Mechanism of Action and Efficacy of Seed Meal-Induced Pathogen Suppression Differ in a Brassicaceae Species and Time-Dependent Manner

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ABSTRACT

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The effect of seed meals derived from *Brassica juncea*, *B. napus*, or *Sinapis alba* on suppression of soilborne pathogens inciting replant disease of apple was evaluated in greenhouse trials. Regardless of plant source, seed meal amendment significantly improved apple growth in all orchard soils; however, relative differences in pathogen suppression were observed. All seed meals suppressed root infection by native *Rhizoctonia* spp. and an introduced isolate of *Rhizoctonia solani* AG-5, though *B. juncea* seed meal often generated a lower level of disease control relative to other seed meal types. When introduction of the pathogen was delayed until 4 to 8 weeks post seed meal amendment, disease suppression was associated with proliferation of resident *Streptomyces* spp. and not qualitative or quantitative attributes of seed meal glucosinolate content. Using the same experimental system, when soils were pasteurized prior to pathogen infestation, control of *R. solani* was eliminated regardless of seed meal type. In the case of *B. juncea* seed meal amendment, the mechanism of *R. solani* suppression varied in a temporal manner, which initially was associated with the generation of allylisothiocyanate and was not affected by soil pasteurization. Among those tested, only *B. juncea* seed meal did not stimulate orchard soil populations of *Pythium* spp. and infection of apple roots by these oomycetes. Although application of *B. napus* seed meal alone consistently induced an increase in *Pythium* spp. populations, no significant increase in *Pythium* spp. populations was observed in response to a composite *B. juncea* and *B. napus* seed meal amendment. Suppression of soil populations and root infestation by *Pratylenchus* spp. was dependent upon seed meal type, with only *B. juncea* providing sustained nematode control. Collectively, these studies suggest that use of a composite *B. juncea* and *B. napus* seed meal mixture can provide superior control of the pathogen complex inciting apple replant disease relative to either seed meal used alone.

Biologically based treatments such as the use of soil organic-residue amendments have been promoted as alternatives to the use of broad-spectrum biocides for the management of soilborne plant pathogens (16,30). Although such an approach has exhibited some practicality in certain environments, such as container-based or greenhouse production systems (27), its application in large-scale soil-based systems has failed to attain a level of efficacy and consistency required for extensive adoption. The production and yield of various bioactive chemistries, including isothiocyanates (ITCs), has encouraged studies exploring the pest control efficacy of Brassicaceae plant residues applied either through soil incorporation of a cover crop or direct amendment of plant residues. ITCs have a broad spectrum of biocidal activity; therefore, investigators have focused on the use of Brassicaceae plant residues as a "biofumigant," where incorporation of plant residue into soil ultimately results in the release of active glucosinolate hydrolysis products (1,6,15). However, a growing body of evidence suggests that certain of these plant residues may operate in the suppression of fungal pathogens via a different, as yet unidentified, mechanism. For example, reports exist of the effective use of *Brassica napus* residues to control soilborne plant pathogens (11,24,25), even though separate reports suggest that these plant residues yield ITCs having relatively low antimicrobial activity (19). Potter et al. (31) observed no relationship between total glucosinolate content or any individual glucosinolate in leaf tissues from different *Brassica* spp. applied as an amendment and suppression of the lesion nematode *Pratylenchus neglectus*. Likewise, the protective effect of Brassicaceae residues against fungal infection of pea plants was found to increase for weeks after ITCs had been lost from the soil by either volatilization or microbial degradation (17,29,35).

Findings from our previous studies demonstrated that attributes other than glucosinolate hydrolysis products contribute to the suppression of certain apple root pathogens in response to *B. napus* seed meal (rapeseed meal [RSM]) amendment (10,11). Control of *Rhizoctonia solani* and *P. penetrans* was obtained via the incorporation of RSM regardless of the glucosinolate content of the amendment (24). RSM is a high-nitrogen-containing product, and suppression of lesion nematodes may be attributed to the often-cited nematicidal or nematistatic effect of nitrogenous amendments (28,32). However, RSM-induced control of *R. solani* does not appear to operate via chemical inhibition of hyphal growth in soil (11) but, rather, through an influence on the resident soil microbial community, as evidenced by the fact that pasteurization of RSM-amended soil eliminated control of apple root infection by an introduced isolate of this fungal pathogen (10). The biological factor mediating disease control apparently varies in a temporal manner. Experimental inhibition of nitrification and the associated emission of nitric oxide (NO) limited disease control immediately after application of the amendment, but did not impact the efficacy of RSM when introduction of the pathogen

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was delayed until well beyond the peak period of NO release (10).

In Washington, apple replant disease is incited by a biological consortium of fungal pathogens and plant-parasitic nematodes (21). Effective use of an individual organic amendment for the control of such a diverse biological complex seems improbable. Although RSM amendment can provide significant control of replant disease in field trials, a post plant application of mefenoxam was required due to the stimulatory impact of the amendment on populations of *Pythium* spp. and infection of apple roots by these oomycetes (25). Given the breadth of glucosinolates produced by Brassicaceae plant species and the corresponding biological activity of the resulting ITCs (6), it is likely that an alternative seed meal exists which will not elicit the stimulatory effect on *Pythium* populations observed in response to RSM amendment.

