Interaction of Brassicaceae Seed Meal Soil Amendment and Apple Rootstock Genotype on Microbiome Structure and Replant Disease Suppression

Likun Wang¹ and Mark Mazzola^{2,†}

¹Department of Plant Pathology, Washington State University, Pullman 99164; and ²United States Department of Agriculture–Agricultural Research Service Tree Fruit Research Lab, 1104 N. Western Ave, Wenatchee, WA 98801
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ABSTRACT

Preplant soil application of a *Brassica juncea*–*Sinapis alba* seed meal formulation (SM) at a rate of 6.6 t ha^{−1} alters composition of the orchard soil microbiome in a manner that yields sustainable long-term suppression of soilborne pathogens in apple production systems. However, the cost of SM amendment has hindered the adoption of this tactic to manage apple replant disease in commercial orchards. Greenhouse trials were conducted to assess the effect of reduced SM application rates in concert with apple rootstock genotype on structure of the rhizosphere microbiome and associated disease control outcomes. At all application rates assessed, SM treatment increased tree growth and reduced disease development relative to the control. In general, total tree biomass and leader shoot length were similar in soils treated with SM at 4.4 or 6.6 t ha^{−1} regardless of rootstock genotype. Equivalent increase in tree biomass when cultivated in soil treated at the lowest and highest SM amendment rate

was attained when used in conjunction with G.41 or G.210 apple rootstocks. Suppression of *Pythium* spp. or *Pratylenchus penetrans* root densities was similar at all SM application rates. When cultivated in nontreated replant orchard soil, Geneva rootstocks (G.41 and G.210) exhibited lower levels of *Pythium* spp. and *P. penetrans* root colonization relative to Malling rootstocks (M.9 and MM.106). For a given rootstock, structure of the rhizosphere microbiome was similar in soils treated with SM at 4.4 and 6.6 t ha^{−1}. G.41 and G.210 rootstocks but not M.9 or MM.106 cultivated in soil treated with SM at 2.2 t ha^{−1} possessed a rhizosphere bacterial community structure that differed significantly from the control. Findings indicate that effective control of apple replant disease may be attained at lower SM amendment rates than employed previously, with lower effective rates possible when integrated with tolerant rootstock genotypes such as G.41 or G.210.

In annual production systems, crop monoculture induces changes in composition of the soil microbiome that may initially result in diminished plant productivity, relative to that attained in crop rotation systems, due to increased intensity of soilborne disease (Shipton 1977). Similarly, changes in the soil microbiome in response to the root system of a perennial plant may impede the successful establishment of new plantings when renovation of an orchard or vineyard system is attempted (Mazzola 1999). In apple, difficulty in the establishment of economically viable orchards on sites previously planted to the same or similar tree fruit species is termed replant disease (Traquair 1984). This disease is widespread in all the major fruit-growing regions of the world (Traquair 1984) and is characterized by uneven tree growth, mortality of young trees, and significant yield reductions that extend over the life of the orchard. A preponderance of evidence indicates that this disease is not defined by a single pathogen but, rather, a pathogen complex. In specific regions, this complex has been reported to consist of fungi (e.g., *Ilyonectria* spp. and *Rhizoctonia* spp.), oomycetes (e.g., *Phytophthora* spp. and *Pythium* spp.), and the root lesion nematode *Pratylenchus penetrans* (Jaffee et al. 1982a,b; Mazzola 1998; Tewoldemedhin et al. 2011). The pathogens that contribute to this disease are persistent in soil long after plants have been removed and rapidly become dominant on sites newly planted to apple (Mazzola 1999).

Control of apple replant disease has long relied upon preplant soil fumigation in conventional and organic production systems (Covey

et al. 1979; Mai and Abawi 1981). Despite advances in technology that have led to a reduction in fumigant emissions (Yates et al. 2002), potential for negative impacts on the environment and human health brought about by use of these compounds continues to be a concern. The fumigants commonly used by growers in commercial orchard production, including 1,3-dichloropropene/chloropicrin, are generally perceived as providing effective control of replant disease (Covey et al. 1979; Mai and Abawi 1981). However, a recent study demonstrated that pathogen populations recover rapidly in fumigated soil, and fumigation had a transient (1-year) effect on composition of the apple rhizosphere microbiome compared with nontreated replant orchard soil (Mazzola et al. 2015).

Organic residue-based soil amendments such as animal composts or plant-based green manures are commonly used in crop production to meet plant nutritional needs and control various soilborne diseases. Brassicaceae plant residues have been applied as a cover crop or soil amendment for the control of soilborne diseases in many plant production systems (Rudolph et al. 2015; Sarwar et al. 1998; Smolinska and Horbowicz 1999) due to the perceived biofumigation effects realized in response to production of inhibitory compounds resulting from glucosinolate hydrolysis, including isothiocyanates. Glucosinolate content is specific to the type of tissue and plant species (Kirkegaard and Sarwar 1998). In general, the biofumigation effect is more efficacious when a Brassicaceae seed meal (SM) product, rather than green manure, is utilized (Borek and Morra 2005). The degree of disease control attained in response to application of SM obtained from a single Brassicaceae species may vary with both the SM source and the target pathogen (Cohen and Mazzola 2006; Mazzola et al. 2007). In previous studies, preplant soil application of specific Brassicaceae SM formulations provided control of apple replant disease in a manner equivalent or superior to that attained in response to preplant soil fumigation (Mazzola et al. 2015).

More recently, mechanisms other than those due to a chemical effect have been proposed to function in the disease control achieved in response to Brassicaceae soil amendments. For

[†]Corresponding author: M. Mazzola; E-mail: mark.mazzola@ars.usda.gov

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example, suppression of charcoal rot of strawberry, caused by *Macrophomina phaseolina*, was obtained using SM sourced from various Brassicaceae species and was not associated with generation of a biologically active compound (Mazzola et al. 2017). Brassicaceae SM soil amendment significantly reduced infection of roots of winter wheat seedlings by *Rhizoctonia solani* AG-8 compared with the nontreated control, regardless of SM glucosinolate content (Handiseni et al. 2013). Studies have indicated that alternative mechanisms, including induction of host defense responses (Cohen et al. 2005) and SM-induced transformation of the soil microbiome to a pathogen-suppressive state (Mazzola and Zhao 2010; Mazzola et al. 2015; Weerakoon et al. 2012), contribute to overall disease control attained in response to soil incorporation of Brassicaceae residues.

