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PROGRESS TOWARDS DEVELOPMENT OF BIOLOGICALLY-BASED STRATEGIES FOR THE MANAGEMENT OF APPLE REPLANT DISEASE

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Abstract

Effective non-fumigant biologically-based methods for control of apple replant disease have been lacking. Studies were conducted to examine the utility of approaches which modulate resident microbial communities as a potential means to provide disease suppression. Cover cropping sequences and use of brassicaceous soil amendments were employed to elicit desired microbial transformations, as well as to provide direct disease suppression. Wheat cropping provided disease control and enhanced fruit yield, but *Brassica napus* seed meal (RSM) amendment in concert with a post-plant mefenoxam soil drench was the sole treatment to provide a growth and yield response equivalent to soil fumigation. RSM-induced control of *Rhizoctonia solani* resulted from the enhanced activity of resident soil microorganisms, specifically *Streptomyces* spp., rather than biofumigation, the mechanism commonly attributed to brassicaceous residue-induced soilborne disease control.

Key words: *Streptomyces*, induced resistance, brassicaceae seed meal, microbial ecology, *Rhizoctonia solani, Pratylenchus, Pythium*

Introduction

Pre-plant soil fumigation has long been the preferred method employed for the control of tree fruit replant diseases. The alleged complex etiology of these diseases (Traquair 1984) has limited investigations into the development of alterative biologically-based strategies for controlling replant diseases. Those attempts that have been made characteristically have monitored the impact of any treatment on plant growth (Slykhuis and Thomas 1986, Wilson et al. 2004), rather than the impact of such treatments on pathogen/parasite populations and disease control.

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Such a constraint limits ones ability to implement control strategies with any capacity to predict the efficacy of such treatments. Studies conducted in Washington state (USA) elucidated the pathogen/parasite complex which incites apple replant disease (ARD), demonstrating that, although the relative contribution of any given agent to disease development differs among sites, the composition of the complex is reasonably uniform among orchards (Mazzola 1998). This information has enabled the generation of control strategies in a biologically-rational method and allowed for the assessment of trials in such a manner as to reveal the basis for disease control success or failure.

Materials and methods

Field trials were established at two sites in Washington; CV and WVC orchards. Disease causal agents at both sites were similar, including *Cylindrocarpon destructans, Phytophthora* spp., *Pythium* spp., and *Rhizoctonia solani*. WVC, but not CV, orchard soils also possessed significant lesion nematode (*Pratylenchus penetrans*) populations. Wheat and *Brassica napus* (rapeseed) were employed as a cover crop and green manure crop, respectively, at the CV orchard. Wheat was cultivated using a seed mixture of the soft white winter wheat cvs. 'Daws', 'Lewjain' and 'Sprague' (1:1:1) over a one-year period, in a series of three short-term plantings, each having a growth duration of approximately nine weeks. Plant material was removed by mowing prior to reseeding of the plots. *Brassica napus* was cultivated for one, two or three years and plant residues were incorporated into the soil profile in September of each year. The site was planted with Gala/M.26 in May 2000. Increase in trunk diameter was recorded periodically through the growing season and fruit was harvested from all trees in August of each year beginning in 2001.

Previous greenhouse trials demonstrated the capacity of *B. napus* seed meal (RSM) soil amendment to control both *R. solani* and *P. penetrans* (Mazzola et al. 2001). Therefore, trials were established to evaluate the capacity of RSM to provide control of ARD at the CV and WVC orchards. RSM was used in conjunction with a post-amendment period of soil solarization or one-year wheat cover crop, or post-tree planting mefenoxam soil drench. These strategies were employed in an attempt to suppress the commonly observed RSM-induced stimulation of *Pythium* spp. (Mazzola et al. 2001). RSM was applied at a rate of 8533 kg/ha and incorporated into soil to a depth of 15–20 cm. A non-treated control and pre-plant soil fumigation with 1,3-dichloropropene-chloropicrin (Telone-C17) were included and the study employed a randomized complete block design consisting of four replicates with eight trees each. CV and WVC orchards were planted to Gala/M.26 and Golden/M.7 trees, respectively, in 2002. Growth and yield were monitored as described above.

