FINAL PROJECT REPORT
WTFRC Project Number: AH-05-508

Project Title: Employing Biological Elements of Orchard Ecosystems

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Agency Name: USDA CSREES
Amount awarded: $303,267
Notes:

Total Project Funding: $166,640

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	22,800	23,940	25,137
Benefits	6,840	7,182	7,541
Wages	12,000	12,000	12,000
Benefits	3,600	3,600	3,600
Equipment	0	0	0
Supplies	8,000	8,000	8,000
Travel	800	800	800
Miscellaneous			
Total	**54,040**	**55,522**	**57,078**

Justification

For sites lacking significant lesion nematode populations, pre-plant *Brassica napus* seed meal amendment used in conjunction with a post-plant mefenoxam (RidomilGold EC) soil drench has provided levels of replant disease control, growth and yield equivalent to pre-plant soil fumigation (Mazzola & Mullinix, 2005). Preliminary studies with alternative seed meals suggest that disease control can be improved upon and may circumvent need for the post-plant mefenoxam application. Realization of this outcome would allow for the implementation of this disease control strategy in organic production systems.

The overall objective of this program is to develop an integrated management method compatible to conventional and organic apple production systems that provides the shortest time frame to initial commercial harvest when re-establishing orchards on sites previously planted to apple. As similar biological entities appear to have a role in replant problems encountered in pear, peach and cherry (Mazzola, unpublished data), it is plausible that such a system would have utility across tree fruit production systems.

Specific objectives:

1.) Examine the capacity of Brassicaceae seed meals to suppress the biological complex inciting replant disease and enhance tree growth in replant orchard soils.

2.) Determine the mechanism(s) by which these soil amendments provide control of the various plant parasites and pathogens that incite replant disease development, with emphasis on *Rhizoctonia solani*.

3.) Assess the influence of rootstock genotype on composition of resident *Streptomyces* populations and the efficacy of RSM-induced disease suppression

SIGNIFICANT FINDINGS:

- Pre-plant brassicaceae seed meal (BSM) soil amendment in conjunction with a post-plant mefenoxam (RidomilGold) soil drench provides initial increase in tree growth & yield equivalent to soil fumigation.

- The biological need for the post-plant mefenoxam soil drench differs with BSM.

- When used as a singular treatment (without mefenoxam), only *Sinapis alba* (yellow mustard) seed meal significantly improved tree performance.

- In field trials, <u>initial</u> tree growth in fumigated soils at times was inferior to or equivalent to other treatments, even those that did not provide disease control.

- The mechanism of pathogen suppression provided by BSM differs by target pathogen species, time and space.

- Growth performance in seed meal amended soils differed by rootstock.

- A composite seed meal compatible with organic production systems was formulated and in the field generated first-year tree growth comparable to soil fumigation.

RESULTS AND DISCUSSION

Tree Growth Performance in Orchard Trials

2005-2007 CV orchard trial

A field trial was established at the Columbia View Research and Demonstration Orchard, and was initiated with tree removal (Red Delicious/Seedling) from the site in October, 2004. In addition to *B. napus* cv. Dwarf Essex (canola), seed meal of *Sinapis. alba* cv IdaGold (yellow mustard) and *B. juncea* cv Pacific Gold (oriental mustard) were employed with or without a post-plant mefenoxam (Ridomil) soil drench. These seed meals were chosen based upon our data obtained in controlled environment studies, and data from the literature, suggesting the relative activity of these materials toward the pathogens and parasites causing replant disease. Seed meal was applied in April 2005 at a rate of 3.08 ton per acre and incorporated into the soil profile by rotovation. The site was planted to Gala/M26 on 19 May with 10 trees per plot with five replicates. A split-plot design was used with five trees per plot receiving a post-plant mefenoxam soil drench on 24 May.