In situations where soil fumigation continues to be a viable disease control method, the cost of the SM product applied at rates utilized in previous studies may preclude grower adoption of the SM disease management strategy. In the earlier trials, SM was applied to orchard soils at a rate of 6.6 metric tons (t) ha⁻¹, resulting in a product cost of U.S.\$5,000 to 13,000 ha⁻¹, depending on the SM formulation and source of the product. Even though SM treatment can result in significant yield increases relative to soil fumigation (Mazzola and Brown 2010; Mazzola et al. 2015), it may be difficult to alter growers' perceptions of this treatment if the initial SM amendment is considerably more expensive compared with soil fumigation. Reducing rates of SM to decrease orchard establishment costs while continuing to provide disease control comparable with soil fumigation may accelerate the commercial adoption of the SM management technique to control replant disease.

The apple rhizosphere microbiome has been shown to differ in composition among apple rootstock genotypes (St. Laurent et al. 2010). *Burkholderia cepacia*, which is known to suppress a variety of pathogens, including *Pythium* spp. and *R. solani*, was detected in the root zone of G.210 but not M.26 apple rootstock grown in the same orchard soil (St. Laurent et al. 2010). As demonstrated in preliminary trials, the efficiency of SM-induced disease control could be influenced by apple rootstock genotype. Tree growth performance in SM-treated soil was greater for Gala apple grafted to G.11 than M.9 rootstock even though trees exhibited identical growth rates in untreated orchard soils after two growing seasons, regardless of rootstock genotype, (Mazzola et al. 2015). Therefore, mechanisms contributing to SM-induced pathogen suppression that operate through activity of the soil or rhizosphere microbiome may be amplified when combined with rootstock genotypes that support microbial elements functional in pathogen suppression.

The objectives of this study were to determine whether (i) the efficacy of a Brassicaceae SM formulation was influenced by rootstock genotype, (ii) effective disease control could be attained at reduced application rates of SM, and (iii) differences in composition of the rhizosphere microbiome were associated with SM application rate or rootstock-dependent differences in disease suppression.

MATERIALS AND METHODS

Soils. Soils used in this study were collected from a commercial orchard (GC) in Manson, WA (N 47°53'05", W 120°09'30"). The orchard was established in 1991 on a site previously planted to apple and was not fumigated prior to replanting of cultivar Golden Delicious on M.7 rootstock. Chelan gravelly sandy loam is the dominant soil type at the GC orchard and characteristics of these soils were previously described (Mazzola 1998; Mazzola et al. 2001). The dominant apple root pathogens resident to this soil are *Pythium attrantheridium*, *P. ultimum*, *P. irregulare*, *R. solani* AG-5 and AG-6, *Ceratobasidium* sp. AG-G, and root lesion nematode *Pratylenchus penetrans* (Mazzola et al. 2007, 2009). Soils were collected within the tree rows at a depth of 10 to 30 cm in October of each year for the three replicate studies conducted in 2014, 2015, and 2016.

Brassicaceae SM. SM utilized in this study were derived from *Brassica juncea* 'Pacific Gold' (Brown et al. 2004) and *Sinapis alba* 'Ida Gold' (Brown et al. 1997). The individual SM were blended together at a ratio of 1:1 and passed through a 1-mm² sieve prior to use. The mineral content and glucosinolate content of these SM were as previously reported (Handiseni et al. 2011; Mazzola et al. 2015).

Soil treatments. SM was applied to moist soil (soil water potential [Ψ] = -63 to -92 kPa) at a rate of 2.2, 4.4, or 6.6 t ha⁻¹. Soil was mixed thoroughly by hand during the process of incorporating the SM and was transferred into 3.8-liter pots after treatment application. Potted soils were sealed in gas-impermeable, transparent Saranex bags (17.8 by 20.3 cm, Bitran Series "S" bags; Com-Pac International, Carbondale, IL) which served as a surrogate for tarping in the field to enhance retention of SM-derived volatile compounds (e.g., allyl isothiocyanate [AITC]). Pots were arranged in a completely randomized design on a greenhouse bench and bags were removed at 48 h posttreatment. Duration of the soil incubation period in the bagged environment was based upon previous studies demonstrating the completion of AITC generation within 24 h post-SM amendment (Mazzola and Zhao 2010). Myrosinase activity and glucosinolate hydrolysis was confirmed by monitoring AITC emission from samples of a soil treated with SM at 2.2 t ha⁻¹ using previously described methods (Mazzola et al. 2007). AITC production was detected within 1 h of SM application and peaked at 2 h post-SM application (0.54 μg ml⁻¹ head space air sample). Bulk soil samples were collected from all pots immediately after removal from the bags and at 6 weeks posttreatment. The 6-week incubation period allowed for degradation of potential herbicidal compounds produced in response to SM application, including ionic thiocyanate that results from hydrolysis of the *S. alba*-derived 4-hydroxybenzyl glucosinolate (Borek and Morra 2005).

Soil pasteurization was used in these studies as a proxy for soil fumigation. Pasteurization was conducted by heating moistened soil in an oven at 80°C for 18 h, followed by cooling for 12 h, prior to heating for an additional 18 h at 80°C. A no-treatment control (NTC) soil was included in all experiments. To compensate for the nitrogen input provided by the application of *B. juncea*-*S. alba* SM, 9.5 g per pot of soybean meal was incorporated into NTC soils, resulting in a nitrogen input equivalent to that of the SM treatment at 6.6 t ha⁻¹.

Plant assay. Apple rootstocks used in these studies—Geneva.41 (G.41), G.210, Malling.9 (M.9), and Malling-Merton.106 (MM.106)—were obtained from Willow Drive Nursery, Ephrata, WA. The G.41 and G.210 rootstocks are considered to possess tolerance to replant disease whereas M.9 and MM.106 are susceptible to the disease (Auvil et al. 2011; Leinfelder and Merwin 2006). Rootstocks possessed a uniform tree stem caliper (diameter) of 0.95 cm. At completion of the 6-week post-SM amendment incubation period, one apple rootstock was planted per pot with five replicates of each rootstock per soil treatment. Plants were grown in the greenhouse at 25 to 28°C with a 14-h photoperiod, watered at 3-day intervals, and harvested 3 months after planting. Rootstock total fresh weight and length of the dominant leader shoot were measured before planting and at harvest. Upon removal from the pot, rootstock rhizosphere soil was collected by shaking loosely adhering soil from roots and carefully removing the remaining firmly adhering soil from root surfaces into collection bags. The total root system was collected at harvest. The experiment was repeated twice (a total of three trials). All experiments possessed SM treatments of 2.2, 4.4, and 6.6 t ha⁻¹; pasteurization; and NTC treatments, with five replicates of each rootstock per treatment. The soybean meal soil amendment treatment was included only in the third experiment.

