ELAS, J.D., TREVORS, J.T. & WELLINGTON, E.M.H., Eds), Marcel Dekker, New York, USA, pp. 525–546.

YUAN, W.M. & CRAWFORD, D.L. (1995) Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Applied and Environmental Microbiology* **61,** 3119–3128.

ZHOU, T. & PAULITIZ, T.C. (1993) In vitro and in vivo effects of *Pseudomonas* spp. on *Pythium aphanidermatum*: zoospore behavior in exudates and on the rhizoplane of bacteria-treated cucumber roots. *Phytopathology* **83,** 872–876.