All seed meal amendments significantly improved tree growth over three growing seasons when used in conjunction with the post-plant mefenoxam soil drench (Figure 1). Among seed meal amendments, only *S. alba* significantly improved growth relative to the control in the absence of the mefenoxam soil drench. Mefenoxam (Ridomil) alone did not improve tree growth over the three year study.

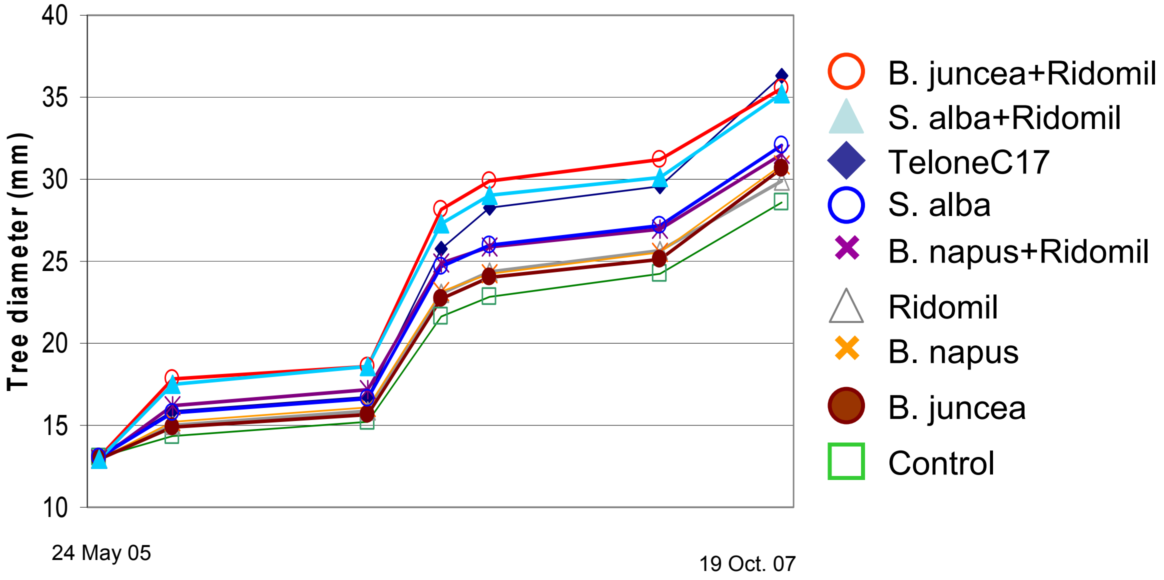

Figure 1. Impact of soil treatments on growth of Gala/M26 at the Columbia View Research and demonstration orchard.

Soil treatments had similar impacts on tree yield as was observed for tree growth. All seed meal treatments that included a post-plant mefenoxam soil drench significantly improved initial fruit yield relative to the control, and yields from *B. juncea* or *S. alba* + mefenoxam treated blocks were significantly greater than the Telone-C17 fumigated blocks. *B. napus* seed meal, *B. juncea* seed meal or mefenoxam alone did not significantly improve yields. As with tree growth, only *S.*

alba was the only seed meal when used singularly significantly improved yields relative to the non-treated control.

Table 1. Impact of soil treatments on fruit yields (kg per tree) from Gala/M26 established at the Columbia View Research and Demonstration orchard in May 2005.

Treatment	2006	2007
Control	0.0	4.71a
Control + mefenoxam	0.17	5.76ab
Telone-C17	0.20	6.94bc
B. napus	0.78	4.70a
B. napus + mefenoxam	0.94	7.83cd
B. juncea	0.95	4.96a
B. juncea + mefenoxam	1.49	9.29d
S. alba	0.45	7.01bc
S. alba + mefenoxam	1.55	8.67d
P=	0.234	0.002

A similar trial was initiated in 2002 at the Columbia View orchard, but included only *B. napus* seed meal. Results were similar to those attained for the trial initiated in 2005. Using the seed meal as a singular treatment did not improve tree growth or yield. Only when used in combination with mefenoxam did this seed meal improve performance of Gala/M26 in replant orchard soil (Table 2). As previously reported, this treatment did not improve tree growth or yield on a site that possessed significant lesion nematode populations (Mazzola and Mullinix, 2005).