Assessment of root infection and microbial community populations. Three separate 1-g subsamples of rhizosphere soil were obtained from each tree and individually placed in tubes containing 10 ml of sterile distilled water. Soil suspensions were prepared by mixing on a vortex mixer for 60 s, followed by serial

dilution and spreading on a *Pythium* semiselective medium (PSSM) (Mazzola et al. 2001) for estimation of populations of *Pythium* spp. For postrhizosphere soil sampling, apple roots were carefully washed in tap water. Nematode extraction was conducted by placing a fine root sample (0.5 g) into a 125-ml flask containing 70 ml of sterile distilled water and incubating at 22°C with a 12-h photoperiod on a gyratory shaker at 140 rpm min⁻¹ for 5 days. Nematodes were collected by passing the wash water through a 38- μ m² sieve twice, then backwashing into a counting Petri dish using distilled water. Numbers of *P. penetrans* were determined by counting using a light microscope ($\times 40$). Root infection by *Pythium* spp. was assessed by plating 10 root segments (0.5 to 1.0 cm) from each rootstock onto PSSM agar, with two replicate plates for each root sample. Plates were incubated at room temperature with a 12-h photoperiod for 2 to 3 days, at which time hyphal growth was examined using a light microscope ($\times 100$). Determination of pathogen root infection and soil or root microbial community composition were measured in all three experiments whereas enumeration of *Pythium* spp. rhizosphere soil populations was only conducted in the first and second experiments.

Terminal-restriction fragment length polymorphism (T-RFLP) analysis was used to profile bacterial and fungal communities in rhizosphere soil, bulk soil, and apple roots. Extraction of DNA from bulk soil, rhizosphere soil, and root samples was conducted using the MO BIO PowerMax Soil DNA Isolation Kit, PowerSoil DNA Isolation Kit, and UltraClean Plant DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), respectively. Fluorescently labeled polymerase chain reaction (PCR) products were generated using labeled internal transcribed spacer (ITS)1F/ITS4 primers for amplifying the fungal ITS region and 8f/907r primers for amplification of the bacterial 16S ribosomal DNA region. Each 50- μ l PCR mixture contained 10 ng of genomic DNA, 10% 10 $\times$ PCR buffer, 1.5 mM MgCl₂, 0.6 mM dNTP mix, 2.5 U of AmpliTaq Gold polymerase (Applied Biosystem, Austin, TX), and 1 mM each labeled primer (Sigma-Aldrich, The Woodlands, TX). PCR were performed in an automated thermal cycler (2720 Thermal cycler; Applied Biosystem). Reaction parameters for the fungal primers were initial denaturation for 5 min at 94°C and 40 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 58°C, and extension at 72°C for 1 min. Reaction parameters for the bacterial primers were initial denaturation for 5 min at 94°C and 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 52°C, and extension at 72°C for 1.5 min. Fungal and bacterial PCR products (10 μ l) were digested using 10 U of *Hae*III and *Hha*I (New England Biolabs, Ipswich, MA) and *Hae*III, respectively, at 37°C for 6 h. Restriction fragments were purified by ethanol precipitation according to Beckman Coulter protocol 608019 (Beckman Coulter, Brea, CA) and resuspended in 40 μ l of sample loading solution and 0.5 μ l of 600 size standard. T-RFLP fragments were separated using the CEQ 8000 Genetic Analysis System (Beckman Coulter) with the following conditions: a capillary temperature of 50°C, denaturation for 120 s at 90°C, injection time of 9 s at 2 kV, and separation for 85 min at 4.8 kV. T-RFLP profiles were analyzed using the fragment analysis module of CEQ 8000 Genetic Analysis Software with settings of a slope threshold of 10, relative peak height threshold of 1%, confidence level of 95%, and quadratic calibration curve with dye mobility calibration PA ver.1 and calculated dye spectra. Fragments were binned with a size range of 1 bp using the amplified fragment length polymorphism tool, with a Y-axis threshold set to 1,000 to exclude background peaks of the sample that occurred in tandem with the DNA standard peaks.

Data analysis. Plant growth data were subjected to analysis of variance (ANOVA) using a general linear model (Proc GLM, SAS version 9.4; SAS Institute, Cary, NC). Means separation was conducted using Tukey's honestly significant difference test ($\alpha = 0.05$). A two-way data analysis was performed for cross-examination of the soil treatments associated with rootstock genotypes.

Nonnormally distributed microbial count data were log₁₀ (count + 1) transformed, and percentage data of *Pythium* spp. root infection were converted to fractions before analyzing using Poisson regression (Gardner et al. 1995) (Proc GENMOD in SAS). Count data of *Pythium* spp. in the soil and *P. penetrans* in the roots were zero inflated and, thus, were analyzed using zero-inflated Poisson regression. Comparison of the means was performed using least-square means ($P \leq 0.05$ considered significant). When an experimental effect was present, data from different experiments were analyzed individually, and the experimental effect was presented.

Differences in bacterial and fungal community composition among soil treatment groups were analyzed by nonmetric multidimensional scaling (NMDS) of the T-RFLP data using PAST ver. 3.16 (Hammer et al. 2001). The Dice similarity coefficient was calculated among groups of samples and used to perform ordination and one-way analysis of similarity (ANOSIM). Results that differed at $P \leq 0.05$ were regarded as significant. Large positive R (up to 1) values indicate dissimilarity among groups (Hammer et al. 2001).

RESULTS

Influence of soil treatment and rootstock genotype on tree growth. In all three independent trials, the ANOVA showed that rootstock total fresh weight differed significantly among soil treatments and rootstock genotypes (Table 1). Furthermore, SM treatments ($P < 0.001$), soybean meal amendment ($P < 0.001$), and soil pasteurization ($P < 0.001$) all promoted tree biomass relative to the NTC 3 months after planting (Table 2). Regardless of rootstock genotype, the increase in total rootstock fresh weight at harvest was greater for trees cultivated in soils amended with SM at 4.4 and 6.6 t ha⁻¹ than the NTC (Table 2). In two of the three experiments, similar rootstock biomass was attained regardless of the application rate of SM when used in conjunction with G.41 or G.210 rootstock. In contrast, M.9 and MM.106 rootstock consistently exhibited a significant increase in total fresh weight when SM application rate was increased from 2.2 to 6.6 t ha⁻¹ (Table 2). The nitrogen input resulting from soybean meal application was equivalent to that of the SM treatment at 6.6 t ha⁻¹; however, soybean meal amendment resulted in an increase in tree biomass less than that attained in response to SM at 4.4 and 6.6 t ha⁻¹ (Table 2). In experiment 3, when comparison was made within the NTC or within the soybean meal treatment, increase in fresh weight did not differ significantly among rootstocks.