The goals of the current study were to (i) investigate the potential of various Brassicaceaous seed meal amendments, or combinations thereof, to suppress multiple elements of the biological complex that incites apple replant disease; (ii) examine and assess whether disease control elicited via these seed meals differed mechanistically; and (iii) determine the temporal nature of disease control yielded through the use of *B. juncea* seed meal, which generates a biologically active volatile isothiocyanate (1,6,20).

MATERIALS AND METHODS

Orchard soils. Soils utilized in these studies were collected from the Columbia View Experimental (CV) orchard, Orondo, WA; the Wenatchee Valley College Research and Demonstration (WVC) orchard, East Wenatchee, WA; and a commercial (GC) orchard, Manson, WA. Characteristics of these soils, both physical and biological, have been described (21,22,24). Soil pH ranged from 6.9 (WVC) to 7.1 (CV) (Cascade Analytical, Wenatchee, WA). Disease development at the CV and WVC orchards has been attributed to the activity of a fungal complex whereas, at the GC orchard, the lesion nematode *P. penetrans* contributed to growth suppression in concert with the same fungal complex. Soils were collected from the root zone of established trees at each site in October 2003 and October 2004 in the manner previously described (24).

Seed meals. Seed meals used in these studies were obtained from *B. napus* cv. Dwarf Essex (DE; Montana Specialty Mills, Great Falls, MT), *B. napus* cv. Athena (AT) (4), *B. juncea* cv. Pacific Gold (PG) (3), and *Sinapis alba* cv. IdaGold (IG) (2). Seed meals varied in glucosinolate profile and content. *B. napus* DE (21.8 μmol g^{-1} of defatted seed meal) and AT (25.4 μmol g^{-1} of defatted seed meal) possessed the lowest content, of which 2-hydroxy-3-butenyl and 4-pentenyl, respectively, were the dominant gluocosinolates. *S. alba* IG and *B. juncea* PG both possessed higher glucosinolate levels, 244 and 303 μmol g^{-1} of defatted seed meal, respectively. 2-Propenyl (allyl) composed >99% of the glucosinolate contained in *B. juncea* PG whereas, for *S. alba* IG, *p*-hydroxylbenzyl glucosinolate represented >95% of the total content. Content of selected minerals were determined for each seed meal (Soiltest Farm Consultants, Inc., Moses Lake, WA). Mineral content for seed meals were as follows; nitrogen, 5.57 to 6.84%; phosphorous, 1.21 to 1.39%; potassium, 1.11 to 1.50%; and sulfur, 0.86 to 1.59%.

Effect of seed meals on growth of apple and disease development. Seed meal was incorporated into soils at a concentration of 0.5% (vol/vol). Soils that were either nontreated (control) or amended with seed meal were decanted into 3.8-liter plastic pots and incubated in the greenhouse for 8 weeks at 22 ± 3°C. This delay between soil amendment and planting was established to circumvent potential phytotoxic effects from volatile allelochemicals produced as a result of glucosinolate hydrolysis. In addition, this protocol allowed for sufficient amplification of resident *Streptomyces* spp., a community previously shown to be associated with pathogen suppression (10,11). Because seed meals contain significant, though variable, additions of plant-available N, 100 ml of Hoagland solution no. 1 (macronutrients only), which supplies N only in the nitrate form, was applied to each control pot. At completion of the incubation period, 8-week-old Gala apple seedlings were planted into soils with five seedlings per pot and four replicate pots per treatment. Plants were harvested after 12 to 15 weeks of growth in the greenhouse. At harvest, seedling root systems were washed under a stream of tap water, and plant height, shoot weight, and root weight were determined. Relative colonization of Gala seedling roots by *Pythium* and *Rhizoctonia* spp. was determined as previously described (11).

Among orchard soils employed in this study, GC is the sole soil possessing significant numbers of the lesion nematode *P. penetrans* (21). Assays were conducted in this soil to determine the efficacy of individual seed meals in nematode suppression and the duration of the response. Soils were treated with the respective seed meals, decanted into 3.8-liter plastic pots with 16 pots per treatment, and incubated in the greenhouse as stated above. At completion of the incubation period, a 50-g soil sample was collected from each pot. Soil populations of *P. penetrans* were determined using the modified pie-pan modification of the Baermann funnel technique (12). One MM106 rootstock, which is highly supportive of *P. penetrans* reproduction (M. Mazzola, *unpublished data*), was planted into each pot and, for each treatment, eight plants were harvested at 3 months and the remaining rootstocks at 6 months after planting. At harvest, plant roots were washed with tap water, two 0.5-g root samples were collected from each tree, and *P. penetrans* populations were determined as described (21).