Table 2. Mean cumulative fruit yields (2003-2006) of Gala/M26 established at the Columbia View orchard in May 2002.

Treatment	Yield (kg/tree)
Control	4.33a
Telone-C17 fumigation	7.53b
B. napus seed meal + mefenoxam	7.86b
B. napus seed meal	4.97a

Interestingly, initial year growth of trees established in fumigated soils tended to under-perform or was not significantly different from that of other treatments, even those treatments that tended to increase the inoculum potential of pathogens known to reside in these soils (e.g. *Pythium* spp.). This has been observed on multiple occasions during our trials conducted in conventional orchard systems and suggests that some resident biological community essential to optimal tree growth was negatively impacted in fumigated soils.

Growth performance attained was directly related to control of the pathogen complex resident to this site. All seed meal amendments significantly reduced root infection by *Rhizoctonia* spp. and both *B. juncea* and *S. alba* significantly reduced infection by *Cylindrocarpon* spp. (Table 3). As expected, *B. napus* and *S. alba* amendments dramatically increased populations of *Pythium* and the effectiveness of the post-plant mefenoxam treatment in these seed meal treated soils is due to the control of this oomycete group. Unexpectedly, our data demonstrated the need for the post-plant mefenoxam drench for effective use of *B. juncea* seed meal, even though *Pythium* spp. were effectively controlled (Table 3). Analysis of root systems demonstrated that the enhanced tree growth resulting from mefenoxam application to *B. juncea* amended soils resulted from control of *Phytophthora cambivora* and *Phytophthora megasperma*.

Table 3. Impact of soil treatments on soil populations and Gala/M26 root infection by fungal pathogens.

Treatment	*Pythium spp.* per g soil	*Cylindrocarpon* root infection (%)	*Rhizoctonia* root infection (%)
Control	265b	19.5a	14.0a
Telone C17	65a	9.0b	11.7ab
Brassica juncea PG	75a	1.9b	5.0b
Brassica napus DE	5320c	18.0a	6.6b
Sinapis alba IG	4515c	7.1b	2.8b

Significance to industry: These findings are very promising due to the fact that the rootstock employed (M26) is highly susceptible to the pathogen complex resident to this site. Thus, it is plausible that further progress could be made towards a viable alternative to fumigation through the addition of host tolerance to the production system.

Based upon these trials, a composite seed meal amendment was formulated with the goal of developing a product that would not require a post-plant mefenoxam soil drench and has capacity to provide lesion nematode control. Such a material may be compatible with organic production systems. These trials are discussed in the following section:

2006 Orchard Rootstock Trial:

A field trial was established at a commercial organic orchard to evaluate the efficacy of a *Brassica napus/Brassica juncea* composite seed meal amendment for control of replant disease, and to assess whether the response was rootstock dependent. Trials were conducted in a commercial organic orchard. Composite seed meal or *B. napus* seed meal alone was applied on May 3 at 3.08 lbs per linear foot of tree row and rotovated into the soil profile. The site was planted with G11, G16, G30, M7, M9, M26 and seedling rootstocks. All G11 rootstocks died irrespective of soil treatment.

B. juncea seed meal suppressed the repeatedly observed *B. napus*-induced proliferation of *Pythium* spp. (Table 4). In assessments completed to date, the *B. napus/B. juncea* composite amendment significantly reduced lesion nematode populations recovered from roots of M26 and M7 rootstocks to a level comparable to or better than that attained through pre-plant soil fumigation. However, the level of nematode control attained was not comparable to what has been achieved with *B. juncea* alone in greenhouse trials (Table 5). Optimization of nematode control with the combination seed meal may require modification of application method to enhance soil retention of the *B. juncea*-derived allyl-isothiocyanate (AITC).