Significant soil treatment and rootstock genotype effects on leader shoot length were observed in each of the three independent

TABLE 1. Effects of *Brassica juncea*-*Sinapis alba* seed meal formulation, rootstock genotype, and their interaction on apple rootstock growth in GC orchard (Manson, WA) replant soil

Experiment, source ^z	Total weight		Shoot length	
	<i>F</i>	<i>P</i> > <i>F</i>	<i>F</i>	<i>P</i> > <i>F</i>
Experiment 1				
Rootstock genotype	13.660	<0.001	8.720	<0.001
Soil treatment	24.430	<0.001	21.900	<0.001
Rootstock genotype $\times$ soil treatment	1.360	0.203	1.250	0.269
Experiment 2				
Rootstock genotype	2.530	0.048	8.250	<0.001
Soil treatment	19.680	<0.001	41.640	<0.001
Rootstock genotype $\times$ soil treatment	0.980	0.474	1.720	0.079
Experiment 3				
Rootstock genotype	6.900	<0.001	28.200	<0.001
Soil treatment	51.560	<0.001	42.290	<0.001
Rootstock genotype $\times$ soil treatment	0.690	0.787	1.420	0.155

^z Data were subjected to analysis of variance using a general linear model.

experiments (Table 1). Tree leader length was greater ($P < 0.05$) for rootstocks cultivated in SM-treated soil relative to the NTC soil for all three experiments (Table 3). Additionally, leader shoot length was similar ($P = 0.121$ and $P = 0.062$) at harvest for rootstocks cultivated in soil treated with SM at 4.4 and 6.6 ha^{-1} , respectively, in experiments 2 and 3, which was significantly ($P < 0.05$) greater than the lowest SM application rate (2.2 t ha^{-1}) for all experiments (Table 3). Leader shoot length of trees grown in soils amended with

TABLE 2. Increase in total apple rootstock fresh weight at 3 months postplanting in GC orchard (Manson, WA) replant soil as affected by soil treatment and rootstock genotype

Experiment, soil treatment ^z	Weight (g) per rootstock ^y			
	MM.106	M.9	G.41	G.210
Experiment 1				
SM at 2.2 t/ha	67.52 ab	46.82 ab	65.52 b	78.28 b
SM at 4.4 t/ha	85.18 b	59.72 bc	87.28 b	124.54 c
SM at 6.6 t/ha	110.28 c	74.62 c	84.50 b	115.10 c
Pasteurization	73.28 ab	43.02 ab	63.42 b	65.60 ab
NTC	54.34 a	29.48 a	27.73 a	41.26 a
Experiment 2				
SM at 2.2 t/ha	82.46 b	60.04 ab	73.28 a	112.02 bc
SM at 4.4 t/ha	122.68 c	95.84 c	119.80 b	143.94 c
SM at 6.6 t/ha	135.30 c	95.74 c	139.08 b	127.20 bc
Pasteurization	56.94 ab	69.92 bc	62.34 a	39.06 a
NTC	41.46 a	33.56 a	46.64 a	63.45 ab
Experiment 3				
SM at 2.2 t/ha	112.64 b	101.14 b	100.44 bc	133.15 bc
SM at 4.4 t/ha	139.52 c	135.00 c	127.44 c	165.80 c
SM at 6.6 t/ha	147.52 c	131.84 c	119.76 c	144.80 c
Soybean meal	119.46 b	100.32 b	96.18 b	100.50 b
Pasteurization	81.48 b	83.84 b	59.98 a	94.74 ab
NTC	48.26 a	40.50 a	42.42 a	56.40 a

^y Values are means of five replicates. Means in the same column for an individual experiment followed by the same letter are not significantly ($P > 0.05$) different according to Tukey's test.

^z SM = *Brassica juncea*-*Sinapis alba* seed meal formulation (1:1) and NTC = no-treatment control.

TABLE 3. Length (cm) of leader shoot at 3 months postplanting in GC orchard (Manson, WA) replant soil as affected by soil treatment and apple rootstock genotype

Experiment, soil treatment ^z	Length (cm) per rootstock ^y			
	MM.106	M.9	G.41	G.210
Experiment 1				
SM at 2.2 t/ha	36.44 ab	32.90 abc	24.66 ab	23.48 b
SM at 4.4 t/ha	37.40 b	38.28 bc	37.16 bc	30.62 bc
SM at 6.6 t/ha	58.30 c	40.22 c	46.04 c	33.72 c
Pasteurization	28.28 a	21.68 a	24.64 ab	24.26 b
NTC	28.12 a	24.22 ab	15.80 a	12.60 a
Experiment 2				
SM at 2.2 t/ha	39.80 b	32.24 ab	22.30 a	36.26 ab
SM at 4.4 t/ha	59.10 c	67.52 c	39.74 b	56.72 bc
SM at 6.6 t/ha	85.24 d	49.26 bc	52.84 b	61.58 c
Pasteurization	35.60 ab	24.64 a	23.28 a	22.86 a
NTC	25.68 a	15.20 a	16.48 a	18.66 a
Experiment 3				
SM at 2.2 t/ha	49.50 b	32.98 b	33.72 ab	31.92 b
SM at 4.4 t/ha	73.40 c	46.50 ab	40.00 bc	51.06 c
SM at 6.6 t/ha	75.68 c	52.60 c	58.06 c	46.15 c
Soybean meal	49.68 b	36.58 b	37.78 b	33.44 b
Pasteurization	39.22 ab	33.36 b	30.52 ab	26.92 ab
NTC	32.34 a	17.38 a	21.32 a	20.04 a

^y Values are means of five replicates. Means in the same column for an individual experiment followed by the same letter are not significantly ($P > 0.05$) different according to Tukey's test.

^z SM = *Brassica juncea*-*Sinapis alba* seed meal formulation (1:1) and NTC = no-treatment control.

SM at 2.2 t ha^{-1} was similar ($P > 0.05$) to that attained in response to soybean meal amendment and pasteurized treatments (Table 3). When comparing leader shoot length among rootstock genotypes, MM.106 cultivated in SM-amended soil consistently had the greatest ($P < 0.05$) shoot length across the three trials, a feature that may be attributed to relative vigor of these rootstock genotypes.

Effect of soil treatment and rootstock genotype on pathogen densities. Poisson regression analysis demonstrated that *Pythium* spp. rhizosphere soil densities at 3 months postplanting did not differ significantly ($P = 0.975$) between experiments 1 and 2; thus, data were pooled for analysis. *Pythium* spp. were not recovered from rootstock rhizosphere soils of the treatments with SM at 4.4 or 6.6 t ha^{-1} (Table 4) and were not detected in the roots of apple cultivated in soils treated with SM at these two rates (Table 5). *Pythium* spp. rhizosphere populations were significantly affected by treatment ($P < 0.001$); however, there was no rootstock genotype effect observed ($P = 0.153$). *Pythium* spp. were detected in rhizosphere soil from the SM treatment at 2.2 t ha^{-1} at 3 months after planting but the population was significantly smaller than in rhizosphere soil from the NTC (Table 4).