Greenhouse and field trials were established to assess the efficacy of additional brassicaceous seed meals for the control of ARD. These included *B. napus* cvs.

'Athena' and 'Dwarf Essex', *B. juncea* cv. 'Pacific Gold', and *Sinapis alba* cv. 'Ida-Gold'. In greenhouse trials, seed meal was applied at 0.3% (vol/vol) while field trials employed the conditions and rates as described above for the 2002 trials. The field study was established at CV orchard in May 2005.

Studies were conducted to assess the mechanism by which RSM provides control of *R. solani*. As RSM soil amendment stimulates resident *Streptomyces* populations, the potential role of these bacteria in the observed disease control response was also evaluated. Both studies employed split-root assays; one half of a Gala seedling root system was established in RSM (0.5% vol/vol) treated or *Streptomyces* inoculated soil, and the remainder of the root system was planted into an adjacent cell infested with *R. solani* AG-5. *Streptomyces* strains used were initially recovered from RSM treated orchard soils (Cohen and Mazzola in press). Plants were harvested after three weeks and 20 segments (0.5 cm length) from the root component established in *R. solani* infested soil were plated on water agar amended with ampicillin. After 72 h incubation, root segments were examined for the presence of *Rhizoctonia* hyphae using a light microscope (×100).

Results

In the cover crop trial established at the CV orchard in 2000, trees grown in soils that received a one-year wheat cover crop attained significantly higher cumulative fruit yields than the control in 2001 to 2003 (Table 1). The fallow treatment and all rapeseed-only treatments did not improve yield during 2002, but two- and three-year rapeseed green manure cropping significantly enhanced cumulative yields as compared to the control. Fruit yields achieved from all fallow, cover crop or green manure crop treated blocks were significantly less than that attained from methyl bromide fumigated blocks.

For the CV orchard planted in 2002, vegetative growth and yield of Gala/M.26 trees established in RSM/mefenoxam-treated plots was equivalent to that attained in Telone-C17 fumigated soil during the three growing seasons monitored, and was superior to all other treatments (Table 2; Mazzola and Mullinix 2005). At the WVC orchard, Golden Delicious/M.7 trees established in Telone-C17, RSM/mefenoxam or RSM/solar treated soils outperformed initial growth of trees established in non-treated soils. However, within 10 months of planting soil fumigation was clearly superior to all other treatments in promoting tree growth at this site, and this status was maintained during the three-year period of observation. Failure of the RSM/mefenoxam treatment to attain a growth increment consistent with that of pre-plant fumigation was directly related to re-colonization of WVC orchard soil and apple roots by *P. penetrans* during the second growing season.

In greenhouse trials, *B. juncea* seed meal amendment provided excellent control of the biological elements that incite replant disease. *Brassica juncea* did not stimulate resident *Pythium* spp. populations, suppressed *Rhizoctonia* root infection, and provided a level of control of *P. penetrans* which was superior to that attained with

Table 1

Cumulative (2001–2003) fruit yields per tree from Gala/M.26 apple established at the CV orchard, Orondo, WA in May 2000 (kg)

Treatment	Yield
Control	5.1 d
Methyl bromide	19.6 a
3-year fallow	5.9 d
1-year wheat cover crop	9.4 b
1-year *B. napus* green manure	6.7 cd
2-year *B. napus* green manure	7.5 c
3-year *B. napus* green manure	8.4 bc

Values in the same column followed by the same letter are not significantly different according to the Tukey test (P = 0.05).

Table 2

Cumulative (2003-2005) fruit yields per tree from Gala/M.26 apple established at the CV orchard, Orondo, WA in May 2002 (kg)

Treatment	Yield
Control	13.0 a
1.3-dichloropropene-chloropicrin	22.6 b
RSM/mefenoxam	23.6 b
RSM/solarization	15.3 a
Wheat/RSM	15.1 a

Values in the same column followed by the same letter are not significantly different according to the Tukey test (P = 0.05).