Table 4. Effect of treatments on *Pythium* soil populations.

	Pythium soil populations (cfu/g soil)	
Treatment	At planting (5.29.06)	At harvest (10.26.06)
Control	550b	604b
Telone-C17 fumigation	135a	350a
B. napus Athena	3890c	3917c
B. napus Athena+*B. juncea* PG	120a	300a

Table 5. Impact of soil treatments on recovery of lesion nematode from roots of apple rootstocks planted at Stormy Mountain Ranch, Chelan, WA

	Pratylenchus penetrans populations (#/g root)	
Treatment	M26	M7
Control	114b	189b
Telone-C17 fumigation	59a	104ab
B. napus Athena	109b	85a
B. napus Athena+*B. juncea* PG	42a	92a

2007 Orchard Field Trial

A further field trial was established at Stormy Mountain Ranch to evaluate the composite seed meal amendment for replant disease control. The site was treated in April 2007 with a *B. juncea/B. napus* (1:1; 3 ton/acre) seed meal amendment and rotovated, fumigated with Telone-C17 or not treated (control). The site was planted with Gala/M26 on 23 May. Root samples were collected 23 October.

Initial tree growth in seed meal treated soil was comparable to that attained in Telone-C17 fumigated soil, and significantly better than the non-treated control (Fig. 2).

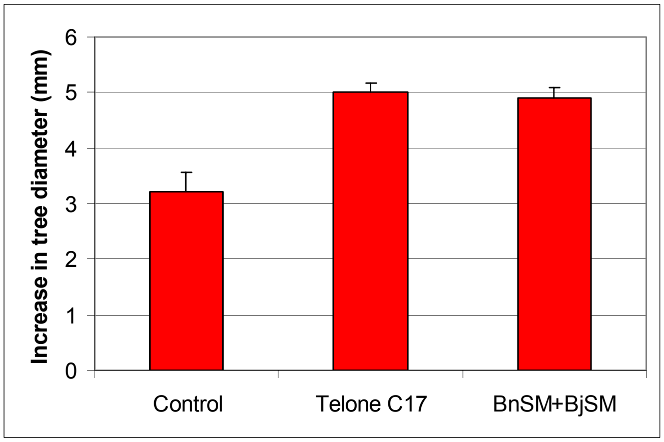

Figure 2. Initial year increase in trunk diameter for Gala/M26 at Stormy Mountain Ranch organic orchard.

As observed in previous trials, increased tree growth was associated with pathogen suppression. The seed meal treatment was as effective as soil fumigation in the initial suppression of lesion nematode root populations (Fig. 3). *Pythium* spp. were not stimulated by the composite seed meal amendment and *Pythium* root infection was not different from the fumigated check, and numerically, but not statistically, lower than the non-treated control (Fig. 4).

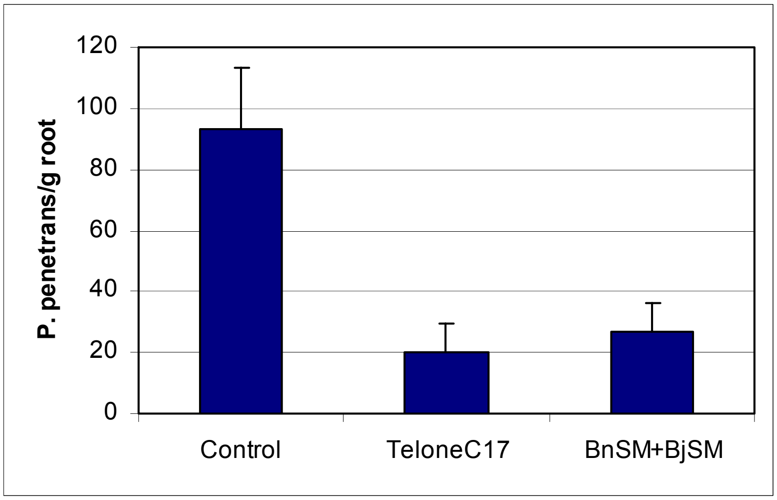

Figure 3. Impact of soil treatments on recovery of lesion nematodes (*P. penetrans*) from the roots of Gala/M26 at the Stormy Mountain Ranch.