Root infection by *Pythium* spp. was significantly affected by soil treatment across the three experiments (experiment 1, $P < 0.001$; experiment 2, $P < 0.001$; and experiment 3, $P < 0.001$). In experiments 1 and 3, *Pythium* spp. were only isolated from roots of M.9 and MM.106 rootstocks grown in soil treated with SM at 2.2 t ha^{-1} , and was not recovered from Geneva rootstocks cultivated in the same soil treatment (Table 5). *Pythium* spp. root infection was significantly ($P < 0.05$) less for all SM treatments relative to the control for all three experiments (Table 5). In the third experiment, *Pythium* spp. root infection at harvest was significantly ($P < 0.001$) greater for apple cultivated in soybean-meal-amended soil relative to the NTC (Table 5). In experiments 1 and 3, *Pythium* spp. root infection for M.9 cultivated in the NTC soil was significantly greater than for G.41 ($P < 0.001$ and $P = 0.047$, respectively) and G.210 ($P < 0.001$ and $P = 0.002$, respectively) rootstocks.

P. penetrans rootstock root density was consistently affected by soil treatment across the three experiments (experiment 1, $P < 0.001$; experiment 2, $P < 0.001$; and experiment 3, $P < 0.001$). *P. penetrans* was recovered from roots of trees grown in the NTC, soybean-meal-amended, and SM at 2.2 t ha^{-1} soils but not in soil treated with SM at 4.4 or 6.6 t ha^{-1} or pasteurized soils (Table 6). Although *P. penetrans* root density was not significantly affected by rootstock genotype in experiments 1 and 2 (experiment 1, $P = 0.893$ and experiment 2, $P = 0.484$), M.9 and MM.106 rootstocks possessed significantly greater *P. penetrans* root densities compared with the Geneva rootstocks when cultivated in the NTC orchard soil ($P < 0.001$) (Table 6). A significant ($P = 0.023$) rootstock genotype effect was found in the third experiment with the soybean meal soil treatment, which resulted in significantly

TABLE 4. Density of *Pythium* spp. recovered from the rhizosphere of apple rootstocks at 3 months postplanting in GC orchard (Manson, WA) replant soil as influenced by soil treatment and rootstock genotype

Soil treatment ^z	Density per rootstock ^y			
	MM.106	M.9	G.41	G.210
SM at 2.2 t/ha	0.83 a	0.83 a	1.30 b	1.82 b
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	2.72 b	2.75 b	2.24 c	2.59 c

^y Pooled data from experiments one (2014) and two (2015) presented in the table were $\log_{10}$ (CFU g^{-1} soil + 1) transformed. Means in the same column followed by the same letter are not significantly ($P > 0.05$; $n = 5$) different based on pairwise comparisons using least square means.

^z SM = *Brassica juncea*-*Sinapis alba* seed meal formulation 1:1 and NTC = no-treatment control.

reduced lesion nematode root density relative to the NTC for the G.41, M.9, and MM.106 rootstocks.

Effect of SM treatment on bulk soil microbiome structure (preplanting). For both fungi (ANOSIM: $R = 0.161$ at 48 h, $R = 0.683$ at 6 weeks) and bacteria ($R = 0.098$ at 48 h, $R = 0.758$ at 6 weeks), differences in community structure among soil treatments became evident between 48 h and 6 weeks posttreatment (Fig. 1). Significant differences in soil fungal community structure

TABLE 5. *Pythium* spp. apple root infection as influenced by soil treatment and rootstock genotype at 3 months postplanting in GC orchard (Manson, WA) replant soil

Experiment, soil treatment ^z	Infection (%) per rootstock ^y			
	MM.106	M.9	G.41	G.210
Experiment 1				
SM at 2.2 t/ha	0.00 a	2.00 a	0.00 a	0.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	4.00 b	24.00 b	4.00 b	6.00 b
Experiment 2				
SM at 2.2 t/ha	4.00 a	0.00 a	6.00 a	16.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	54.00 b	52.00 b	58.00 b	42.00 b
Experiment 3				
SM at 2.2 t/ha	4.00 a	4.00 a	0.00 a	0.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Soybean meal	35.00 c	49.00 c	63.00 b	27.00 b
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	13.00 b	28.00 b	2.00 a	3.00 a

^y For each experiment, means in the same column followed by the same letter are not significantly ($P > 0.05$; $n = 5$) different based on pairwise comparisons using least square means.

^z SM = *Brassica juncea*–*Sinapis alba* seed meal formulation 1:1 and NTC = no-treatment control.

TABLE 6. Density of *Pratylenchus penetrans* recovered from apple rootstock roots as influenced by soil treatment and rootstock genotype at 3 months postplanting in GC orchard (Manson, WA) replant soil

Experiment, soil treatment ^z	Density (nematodes g ⁻¹ of root) per rootstock ^y			
	MM.106	M.9	G.41	G.210
Experiment 1				
SM at 2.2 t/ha	0.10 a	0.40 a	0.00 a	0.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	454.90 b	738.40 b	266.10 b	15.60 b
Experiment 2				
SM at 2.2 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	27.60 b	23.20 b	37.60 b	31.50 b
Experiment 3				
SM at 2.2 t/ha	0.40 a	0.00 a	0.40 a	0.00 a
SM at 4.4 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
SM at 6.6 t/ha	0.00 a	0.00 a	0.00 a	0.00 a
Soybean meal	13.20 b	24.40 b	43.80 b	28.00 b
Pasteurization	0.00 a	0.00 a	0.00 a	0.00 a
NTC	527.80 c	783.20 c	351.80 c	67.60 b

^y For each experiment, means in the same column followed by the same letter are not significantly ($P > 0.05$; $n = 5$) different based on pairwise comparisons using least square means.

^z SM = *Brassica juncea*–*Sinapis alba* seed meal formulation 1:1 and NTC = no-treatment control.

existed between the treatments with SM at 2.2 t ha⁻¹ and at 4.4 or 6.6 t ha⁻¹ at 48 h ($P = 0.008$ and $P = 0.009$, respectively) but composition of the community in the soil treated with SM at 2.2 t ha⁻¹ was similar ($P = 0.947$) to the NTC (Fig. 1A). At 6 weeks post-SM amendment, fungal communities in all treatments were distinct and significantly ($P < 0.05$) different from each other (Fig. 1B). Bacterial community structure did not exhibit distinct differences among soil treatments at 48 h post-SM application (Fig. 1C). After 6 weeks, bacterial community structures in soils treated with SM at 2.2, 4.4, and 6.6 t ha⁻¹ were similar ($P \geq 0.069$) but differed significantly ($P \leq 0.008$) from the NTC soil (Fig. 1D).