Effect of seed meals on *R. solani* AG-5 apple root infection. The capacity of Brassicaceae seed meals to suppress apple root infection by *R. solani* AG-5 was examined in artificially infested soils. *R. solani* AG-5 strain 5-104 initially was recovered from the roots of apple (Gala/M26) cultivated in a commercial orchard near Moxee, WA (21). Each seed meal (*B. napus* cvs. AT and DE, *S. alba* IG, and *B. juncea* PG) was applied individually to CV orchard soil at a rate of 0.5% (vol/vol). Nontreated soil and seed meal-amended soils were placed in 3.8-liter plastic pots and incubated on a greenhouse bench. After 6 weeks, 10 *R. solani*-infested oat seed, prepared as previously described (26), were placed in a circular pattern at a soil depth of 8 cm. Soils were incubated overnight, and each pot then was planted with five 8-week-old Gala apple seedlings. Each treatment was represented by a total of five pots. Seedlings were harvested after 8 weeks, at which time plants were processed as described above and 10 root segments from each plant were plated onto water agar amended with ampicillin (100 μg ml^{-1}) and streptomycin (100 μg ml^{-1}). Agar plates were incubated at room temperature and examined after 48 h using a light microscope (×100) for hyphal growth of *R. solani.*

Experiments were conducted to evaluate the role of indigenous soil microorganisms in the seed meal-induced suppression of Rhizoctonia root rot. WVC orchard soil was amended with the individual seed meals as described above and incubated at room temperature for 6 weeks. Pasteurized treatments were prepared by placing one-half of each of the respective soils in heat-resistant bags and exposing them to steam at 102°C for 3 h. Soils were cooled overnight prior to repeating the steaming cycle. Infestation of native and pasteurized soils, growth conditions, plant processing, and assessment of root infection were conducted as above.

Streptomyces spp. numbers in seed meal-amended and nontreated soils were determined immediately prior to planting in all trials. Two 0.5-g bulk soil samples were arbitrarily collected from each pot just prior to planting and at harvest. Soils were resuspended in 10 ml of sterile distilled water and vortexed at highest speed for 60 s. Serial dilutions of the soil suspension were

plated onto 1/50th-strength trypticase soy agar. After 72 h of incubation at room temperature, colonies exhibiting a growth characteristic representative of members belonging to the genus *Streptomyces* were subjected to microscopic examination (×100) for confirmation of identity and enumeration.

Temporal dynamic of *B. juncea* PG-induced control of *R. solani* AG-5. The volatile isothiocyanate emanating from hydrolysis of the dominant glucosinolate (allyl-glucosinolate) produced by *B. juncea* PG is known to inhibit growth of *R. solani* (18). In studies described above, volatile isothiocyanates likely would have evacuated the soil system prior to introduction of the pathogen 6 weeks after seed meal amendment. Therefore, additional studies were conducted to assess the temporal nature of pathogen suppression and disease control in response to *B. juncea* PG soil amendment.

CV soil was amended with *B. napus* DE, *S. alba* IG, or *B. juncea* PG at a rate of 0.5% (vol/vol), and 7 g of amended or nontreated soil was placed in the base of a 5-cm-diameter petri plate. Three *R. solani* AG-5 colonized oat grains were attached to the lid of the petri plate using double-sided adhesive tape and the plate was sealed using parafilm. Each treatment was represented by three replicate plates which were incubated at room temperature. After 24 h, plates were unsealed, and oat grains were removed and placed on individual water agar plates amended with ampicillin (100 μg ml^{-1}). Viability of *R. solani* was determined after 24 h of incubation at 22°C by measuring linear hyphal growth. Plates containing CV soils remained open for 15 min prior to attaching new oat inoculum to the lids and resealing the plates with parafilm. Plates again were incubated for 24 h, at which time oat grains were removed and placed on water agar, with viability of *R. solani* determined as above.

CV orchard soil was amended with *B. juncea* seed meal as above 0 h, 24 h, or 4 weeks prior to infestation with *R. solani* AG-5. Amended and nontreated soils were decanted into conical tubes (21 cm in length by 4 cm in top diameter) with 10 tubes per soil treatment. Each tube was planted with one 4-week-old Gala apple seedling, with the lowest root being placed 3 cm above a single *R. solani*-infested oat grain. Plants were placed in an environmental growth chamber at a temperature regime of 24°C, day, and 16°C, night, with a 16-h photoperiod. Plants were harvested after 2 weeks and infection rates were determined by plating 20 root segments from each plant on water agar and monitoring hyphal growth as described above. A study employing the 0- and 24-h incubation periods prior to pathogen infestation also was conducted in pasteurized CV orchard soil using the same growth conditions.

Emission of allyl-isothiocyanate from *B. juncea* PG-amended soil. The temporal pattern of allyl-ITC emission from *B. juncea* PG-amended CV orchard soil was monitored. *B. juncea* PG was added to 250 ml of soil at a concentration of 0.5% (vol/vol; seed meal at 3.7 mg ml^{-1} of soil) and soil was placed in a 0.946-liter mason jar, with three replicate sample jars. One hour prior to each sampling, the jar tops were fastened with a lid containing a rubber septum. The head space was sampled by piercing the septum with a 3.8-cm-long 18-gauge needle and extracting a 1-ml volume which was injected into a Hewlett-Packard 5880A series gas chromatograph. The gas chromatograph was equipped with a CP-PoraBond Q fused silica capillary column (10 m by 0.32 mm; Varian, Palo Alto, CA) with injector and detector temperatures set at 100 and 250°C, respectively. Allyl-ITC was eluted using a program which increased column temperature from 125 to 175°C so that the peak was emitted within 3 min. Concentrations were determined by comparison to a standard curve generated using a pure allyl-ITC solution (Sigma-Aldrich, St. Louis).