Significance to industry: These preliminary findings indicate that this alternative treatment may be of value for control of replant disease in organic orchard systems. As this material also provides a significant source of N, P, K and S, the economic value of this strategy may surpass that resulting from disease suppression.

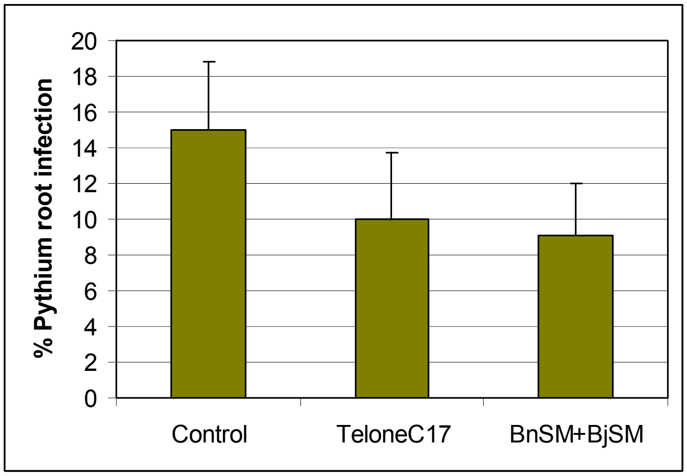

Figure 3. Impact of soil treatments on recovery of *Pythium* spp. from the roots of Gala/M26 at the Stormy Mountain Ranch.

Greenhouse rootstock trials

Multiple rootstock trials were performed in the greenhouse in addition to the field trial cited above. In general, rootstocks from the USDA-Geneva/Cornell breeding program demonstrated lower levels of susceptibility to the pathogens (e.g. *Pythium* spp.) and parasites (e.g lesion nematodes) than those from the Malling series. G11 and G30 rootstock, in general, supported lower lesion nematode reproduction relative to other rootstocks evaluated (Table 6). Likewise, these rootstocks exhibited higher tolerance to infection by *Pythium* spp. relative to M26, MM106 or MM111.

Significance to industry: These findings support previous and ongoing trials (conducted by G. Fazio, T. Auvil) which indicate that apple rootstocks differ in relative performance on orchard replant sites, and by association tolerance to the causal pathogen complex. Thus, use of the Geneva series rootstocks is likely to provide growers with improved productivity on orchard replant sites.

Table 6. Impact of rootstock on recovery of *Pratylenchus penetrans* (lesion nematode) and *Pythium* spp. from apple roots when grown in GC orchard replant soil

Rootstock	# *P. penetrans*/g root Experiment			% *Pythium* root infection
	A	B	C	
Bud9	374b[z]	142a	578a	16.6b
G11	113a	-	434a	13.4b
G16	292ab	196a	1269cd	9.5b
G30	136a	-	472a	10.7b
M7	535b	710c	930	23.0ab
M9 Pajam	416b	254a	672ab	15.0b
M9 Nic29	430b	284a	1556d	13.2b
M26	562b	480b	393a	35.0a
MM106	478b	1754d	499a	34.8a
MM111	597b	850c	1085c	39.1a
Seedling	380b	371ab	956bc	20.5b

[z] Values in the same column followed by the same letter are not significantly ($P>0.05$) different.

Brassica seed meal mechanism(s) of action

Knowledge concerning the means by which any treatment provides disease control is imperative for the effective use of a practice beyond the site in which initial experiments are conducted. The inability of producers to effectively, consistently and predictably utilize many ‘alternative’ treatments, such as composts or compost teas’, for disease control results from an absence of information relative to why disease control was attained, or conversely, why a treatment failed. Numerous studies have reported that the efficacy of brassica plant residues, applied either as a green manure or in the seed meal form used in these studies, is due to the process termed biofumigation. This process relies upon the hydrolysis of chemicals (glucosinolates) in brassica tissues that when hydrolyzed yield various active compounds including isothiocyanates, related to the active compound in the fumigant metam sodium.