Effect of soil treatment and rootstock genotype on rhizosphere microbiome composition (at harvest). NMDS analysis revealed shifts in rhizosphere microbial community structure related to soil treatment and rootstock genotype (Fig. 2). At harvest, the MM.106 rhizosphere fungal community composition did not differ between the control and SM treatment at 2.2 t ha⁻¹ ($P = 0.420$) but differed significantly ($P < 0.05$) among all other soil treatment comparisons (Fig. 2A). M.9 rhizosphere fungal community composition was dissimilar among all soil treatments ($P \leq 0.033$). For a given Geneva rootstock (G.41 or G.210), structure of the rhizosphere fungal communities was dissimilar among all soil treatments, with two exceptions; rhizosphere fungal communities from G.41 cultivated in SM-treated soil at 4.4 and 6.6 t ha⁻¹ were similar ($P = 0.065$) (Fig. 2B), as were the communities from G.210 planted in soil treated with SM at 2.2 or 4.4 t ha⁻¹ ($P = 0.062$).

SM treatments altered rhizosphere bacterial community structure in a rootstock-dependent manner. For M.9 and MM.106 (Fig. 2C) rootstocks, rhizosphere bacterial communities were similar in composition between the NTC and SM treatments at 2.2 t ha⁻¹ (M.9: $P = 0.214$, MM.106: $P = 0.391$). When cultivated in SM at 4.4 and 6.6 t ha⁻¹, these same rootstocks possessed rhizosphere bacterial communities that were dissimilar ($P < 0.05$) in composition from that detected in the NTC. In contrast, regardless of SM input rate, bacterial community structure in the rhizosphere of G.41 (Fig. 2D)

Fig. 1. Influence of *Brassica* seed meal application rate on soil fungal community composition at A, 48 h and B, 6 weeks and soil bacterial community composition at C, 48 h and D, 6 weeks posttreatment. Ordination of soil microbiomes was conducted by nonmetric multidimensional scaling of terminal restriction fragment length polymorphism-derived data and distance was based on Dice dissimilarity coefficient. Data shown are from experiment 2, initiated in October 2015. SM = *Brassica juncea*–*Sinapis alba* seed meal formulation (1:1) and NTC = no-treatment control.

and G.210 differed significantly from the NTC. For a given rootstock, rhizosphere bacterial communities exhibited greater dissimilarity across soil treatments, based upon ANOSIM R value, for rootstocks G.41 ($R = 0.780$) and G.210 ($R = 0.715$) relative to MM.106 ($R = 0.563$) and M.9 ($R = 0.432$).

Consideration of rootstock genotype effects, independent of SM amendment, on the rhizosphere microbiome was defined by comparing fungal and bacterial communities detected in the rootstock rhizosphere when cultivated in the NTC soil. G.41 and G.210 rhizosphere fungal communities did not differ significantly ($P = 0.063$) in composition but were distinct ($P < 0.05$) from the community in the rhizosphere of rootstock M.9 (Fig. 3A). The rhizosphere bacterial community from G.41 was distinct from MM.106 ($P = 0.010$) and M.9 ($P = 0.008$) but did not differ significantly ($P = 0.096$) from G.210 (Fig. 3B).

Effect of soil treatment and rootstock on composition of the root microbiome. For a given rootstock (G.41 or MM.106), the root fungal community composition was similar when planted in soils amended with SM at 4.4 and 6.6 t ha⁻¹ (G.41, $P = 0.161$; MM.106, $P = 0.306$) but distinct ($P < 0.05$) from the NTC. Additionally, composition of the MM.106 root fungal community when cultivated in pasteurized soil was similar to that of the SM treatments ($P > 0.05$) but differed significantly from that of the NTC ($P = 0.007$). For rootstock G.41, root fungal community composition in soil treated with SM at 2.2 t ha⁻¹ and pasteurized soil were similar to SM at 6.6 t ha⁻¹ ($P = 0.078$ and $P = 0.193$, respectively).

Root bacterial community composition in the NTC differed ($P < 0.05$) from that of the SM treatments at 4.4 and 6.6 t ha⁻¹ for rootstocks MM.106 and G.41; however, no significant difference ($P = 0.669$ and $P = 0.069$) was observed between SM at 4.4 and 6.6 t ha⁻¹. Bacterial community composition in roots of MM.106 cultivated in pasteurized soil differed ($P = 0.032$) from the NTC but was not different from the treatments with SM at 2.2 ($P = 0.227$),

4.4 ($P = 0.051$), and 6.6 t ha⁻¹ ($P = 0.077$). In roots of G.41, bacterial community composition was distinct between the treatments with SM at 2.2 and 4.4 t ha⁻¹ ($P = 0.007$), whereas no difference was observed between SM at 2.2 and 6.6 t ha⁻¹ ($P = 0.076$) or between SM at 2.2 t ha⁻¹ and the pasteurized treatment ($P = 0.541$).

Although significant differences in composition of the root microbiome were detected among treatment groups, in general, the microbiome detected in apple rootstock roots demonstrated greater degrees of overlap across soil treatments based on ANOSIM. For root fungal communities, R_{ANOSIM} across soil treatments was 0.214 for MM.106 and 0.302 for G.41, indicating that the MM.106 root fungal communities were highly similar and the G.41 communities had significant overlap among soil treatments (Ramette 2007). For root bacterial communities, across soil treatments, $R = 0.283$ and 0.237 for MM.106 and G.41, respectively.

DISCUSSION

Various preplant soil amendments have been utilized as a measure to control replant disease and improve growth of apple, including composts (van Schoor et al. 2009) and monoammonium phosphate fertilizer (Slykhuis and Li 1985). However, these strategies had inconsistent effects on tree growth and yield, and generally did not diminish root infection by disease-causing agents (Nielsen and Yorston 1991; Yao et al. 2006). However, reliable promotion of apple growth and yield on replant orchard sites in response to SM amendment has been reported in various orchard trials conducted over several growing seasons (Mazzola and Brown 2010; Mazzola et al. 2015). Vegetative growth and fruit yield over four growing seasons was superior when trees were cultivated in soil receiving preplant *B. juncea*-*S. alba* SM amendment at a rate of 6.6 t ha⁻¹ relative to fumigated soil at multiple orchard locations (Mazzola et al. 2015). In the same study, enhanced tree performance in SM-amended soil was directly associated with persistent suppression of root infection by multiple replant pathogens. The current study was conducted to examine potential integration of host genetics with lower SM application rates as a means to provide effective control of apple replant disease while reducing overall treatment costs. A significant increase in vegetative tree growth was observed for all SM treatments relative to the control, and tree growth attained in SM-treated soil was comparable with or superior to that in pasteurized soil regardless of amendment rate.