Impact of composite seed meal amendment on *Pythium* spp. populations and root infection. Amendment of soil with *B. napus* seed meal has consistently induced a stimulation of resident *Pythium* populations (10,24). Studies were conducted to determine whether a composite amendment consisting of *B. napus* DE and *B. juncea* PG altered the response in *Pythium* spp. populations and infection of apple planted in treated soils. CV and GC soils were amended with seed meal at a total concentration of 0.5% but at different ratios of *B. napus* DE:*B. juncea* PG, as follows: 1:0, 2:1, 1:1, 1:2, and 0:1. Soils did not receive an extended incubation period as in previous trials but, rather, were dispensed immediately into conical tubes, and each cone was planted with one 8-week-old apple seedling. Nontreated soil was included as the control and each treatment was represented by 10 plants arranged in a completely randomized design. Plants were grown in controlled environment chambers with a 16-h photoperiod and a day-and-night temperature regime of 24 and 16°C, respectively.

Pythium spp. soil populations were determined at 0, 3, 7, and 14 days post planting. A 1-g soil sample was collected from four randomly selected tubes per treatment. Soil was resuspended in 10 ml of sterile distilled H_2O, vortexed for 60 s, and serial dilutions were plated onto *Pythium* semiselective medium (PSSM) (24). Plants were harvested after 2 weeks and processed as described above. Ten root segments, 0.5 to 1.0 cm in length, from each seedling were excised randomly from each Gala seedling and plated on PSSM. Plates were incubated at room temperature and examined after 24 and 48 h to determine relative root infection and estimate soil populations of *Pythium* spp. From the remaining root tissues, lesion nematode root populations were determined as described (21).

Data analysis. Data were analyzed using SigmaStat (version 3.1; Systat Software Inc., Point Richmond, CA). Percent root infection data and soil population data were transformed to arcsine-square root and $\log_{10}$ values, respectively, prior to conducting analysis of variance, and means separation was performed using the Student-Newman-Keuls method. All experiments were conducted twice. Data reported are from the initial experiment, and significant differences between experiments are described in the text.

RESULTS

Impact of amendments on apple growth and disease suppression. Individual seed meal amendments enhanced Gala apple seedling root and shoot biomass relative to the nontreated control

TABLE 1. Effect of Brassicaceae seed meal amendment on growth of 'Gala' apple seedlings in replant orchard soils

	Orchard[z]					
	CV		GC		WVC	
Treatment[y]	Root wt (g)	Shoot wt (g)	Root wt (g)	Shoot wt (g)	Root wt (g)	Shoot wt (g)
Control	0.49 a	1.13 a	0.84 a	2.06 a	0.68 a	0.94 a
DE	0.97 b	3.98 b	1.47 b	6.13 c	0.71 a	2.07 b
AT	0.94 b	3.67 b	1.26 b	4.94 bc	0.69 a	2.34 b
IG	1.04 b	4.26 b	1.45 b	5.73 bc	0.67 a	2.49 b
PG	0.75 ab	4.03 b	1.28 b	4.60 b	1.13 b	2.68 b

[y] DE = *Brassica napus* cv. Dwarf Essex, AT = *B. napus* cv. Athena, IG = *Sinapis alba* cv. IdaGold, and PG = *B. juncea* cv. Pacific Gold.

[z] Orchard designations: CV, Columbia View Experimental Orchard, Orondo, WA; GC, commercial orchard, Manson, WA; and WVC, Wenatchee Valley College Research and Demonstration Orchard, E. Wenatchee, WA. Means in a column followed by the same letter are not significantly different ($P > 0.05$; $n = 4$).

regardless of soil in which assays were conducted (Table 1). Although shoot growth was enhanced consistently across orchard soils tested, in certain instances plants grown in *B. juncea* PG-amended soils exhibited superior root biomass production relative to *B. napus* or *S. alba* seed meals. In one experiment conducted in the WVC orchard, a significant increase in seedling root biomass was observed only in response to *B. juncea* PG amendment (Table 1). When repeated in WVC soil, all seed meal amendments significantly improved root and shoot biomass relative to the control (data not shown). A similar finding was obtained in CV soil, in which seedling root biomass did not differ among seed meal treatments in one experiment (Table 1); however, in a second experiment, *B. juncea* PG amendment resulted in seedling root biomass that was significantly greater than that obtained in *B. napus* AT- ($P = 0.009$) or *S. alba* IG- ($P = 0.005$) amended soil.