RSM – *Brassica napus* seed meal.

either *S. alba* or *B. napus* (Table 3). In the field, post-plant application of mefenoxam enhanced first-year growth of Gala/M.26 established in *B. juncea* seed meal amended soils (Table 4). This growth promotion was realized even though seed meal amendment did not stimulate soil populations nor increase root infection by resident *Pythium* spp. Molecular analysis of the oomycete community inhabiting roots of Gala/M.26 revealed that *P. ultimum* dominated this population in roots of trees grown in non-treated CV orchard soil. In contrast, *Phytophthora cambivora* was detected in all trees grown in *B. juncea* seed meal amended soils, but not trees grown in the same soils receiving a post-plant mefenoxam soil drench. *Pythium ultimum* was not detected in roots of any tree cultivated in *B. juncea* seed meal amended soil.

Table 3

Impact of brassicaceous seed meals on *Rhizoctonia* root infection, lesion nematode populations and growth of Gala apple seedlings in replant orchard soil

Treatment	Root weight (g)	Shoot weight (g)	*P. penetrans* per root (g)	*Rhizoctonia* root infection (%)
Control	0.49 a	1.13 a	370 c	31.5 b
RSM	0.97 b	3.98 b	79 ab	6.1 a
ATHSM	0.94 b	3.67 b	111 b	9.7 a
IGSM	1.04 b	4.26 b	105 b	9.0 a
PGSM	0.75 ab	4.03 b	2 a	16.0 a

Values in the same column followed by the same letter are not significantly different according to the Tukey test (P = 0.05).

RSM – *Brassica napus* cv. 'Dwarf Essex', ATHSM – *B. napus* cv. 'Athena', IGSM – *Sinapis alba* cv. 'IdaGold', PGSM – *B. juncea* cv. 'Pacific Gold' seed meals.

Table 4

Impact of pre-plant seed meal amendment and post-plant mefenoxam soil drench on first-year increase in trunk diameter of Gala/M.26 apple established at the CV orchard, Orondo, WA in May 2005 (mm)

Pre-plant soil treatment	No post-plant treatment	Post-plant mefenoxam
Control	1.32 d	2.04 cd
Telone-C17	2.75 b	–
Brassica juncea	1.96 cd	4.75 a
Brassica napus	2.34 c	3.29 b
Sinapis alba	2.71 bc	4.49 a

Values followed by the same letter are not significantly different according to the Tukey test ($P = 0.05$).

In split-root assays, establishing a component of a Gala seedling root system in RSM amended soil significantly suppressed root infection on an adjacent but physically separated component of the same seedling root system planted in *R. solani* AG-5-infested soil; from 63.6% in non-treated soil to 36.2% in RSM amended soil ($P < 0.001$). Pasteurization of RSM-amended soil prior to pathogen infestation abolished control of *R. solani*. When introduced into pathogen infested soils, individual *Streptomyces* isolates provided levels of disease control similar to that obtained with RSM (Table 5). In subsequent split-root assays, inoculation of soils with some but not all of these same *Streptomyces* spp. isolates suppressed root infection by *R. solani* in a similar fashion (Table 5; Cohen and Mazzola in press).

Table 5

Impact of *Brassica napus* seed meal (RSM) amendment or *Streptomyces* spp. soil inoculation on infection of apple roots by an introduced isolate of *Rhizoctonia solani* AG-5

Treatment	Root infection* (%)	Root infection – split-root assay (%)
Control	27.8 b	47.1 c
RSM**	11.1 a	22.1 a
Streptomyces CR2***	3.3 a	17.1 a
Streptomyces SCV22***	12.2 a	25.7 ab
Streptomyces CVR44***	17.9 ab	28.6 ab
Streptomyces RR2***	10.0 a	32.1 b

**Streptomyces* isolate or RSM was applied to the same soil which had been infested with inoculum of *Rhizoctonia solani* AG-5.