Findings from this study support those of previous reports (Cohen et al. 2005; Mazzola et al. 2001; Weerakoon et al. 2012), indicating that enhanced tree growth and disease control attained in response to SM amendment is not exclusively dependent on nutrient input or

Fig. 2. Influence of a *Brassica* seed meal formulation on composition of the fungal and bacterial community in rhizosphere soils of rootstock MM.106 **A**, fungal community and **C**, bacterial community and G.41 **B**, fungal community and **D**, bacterial community at harvest (3 months postplanting). Ordination of soil microbiomes was conducted by nonmetric multidimensional scaling of terminal restriction fragment length polymorphism-derived data and distance was based on Dice dissimilarity coefficient. Data shown are from experiment 2, initiated in October 2015. SM = *Brassica juncea*-*Sinapis alba* seed meal formulation (1:1) and NTC = no-treatment control.

Fig. 3. Effect of rootstock genotype on **A**, fungal and **B**, bacterial community composition detected in rhizosphere soil at harvest (3 months after planting) when cultivated in untreated orchard soil. Ordination of soil microbiomes was conducted by nonmetric multidimensional scaling of terminal restriction fragment length polymorphism-derived data and distance was based on Dice dissimilarity coefficient. Data shown are from experiment 2, initiated in October 2015.

glucosinolate hydrolysis product concentrations. In fact, soybean meal, applied to provide plant-available N at a level equivalent to that of the treatment with SM at 6.6 t ha⁻¹, resulted in significantly greater apple root infection by *Pythium* spp. in this and previous studies (Cohen et al. 2005), and a corresponding reduction in growth relative to that attained in response to SM applied at a rate of 4.4 or 6.6 t ha⁻¹. SM-induced control of *Pythium* spp. apple root infection relies upon the generation of biologically active compounds and SM-induced changes in the soil microbiome, including proliferation of *Trichoderma* spp. (Weerakoon et al. 2012). Based on ANOSIM of T-RFLP-derived data, bulk soil fungal community composition at 6 weeks posttreatment in soybean-meal-amended soil was not distinguishable from the NTC ($P = 0.097$), suggesting that increased nitrogen is not the sole cause of SM-induced shifts in the fungal microbiome that were observed here and previously.

P. penetrans densities were significantly less in roots of trees cultivated in SM- and soybean-meal-amended soil relative to the NTC, regardless of glucosinolate input levels used in this study. Similarly, equivalent suppression of *P. penetrans* was attained in response to soil application of *B. napus* SM or soybean meal (Cohen et al. 2005) and was attributed to the nematicidal effects of ammonia generated in response to these organic nitrogen amendments (Oka and Pivonia 2003; Rodríguez-Kábana 1986). Although chemistries derived through transformation of nitrogenous amendments can yield suppression of plant-parasitic nematodes, suppression of *P. penetrans* resulting from such treatment (e.g., *B. napus* SM) was transient relative to that attained in response to *B. juncea* SM amendment (Mazzola et al. 2009), which yields the nematicidal volatile AITC (Hewavitharana et al. 2014; Yu et al. 2005). Overall, these and previous findings indicate that control of replant disease due to addition of SM to soil is reliant on multiple factors, including production of AITC generated in response to application of *B. juncea* SM, that may interact or change in dominance depending upon composition of the target pathogen population.

Replant-disease-tolerant apple rootstocks have been identified (Auvil et al. 2011; Isutsa and Merwin 2000) and evaluated as a disease management tool in commercial production systems. In the absence of soil fumigation, disease-tolerant rootstock genotypes have demonstrated large site-to-site variation in relative growth (Auvil et al. 2011), indicating that application as a standalone replant disease management option may not be suitable. In a previous study, preplant *B. juncea*–*B. napus* SM soil amendment resulted in significantly greater tree growth and yield when used in conjunction with Gala-G.11 relative to Gala-M.9 (Mazzola et al. 2015). Rootstock genotype effects on tree growth were also attained in the current study when grown in SM-amended soil. For instance, increase in biomass of G.210 rootstock commonly was greater than M.9 and G.41 when cultivated in soils treated with SM at 2.2 (experiments 2 and 3) and 4.4 t ha⁻¹ but no significant difference in plant biomass was observed among rootstocks when cultivated in the NTC or soybean-meal-amended soils. In addition, G.210 rootstock consistently demonstrated maximum growth promotion when cultivated in soil amended with SM at 4.4 t ha⁻¹ while MM.106 rootstock generally exhibited a maximum growth response at the highest SM amendment rate (6.6 t ha⁻¹). This indicates that efficacy of Brassicaceae SM amendment for improvement of tree growth may also be influenced by rootstock genotype.

Findings from this and previous studies (St. Laurent et al. 2010; Zhu et al. 2014) support a role for rootstock genotype in determining, in part, structure of the rhizosphere microbiome. In addition, differences in composition of the rhizosphere microbiome have been reported among disease-tolerant and -susceptible apple rootstock genotypes when planted into orchard replant soils (Rumberger et al. 2004, 2007; St. Laurent et al. 2010; Yao et al. 2006). Specific plant root metabolites can alter the chemical environment of the rhizosphere, leading to the recruitment of a unique population of microorganisms (Lareen et al. 2016). Rhizodeposit metabolic composition varied among apple rootstock

genotypes, with metabolites including benzoic acid, rutin, and various untargeted metabolites differentiating rootstocks based upon tolerance to replant disease (Leisso et al. 2017). Because distinctive changes in the rhizosphere microbiome have a role in SM-induced control of various pathogens that contribute to the development of apple replant disease (Cohen et al. 2005; Mazzola et al. 2015; Weerakoon et al. 2012), rootstock genotype effects on microbial recruitment to the rhizosphere may influence overall efficacy of the SM amendment as a disease control alternative. Similarly, rootstocks that support diminished reproduction or are tolerant or resistant to the pathogen would potentially yield greater growth response and disease control outcomes at lower SM application rates.

In the current study, composition of the rhizosphere microbiome was mediated by both rootstock genotype and soil treatment. The specific effect of plant genotype on rhizosphere microbiome composition was evident when comparison was made across rootstocks cultivated in the NTC orchard soil. SM effects on the soil microbiome were evident because fungal and bacterial community composition in bulk soils treated with SM were distinct from the NTC orchard soil at 6 weeks posttreatment. Interactions between rootstock genotype and SM application rate were observed, suggesting possible impacts on overall disease control efficacy; however, the influence of rootstock genotype tended to be masked at the higher SM amendment rates. At the highest amendment rate (6.6 t ha⁻¹), rhizosphere microbial community composition differed significantly between the SM treatment and NTC for all rootstock genotypes. In contrast, when cultivated in soil treated at the lowest SM amendment rate (2.2 t ha⁻¹), the microbial community detected in the rhizosphere of G.41 and G.210 differed significantly from the NTC but the rhizosphere microbial community from M.9 and MM.106 was indistinguishable from the control. This finding, in concert with maximal growth performance of G.41 and G.210, often attained at a lower SM amendment rate than M.9 or MM.106, supports the premise that rootstock genotype may influence biologically mediated disease control processes that are SM dependent.