Among those examined, only GC orchard soil possessed lesion nematode populations considered capable of suppressing apple growth (21). Application of any of the three Brassicaceae seed meals significantly reduced numbers of *P. penetrans* recovered from GC orchard soil immediately prior to planting (Table 2). Lesion nematode populations recovered at 3 months post planting from the roots of MM106 rootstock grown in seed meal-amended soils were significantly lower than those recovered from MM106 rootstock planted in nontreated orchard soil, and there were no significant differences among seed meal treatments. However, at 6 months post planting, lesion nematode numbers recovered from roots of MM106 rootstock grown in *B. juncea* PG soil were significantly lower than those obtained from plants grown in *B. napus* DE-, *S. alba* IG-, or *B. napus* AT-amended soils (Table 2).

TABLE 2. Effect of Brassicaceae seed meal amendment on recovery of *Pratylenchus penetrans* recovered from soil and roots of MM106 rootstock in a commercial orchard in Manson, WA

	P. penetrans g^{-1} of soil[z]		
Treatment[y]	Preplant	3 months post plant	6 months post plant
Control	217 b	115 b	643 c
DE	19 a	16 a	281 b
AT	5 a	11 a	177 b
IG	7 a	1 a	246 b
PG	1 a	4 a	2 a

[y] DE = *Brassica napus* cv. Dwarf Essex, AT = *B. napus* cv. Athena, IG = *Sinapis alba* cv. IdaGold, and PG = *B. juncea* cv. Pacific Gold.
[z] Means in a column followed by the same letter are not significantly different ($P > 0.05$; soil populations, $n = 16$; root populations, $n = 8$).

TABLE 3. Effect of Brassicaceae seed meal amendment on percent root infection by resident *Pythium* spp. for 'Gala' seedlings grown in replant orchard soils

	Orchard[z]		
Treatment[y]	CV	GC	WVC
Control	12.5 a	13.0 a	15.0 a
DE	45.0 b	43.5 b	55.5 b
AT	51.0 b	68.5 c	68.5 bc
IG	49.5 b	77.5 c	78.0 c
PG	7.0 a	11.0 a	20.5 a

[y] DE = *Brassica napus* cv. Dwarf Essex, AT = *B. napus* cv. Athena, IG = *Sinapis alba* cv. IdaGold, and PG = *B. juncea* cv. Pacific Gold.
[z] Orchard designations: CV, Columbia View Experimental Orchard, Orondo, WA; GC, commercial orchard, Manson, WA; and WVC, Wenatchee Valley College Research and Demonstration Orchard, E. Wenatchee, WA. Means in a column followed by the same letter are not significantly different ($P > 0.05$; $n = 4$).

With the exception of *B. juncea* PG, populations of *Pythium* spp. in orchard soil increased significantly in response to seed meal amendments. For all three orchards, *Pythium* spp. numbers increased from <60 to >500 propagules g^{-1} of soil in response to amendment with either *B. napus* AT, *B. napus* DE, or *S. alba* IG, with the greatest increase observed in GC orchard soils where populations were >4,000 CFU g^{-1} of soil at planting. *Pythium* spp. numbers in *B. juncea* PG-amended soils were <50 CFU g^{-1} of soil, which was not significantly different from the nontreated controls. Relative root infection of apple seedlings grown in these orchard soils corresponded with the increase in *Pythium* spp. soil populations induced by seed meal amendment. Root infection frequency by *Pythium* spp. increased from ≈13% for seedlings grown in nontreated soils to ≈45% or higher for seedlings grown in *B. napus* AT-, *B. napus* DE-, or *S. alba* IG-amended soil (Table 3). *Pythium* spp. root infection for plants grown in *B. juncea* PG-amended soils was not altered relative to the nontreated control in any of the orchard soils examined.

Rhizoctonia spp. were recovered consistently from roots of Gala seedlings planted in CV orchard soil, infrequently from seedlings grown in WVC orchards oils, and were not isolated from those grown in GC orchard soil. *Rhizoctonia* spp. were recovered at a frequency of 25% from seedlings grown in nontreated CV orchard soil. All seed meal amendments significantly ($P < 0.001$) suppressed *Rhizoctonia* spp. root infection of seedlings grown in CV orchard soil, with the reduction in root infection ranging from 48% (*B. juncea* PG) to 80% (*S. alba* IG), and there were no significant differences among seed meal treatments (data not shown).

Effect of seed meal amendments on apple root infection by *R. solani* AG-5. In assays conducted in CV orchard soil, application of seed meal 6 weeks prior to pathogen infestation and planting resulted in elevated *Streptomyces* spp. numbers and consistently suppressed infection of apple seedling roots by *R. solani* AG-5 (Table 4). However, when the assay was conducted in WVC orchard soil, although all seed meal amendments suppressed root infection relative to the nontreated control, *B. juncea* PG treatment was less effective than *B. napus* DE soil amendment. Regardless of seed meal type, pasteurization of amended soil prior to introduction of *R. solani* AG-5 inoculum

TABLE 4. Effect of Brassicaceae seed meal amendment on soil populations of *Streptomyces* spp. and infection of 'Gala' seedling roots by *Rhizoctonia solani* AG-5 in native or pasteurized orchard soils artificially infested with the pathogen[y]