**RSM was added to soil at a rate of 0.5% (vol/vol).

***Individual *Streptomyces* spp. were established at a final density of approximately 5×10^8 cfu/g of soil.

Discussion

The incorporation of brassicaceous green manures in disease management programs has received significant consideration due to the perception that these organic materials possess the capacity to actively suppress soilborne pathogens and parasites through the release of glucosinolate hydrolysis products (Brown and Morra 1997). However, in certain instances brassicaceous plant residues have been shown to provide disease control regardless of glucosinolate content (Mazzola et al. 2001) or to be no more effective than a fallow period or non-*Brassica* break crops for control of soilborne diseases in annual cropping systems (Gardner et al. 1998). In our studies, a one- or two-year *B. napus* green manure crop had little or no impact on ARD development. Although a three-year rapeseed green manure crop significantly diminished root infection by *Rhizoctonia* spp. and enhanced yields at the third year of fruit harvest, this treatment was no more effective than a one year wheat cover crop. The growth response achieved with any of the cover or green manure crop treatments was inferior to pre-plant soil fumigation with methyl bromide, and resulting yields did not approach that likely to be necessary for adoption of these practices by commercial producers.

The findings from these studies indicate that RSM/mefenoxam treatment is a suitable alternative to pre-plant fumigation for the control of apple replant disease on sites where lesion nematode does not contribute to disease severity. This integrated soil treatment provided control of *Cylindrocarpon, Pythium* and *Rhizoctonia* at the CV orchard resulting in yields which were equivalent to that obtained in response to pre-plant soil fumigation through four growing seasons. Modification of this treatment will be necessary on sites possessing significant lesion nematode populations. At the WVC site, root populations of *Pratylenchus* had increased to damaging levels for all treatments, with the exception of soil fumigation, by the end of the second growing season (Mazzola and Mullinix 2005). Nematode suppression induced by the low glucosinolate content RSM is likely a result of the nematicidal nature of nitrogenous amendments rather than production of a volatile glucosinolate hydrolysis product (Mazzola and Mullinix 2005). Thus, nematode populations at depths greater than that at which materials were incorporated (15–20 cm) likely were not affected by RSM application.

In addition to superior growth and yield promotion, grower adoption of the RSM/mefenoxam treatment is more feasible than any cover crop or green manure strategy due to the ease at which the method can be integrated into current orchard management practices. Disease suppression in response to *B. napus* green manure required a three year period of lost orchard productivity while the RSM/mefenoxam treatment can be executed in a time frame characteristic of that required for orchard fumigation. Seed meal amendment in the autumn prior to orchard planting or in spring as little as six weeks prior to planting in concert with a post-plant mefenoxam soil drench both have been effective in the control of apple replant disease.

Brassica juncea seed meal was selected for inclusion in the 2005 field trial due to the observation in controlled environment experiments that application of this

material did not result in amplification of resident *Pythium* spp. populations. Such an outcome is of significance for the use of this disease management strategy in organic production systems where the post-plant mefenoxam soil drench is not feasible. Rather unexpectedly, post-plant mefenoxam application dramatically improved growth of trees in *B. juncea* seed meal amended soil, and this response was directly associated with suppression of root infection by *P. cambivora*. As the rootstock used in this trial, M.26, is susceptible to *Phytophthora* spp., a possible strategy to avert the need of the post-plant mefenoxam soil drench may be to employ a *Phytophthora* resistant rootstock such as Bud.9 or M.9.

Results from split-root assays indicate that the suppression of apple root infection by *R. solani* in response to RSM amendment or individual *Streptomyces* isolates is a plant-mediated phenomenon. The vast majority of *Streptomyces* strains isolated from the apple rhizosphere when grown in RSM amended soils produce nitric oxide (NO) and possess a nitric oxide synthase homolog (Mazzola et al. unpublished). NO is known to prime plant defense responses by stimulating the production of phenylpropanoids including salicylic acid. Thus, it is plausible that the induction of plant resistance observed in response to RSM soil amendment operates through the resident *Streptomyces* spp. population.

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