Our studies demonstrated with absolute certainty that the mechanism by which brassica residues induce disease control can be pathogen specific, involve a biological rather than a chemical mechanism, and can change over time. This can be demonstrated in the example concerning control of *Rhizoctonia solani* in response to *B. juncea* amendment. Allyl-isothiocyanate (AITC) is generated in response to *B. juncea* seed meal amendment. Production and release of AITC from a test soil was complete within 24 of seed meal amendment (Fig. 4A). When *R. solani* was introduced into soil at the same time as seed meal application (0h), disease was controlled. When introduction of the pathogen was delayed for 24 h, disease in seed meal amended soils is as severe in the control (Fig. 4B). This demonstrated that **initial** disease control was dependent upon production of AITC.

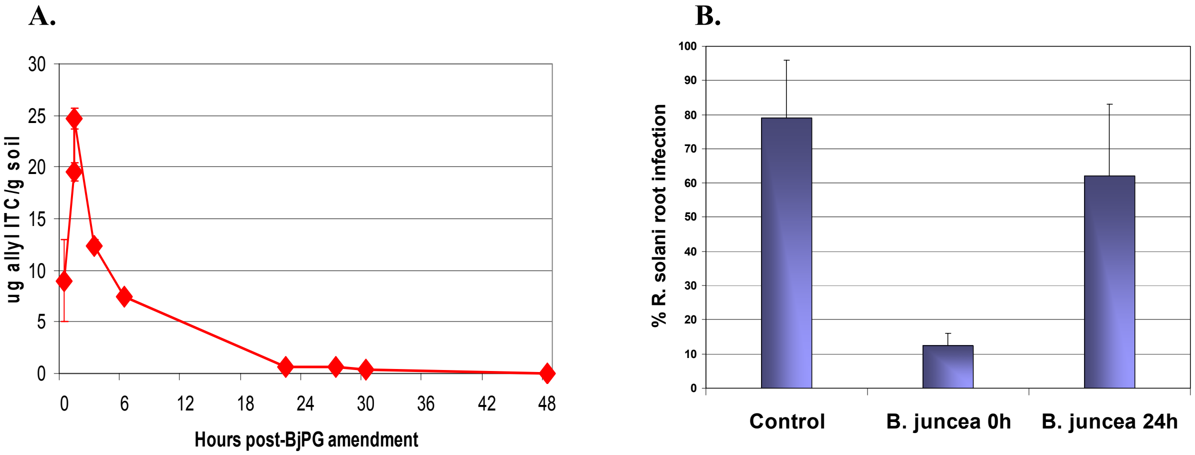

Fig. 4. Release of allyl isothiocyanate from *Brassica juncea* seed meal amended soil over a 48 h period (A). Relative control of *Rhizoctonia solani* when the pathogen was added to soil at the time of seed meal amendment (0h) or when delayed until 24 h after seed meal amendment.

However, the active mechanism changed with time, and disease suppression was restored simply by incubating soils for 4-6 weeks (Table 7). Restoration of disease suppression was dependent, in part, upon proliferation of bacteria belonging to the genus *Streptomyces*, and these bacteria are also responsible for control of *R. solani* attained with *B. napus* or *S. alba* seed meal (Mazzola et al., 2007). Sterilization of the soil prior to adding the pathogen abolished the disease control ability of these seed meals (Cohen and Mazzola, 2005; 2006), providing further support for the role of the resident soil biology in seed meal-induced suppression of *R. solani*. Numerous *Streptomyces* isolates were found to control *R. solani*, and did so through the induction of host defense responses (Cohen and Mazzola, 2006).