	CV native soil		WVC native soil		WVC pasteurized soil	
Treatment[z]	Population ($\log_{10}$ CFU g^{-1})	Infection (%)	Population ($\log_{10}$ CFU g^{-1})	Infection (%)	Population ($\log_{10}$ CFU g^{-1})	Infection (%)
Control	5.38 a	32 c	5.52 a	58 c	1.69	81 a
DE	7.14 b	14 ab	7.86 c	8 a	ND	89 a
AT	6.75 b	10 ab	–	–	–	–
IG	7.42 b	9 a	7.30 bc	14 ab	2.08	91 a
PG	6.87 b	16 b	6.79 b	30 b	ND	96 a

[y] Orchard designations: CV, Columbia View Experimental Orchard, Orondo, WA; and WVC, Wenatchee Valley College Research and Demonstration Orchard, E. Wenatchee, WA. Population = *Streptomyces* spp. ($\log_{10}$ CFU g^{-1} of soil) and Infection = percent root infection. Means in a column followed by the same letter are not significantly different ($P > 0.05$; $n = 5$); – indicates not determined; ND = not detected (detection limit was 100 CFU g^{-1} of soil).
[z] DE = *Brassica napus* cv. Dwarf Essex, AT = *B. napus* cv. Athena, IG = *Sinapis alba* cv. IdaGold, and PG = *B. juncea* cv. Pacific Gold. Seed meal was incorporated into soil 6 weeks prior to introduction of *R. solani* AG-5 inoculum.

resulted in suppression or eradication of *Streptomyces* populations and elimination of disease control (Table 4).

Temporal dynamic of *B. juncea* PG-induced control of *R. solani* AG-5. The efficacy of *B. juncea* PG for the control of *R. solani* AG-5 was dependent upon the timing of pathogen infestation relative to application of the amendment. When *B. juncea* PG amendment was incorporated into soil at the same time as pathogen infestation, the treatment significantly ($P < 0.001$) reduced *R. solani* infection of Gala seedling roots (Table 5). However, when introduction of the pathogen was delayed until 24 h post seed meal amendment, root infection was similar in the *B. juncea* PG-amended and nontreated soils (Table 5). Incubation of *B. juncea* PG-amended soil for 4 weeks prior to infestation with *R. solani* AG-5 resulted in the restoration of disease suppression. This reestablishment of disease control in *B. juncea* PG-amended soil was associated with an increase in resident *Streptomyces* spp. numbers from 1.25×10^5 CFU g^{-1} of soil at day one to 3.75×10^7 CFU g^{-1} of soil in soils incubated for 4 weeks.

TABLE 5. Effect of duration of period between *Brassica juncea* cv. Pacific Gold seed meal (PG) amendment (0.5% vol/vol) and pathogen introduction on infection of 'Gala' seedlings grown in Columbia View orchard soil artificially infested with *Rhizoctonia solani* AG-5

	R. solani infection frequency (%)[z]	
Treatment	Native soil	Pasteurized soil
Control	79 b	44 b
PG incubation		
0 h	13 a	2 a
24 h	62 b	37 b
4 weeks	28 a	–

[z] Means in a column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$); – indicates not determined.

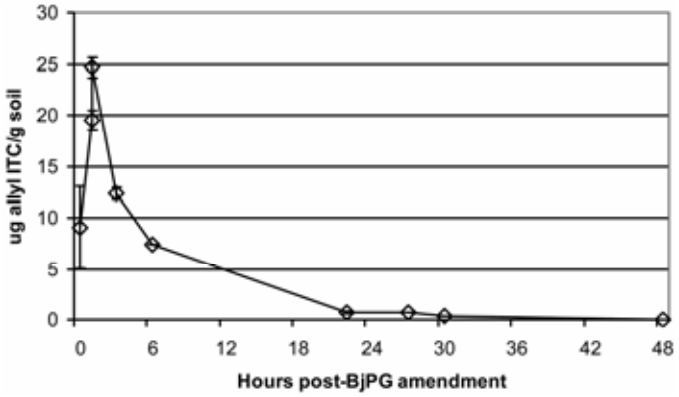

Fig. 1. Temporal pattern of allyl-isothiocyanate emission from Columbia View orchard soil amended with *Brassica juncea* cv. Pacific Gold seed meal (BjPG) as determined by monitoring concentration in the headspace of a chamber by gas chromatography. Seed meal was added to soil at a concentration of 0.5% (vol/vol). Bars = standard deviation of the mean.

Pasteurization of soil prior to pathogen infestation did not diminish the initial disease control obtained in response to *B. juncea* PG amendment. When inoculum of *R. solani* AG-5 was introduced into pasteurized soil at the time of seed meal amendment, the infection rate for Gala seedlings cropped in this soil was significantly ($P = 0.001$) less than when the pathogen was introduced 24 h post seed meal amendment of pasteurized soil or in the no-seed meal control (Table 5).

When *R. solani* AG-5 oat grain inoculum was exposed to volatiles emanating from *B. juncea* PG-amended soil for the period 0 to 24 h post amendment, hyphal growth was completely inhibited. In contrast, hyphal growth from oat grain inoculum incubated in the presence of *B. napus* DE- or *S. alba* IG-amended soil was not significantly ($P > 0.05$) different from the control. When exposed to volatiles from the same soil during the period 24 to 48 h post seed meal amendment, hyphal growth of *R. solani* was equivalent for all treatments (data not shown). The temporal nature of fungal growth inhibition corresponded with the dynamics of allyl-isothiocyanate emission from *B. juncea* PG-treated soil (Fig. 1).

Effect of composite seed meal amendment on *Pythium* and *Pratylenchus* spp. Amendment of either CV or GC orchard soil with *B. napus* DE resulted in a rapid increase in populations of *Pythium* spp., with the response of greater magnitude in GC soil (Table 6). Co-application of *B. napus* with *B. juncea* seed meal effectively suppressed the *Pythium*-stimulating effect of *B. napus* DE. An equivalent response with regard to populations of *Pythium* spp. and root infection of seedlings grown in amended soils was observed whether *B. juncea* PG represented one-third or two-thirds of the total composite seed meal amendment applied to soils (Table 7). All seed meal amendments resulted in a similar suppression of lesion nematode numbers over the course of this short 2-week plant growth period (Table 7).

DISCUSSION

The formulation of economically viable and horticulturally acceptable nonfumigant measures for the control of tree fruit replant diseases is confounded by the biological complexity of these disease phenomena. However, once orchards are established, few other impediments to production, such as weed control, are difficult to resolve with the use of standard practices in conventional systems. The preplant application of *B. napus* seed meal in concert with a post plant application of mefenoxam for the control of apple replant disease previously was shown to be effective on sites where the causal fungal complex (21) acted alone to incite disease rather than in concert with the lesion nematode (25). The current studies were undertaken as a first step to identify an alternative seed meal or composite seed meal amendment capable of providing a broader level of activity against the spectrum of agents contributing to replant disease, and suitable for use in both organic and conventional orchard management systems.

TABLE 6. Effect of *Brassica napus* cv. Dwarf Essex (DE), *Brassica juncea* cv. Pacific Gold (PG), and composite seed meal amendments on populations of *Pythium* spp. (propagules g^{-1} of soil) recovered from replant orchard soils[z]

	CV orchard				GC orchard			
Treatment	Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7	Day 14
Control	25 a	25 a	0 a	75 a	0 a	50 a	25 a	25 a
DE	25 a	525 b	400 b	275 b	0 a	2,425 b	3,500 b	3,525 b
PG	0 a	0 a	0 a	0 a	0 a	0	50 a	0 a
DE+PG 1:1	0 a	0 a	0 a	0 a	0 a	125 a	125 a	25 a
DE+PG 1:2	0 a	25 a	0 a	0 a	0 a	75 a	0 a	75 a
DE+PG 2:1	0 a	75 a	25 a	25 a	50 a	50 a	0 a	150 a

[z] Orchard designations: CV, Columbia View Experimental Orchard, Orondo, WA; and GC, commercial orchard, Manson, WA. Means in a column followed by the same letter are not significantly different ($P > 0.05$; $n = 4$). Limit of detection for any individual soil sample equals 100 propagules g^{-1} of soil.

Control of Rhizoctonia root rot of apple in response to *B. napus* seed meal amendment previously was shown to operate through the resident soil microbial community, with *Streptomyces* spp. likely possessing a functional role (10,11). In the current study, capacity of each Brassicaceae seed meal examined to suppress root infection by *R. solani* AG-5 required activity of the native orchard soil biology. This included *B. juncea* seed meal, which also produces glucosinolate hydrolysis products previously shown to inhibit this fungal pathogen (18,37), and provide control of damping-off incited by *R. solani* AG-4 (9). In the case of *B. juncea* PG, the requirement for an active microbial community and the functional mode of action leading to disease control varied in a temporal manner. Disease control was achieved when *B. juncea* PG and *R. solani* were introduced into a soil system simultaneously, but no disease control was detected if pathogen infestation was delayed until 24 h post seed meal amendment. Suppression of *R. solani* during the initial 24 h period post amendment was not dependent upon the resident microbial community because the same temporal relationship in disease control was exhibited in both native and pasteurized soils. This pattern corresponded directly with the pattern of allyl isothiocyanate (AITC) release from *B. juncea* PG-treated soil; >99% of AITC emission was recorded within 24 h of seed meal amendment, and growth of the pathogen was not impeded when exposed to volatiles released from the same soil 24 to 48 h after amendment. The *B. juncea* PG-treated soil, which was conducive to disease development when pathogen introduction was delayed until 24 h post amendment, reverted to the suppressive state when soil was incubated for 4 weeks prior to introduction of the pathogen. This event, as well as the control of *R. solani* AG-5 conferred by *B. napus* DE, *B. napus* AT, or *S. alba* IG soil amendment, was associated with an increase in *Streptomyces* spp. soil population. These findings are in agreement with previous, more definitive studies demonstrating the capacity of *Streptomyces* spp. to suppress infection of apple roots by *R. solani* AG-5 (10).