Table 7. Control of Rhizoctonia root infection and populations of *Streptomyces* in *B. juncea* seed meal amended soil incubated for four weeks prior to addition of the pathogen.

Treatment	% *R. solani* root infection	*Streptomyces* soil population
Control	79b	1.25×10^5
B. juncea seed meal	28a	3.75×10^7

Significance to industry: These findings are important as they have identified a biological indicator (*Streptomyces* spp.) which can now be monitored to determine when seed meal treated soil has developed suppressiveness to *Rhizoctonia solani*. Likewise, this "indicator species" can now be used in attempts to identify other materials that may be suitable for the control of *Rhizoctonia*. In contrast to *R. solani*, AITC production by *B. juncea* seed meal is the dominant mechanism responsible for control of *Pythium* spp. However, emerging data from this program indicate that an as yet unidentified biological mechanism is responsible for long-term suppression of this pathogen.

Impact of rootstock on Streptomyces populations and seed meal disease control

As cited above, rootstock performance in seed meal amended soils was variable, both in the greenhouse and in the field. In the field, root biomass production for G30 was superior to all other rootstocks examined. However, the short-term duration of these trials and variability among trials preclude the formulation of additional firm conclusions.

Rootstocks did vary in the capacity to support resident populations of *Streptomyces*. G30, Bud9, and G11 supported the largest populations on a per gram root basis, while populations recovered from the roots of M9 were significantly lower (Fig. 5). This is a clear association, but at this time there is not sufficient data to conclusively state a causal role for this association in the relative differences in disease tolerance observed amongst rootstocks.

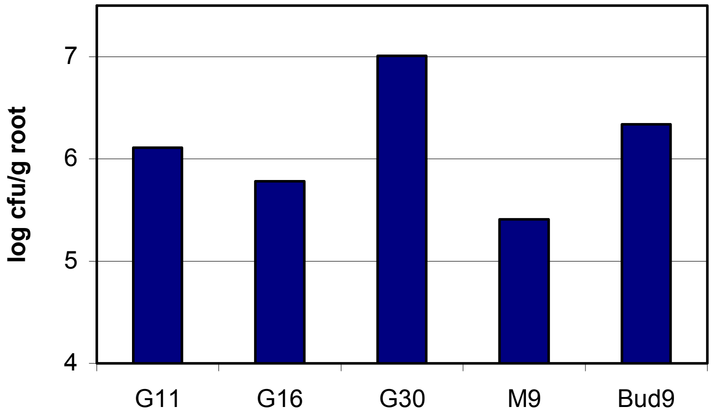

Figure 5. Relative recovery of *Streptomyces* spp. (log cfu/g root) from apple rootstocks grown in GC orchard soil.

Significance to industry: *Streptomyces* are a known to produce a broad-spectrum of antifungal and antibacterial compounds. In our work, we have demonstrated that these bacteria can also induce apple host defense responses. Should additional studies support these initial findings, as well as the importance of these bacteria in disease control, the capacity to support *Streptomyces* populations could serve as a relevant bio-marker for the selection of disease-tolerant rootstocks.

Citations:

Cohen M. F., and **Mazzola, M.** 2006. Impact of resident bacteria, nitric oxide emission and particle size on root infection by *Pythium* spp. and *R. solani* AG-5 in *Brassica napus* seed meal amended soils. Plant and Soil 286:75-86.

Cohen, M. F., Yamasaki, H., and **Mazzola, M.** 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of Rhizoctonia root rot. Soil Biology & Biochemistry 37:1215-1227.

Mazzola, M., Brown, J., Izzo, A, and Cohen, M. F. 2007. Mechanism of action and efficacy of seed meal-induced suppression of pathogens inciting apple replant disease differ in a Brassicaceae species and time-dependent manner. Phytopathology 97:454-460.

Mazzola, M., and Mullinix, K. 2005. Comparative field efficacy of management strategies containing *Brassica napus* seed meal or green manure for the management of apple replant disease. Plant Disease 89:1207-1213.