Suppression of root infection by *R. solani* in response to application of any of the seed meal amendments was fairly uniform across soils examined in this study. However, when studies were conducted in a manner that circumvented the period of pathogen exposure to AITC produced in response to soil amendment with *B. juncea* seed meal, the level of disease control achieved with *B. juncea* PG commonly was lower than one or more of the alternative seed meal amendments. Although populations of *R. solani* resident in the orchard site are not likely to evade exposure to AITC, sclerotia of *R. solani* are relatively tolerant of volatiles emanating from decomposing Brassicaceae residues (37) and may serve as a persistent reservoir of inoculum in *B. juncea* PG-treated soils. The resident soil microbial community also will recover (23), and the pattern of microbial succession ultimately may influence overall control of *R. solani* achieved with *B. juncea* PG amendment. The rapid emission of AITC from *B. juncea* PG-treated soils will be of significance in the post plant setting because *R. solani* commonly is associated with rootstocks of planting material used to establish the orchard (7). This potential source of pathogen inoculum would not be exposed to AITC generated in response to *B. juncea* seed meal amendment and, in this instance, *B. napus* seed meal could prove a more effective material for control of *R. solani*.

Although all seed meals suppressed initial lesion nematode soil populations, only *B. juncea* PG amendment resulted in a sustained suppression of root populations throughout a 6-month growth period. Lesion nematode population trends in *B. napus* seed meal-treated soils in this greenhouse study were similar to that observed in previous field studies, where initial nematode suppression was not maintained into the second growing season (25). Initial nematode suppression realized in response to all seed meal amendments could be attributed to the nematistatic properties of this and other high-N-content organic amendments (27,31). The prolonged and sustained suppression of *P. penetrans* populations in *B. juncea* PG relative to *B. napus* DE or *S. alba* IG amendment is consistent with the known nematicidal activity of AITC produced by incorporation of this seed meal (38), and the lack of active ITCs produced in response to residues of *B. napus* (14) or *S. alba* (8).

Due to the associated increase in *Pythium* spp. root infection, effective application of *B. napus* DE for control of apple replant disease required a post plant application of mefenoxam (25), an approach not suitable for use in organic production systems. *B. juncea* PG was the only amendment that did not stimulate *Pythium* spp. populations and, when used in conjunction with *B. napus* seed meal, it suppressed the characteristic *B. napus* DE-induced stimulation of this community. Such a finding would suggest that *B. juncea* PG amendment, in and of itself, could be appropriate in organic systems. However, *B. juncea* PG was found to stimulate apple root infection by *Phytophthora cambivora* and *P. megasperma* in the field (M. Mazzola, *unpublished data*) whereas *B. napus* DE did not, and *B. napus* seed meals appear to provide superior control of *R. solani*. As such, based on these preliminary trials, a composite *B. juncea* PG and *B. napus* DE amendment may be more suitable for application in organic systems.

Findings from the current study suggest that the use of Brassicaceae seed meal amendments has promise as an alternative strategy for the control of apple replant disease, and that disease control can be realized in a predictable manner. Amendment rates employed in this and previous studies (10,11,24,25) are equivalent to the application of seed meal at 8 to 10 t ha^{-1}. These rates equate to an amount that ranges from <1 to 25% of that recently used in the evaluation of composts in greenhouse, apple orchard, and other field-level production systems (5,33,36,37). Seed meal sourced from the same *Brassica* sp. (*B. napus*) but different cultivars (*B. napus* DE and *B. napus* AT) or of the same cultivar (*B. napus* DE) over multiple years consistently has provided the same level and spectrum of disease suppression (24,25). In addition, although these seed meals operate in part through the resident soil microbial community, a similar spectrum of pathogen control has been observed in all orchard soils examined. Biological function of amendments in only specific soils (34) can, at times, limit their value as a disease control option. Populations of one proposed functional microbial group active in control of *R. solani*, resident streptomycetes, were consistently amplified in these studies regardless of orchard soil, and have been maintained in the field for upwards of two full growing seasons (11). Such consistency in product, both in terms of composition and activity, an attribute often lacking in organic amendments (13), may enhance the adoption of their use in commercial orchard production systems upon demonstration of functionality in field trials.

TABLE 7. Effect of *Brassica napus* cv. Dwarf Essex (DE) and *B. juncea* cv. Pacific Gold (PG) seed meal amendments or their composite at varying ratios on percent *Pythium* spp. root infection and recovery of *Pratylenchus* spp. (number g^{-1} of root) from 'Gala' apple seedlings[z]

	Pythium root infection (%)		
Treatment	CV orchard	GC orchard	GC, *Pratylenchus* spp.
Control	7 a	8 a	252 b
DE	31 b	45 b	56 a
PG	0 a	3 a	4 a
DE+PG 1:1	1 a	8 a	21 a
DE+PG 1:2	6 a	5 a	3 a
DE+PG 2:1	1 a	7 a	25 a

[z] Orchard designations: CV, Columbia View Experimental Orchard, Orondo, WA; and GC, commercial orchard, Manson, WA. Means in the same column followed by the same letter are not significantly different ($P > 0.05$; $n = 10$).

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