In summary, findings from this study indicate that reduced Brassicaceae SM application rates, relative to that employed in previous studies (Mazzola et al. 2015), may provide effective control of apple replant disease when integrated with an appropriate apple rootstock genotype. SM treatment resulted in significantly greater vegetative growth and suppressed soilborne pathogens relative to the control treatment. All SM application rates provided significant disease control and improved growth of apple rootstocks relative to the NTC. No significant difference was obtained between SM soil treatments of 4.4 and 6.6 t ha⁻¹ in terms of promoting tree growth, regardless of rootstock genotype. Although tree growth at the higher SM amendment rates was consistently superior to that attained in response to application of SM at 2.2 t ha⁻¹ for M.9 and MM.106 rootstock, similar plant biomass was commonly attained for G.41 and G.210 at all SM rates. Additionally, root infection by *P. penetrans* and *Pythium* spp. was lower in rootstock G.41 and G.210 relative to MM.106 and M.9 when cultivated in the control soil. Thus, among the treatments evaluated, use of G.41 or G.210 rootstock with SM applied at a rate 4.4 t ha⁻¹ may be the most economically viable alternative to preplant soil fumigation for control of apple replant disease.

LITERATURE CITED

- Auvil, T. D., Schmidt, T. R., Hanrahan, I., Castillo, F., McFerson, J. R., and Fazio, G. 2011. Evaluation of dwarfing rootstocks in Washington apple replant sites. *Acta Hort.* 903:265-271.
- Borek, V., and Morra, M. J. 2005. Ionic thiocyanate (SCN⁻) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. *J. Agric. Food Chem.* 47:3837-3842.
- Brown, J., Davis, J. B., Erickson, D. A., Brown, A. P., and Seip, L. 1997. Registration of 'IdaGold' mustard. *Crop Sci.* 38:541.

- Brown, J., Davis, J. B., Erickson, D. A., and Gosselin, T. K. 2004. Registration of 'Pacific Gold' oriental condiment mustard. *Crop Sci.* 44:2271-2272.
- Cohen, M. F., and Mazzola, M. 2006. Resident bacteria, nitric oxide emission and particle size modulate the effect of *Brassica napus* seed meal on disease incited by *Rhizoctonia solani* and *Pythium* spp. *Plant 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 Biol. Biochem.* 37: 1215-1227.
- Covey, R. P., Benson, N. R., and Haglund, W. A. 1979. Effect of soil fumigation on the apple replant disease in Washington. *Phytopathology* 69: 684-686.
- Gardner, W., Mulvey, E. P., and Shaw, E. C. 1995. Regression analyses of counts and rates: Poisson, overdispersed Poisson, and negative binomial models. *Psychol. Bull.* 118:392-404.
- Hammer, Ø., Harper, D. A. T., and Paul, D. R. 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontol. Electronica* 4:1-9.
- Handiseni, M., Brown, J., and Mazzola, M. 2013. Effect of Brassicaceae seed meals with different glucosinolate profiles on *Rhizoctonia* root rot in wheat. *Crop Prot.* 48:1-5.
- Handiseni, M., Brown, J., Zemetra, R., and Mazzola, M. 2011. Herbicidal activity of Brassicaceae seed meal on wild oat (*Avena fatua*), Italian ryegrass (*Lolium multiflorum*), redroot pigweed (*Amaranthus retroflex*) and prickly lettuce (*Lactuca serriola*). *Weed Technol.* 25:127-134.
- Hewavitharana, S. S., Rudell, D., and Mazzola, M. 2014. Carbon source-dependent antifungal and nematicidal volatiles derived during anaerobic soil disinfection. *Eur. J. Plant Pathol.* 140:39-52.
- Isutsa, D. K., and Merwin, I. A. 2000. Malus germplasm varies in resistance or tolerance to apple replant disease in a mixture of New York orchard soils. *HortScience* 35:262-268.
- Jaffee, B. A., Abawi, G. S., and Mai, W. F. 1982a. Fungi associated with roots of apple seedlings grown in soil from an apple replant site. *Plant Dis.* 66: 942-944.
- Jaffee, B. A., Abawi, G. S., and Mai, W. F. 1982b. Role of soil microflora and *Pratylenchus penetrans* in an apple replant disease. *Phytopathology* 72: 247-251.
- Kirkegaard, J. A., and Sarwar, M. 1998. Biofumigation potential of brassicas. *Plant Soil* 201:71-89.
- Lareen, A., Burton, F., and Schäfer, P. 2016. Plant root-microbe communication in shaping root microbiomes. *Plant Mol. Biol.* 90:575-587.
- Leinfelder, M. M., and Merwin, I. A. 2006. Rootstock selection, preplant soil treatments and tree planting positions as factors in managing apple replant disease. *HortScience* 41:394-401.
- Leisso, R., Rudell, D., and Mazzola, M. 2017. Metabolic composition of apple rootstock rhizodeposits differs in a genotype-specific manner and affects growth of subsequent plantings. *Soil Biol. Biochem.* 113:201-214.
- Mai, W. F., and Abawi, G. S. 1981. Controlling replant disease of stone fruits in northeastern United States by preplant fumigation. *Plant Dis.* 65: 859-864.
- Mazzola, M. 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. *Phytopathology* 88:930-938.
- Mazzola, M. 1999. Transformation of soil microbial community structure and *Rhizoctonia*-suppressive potential in response to apple roots. *Phytopathology* 89:920-927.
- Mazzola, M., Agostini, A., and Cohen, M. F. 2017. Incorporation of *Brassica* seed meal soil amendment and wheat cultivation for control of *Macrophomina phaseolina* in strawberry. *Eur. J. Plant Pathol.* 149:57-71.
- Mazzola, M., and Brown, J. 2010. Efficacy of Brassicaceae seed meal formulations for the control of apple replant disease in conventional and organic production systems. *Plant Dis.* 94:835-842.
- Mazzola, M., Brown, J., Izzo, A. D., and Cohen, M. F. 2007. Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a Brassicaceae species and time-dependent manner. *Phytopathology* 97: 454-460.
- Mazzola, M., Brown, J., Zhao, X., Izzo, A. D., and Fazio, G. 2009. Interaction of Brassicaceae seed meal and apple rootstock on recovery of *Pythium* spp. and *Pratylenchus penetrans* from roots grown in replant soils. *Plant Dis.* 93: 51-57.
- Mazzola, M., Granatstein, D. M., Elfving, D. C., and Mullinix, K. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. *Phytopathology* 91:673-679.
- Mazzola, M., Hewavitharana, S., and Strauss, S. L. 2015. *Brassica* seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. *Phytopathology* 105:460-469.
- Mazzola, M., and Zhao, X. 2010. *Brassica juncea* seed meal particle size influences chemistry but not soil biology-based suppression of individual agents inciting apple replant disease. *Plant Soil* 337:313-324.
- Neilsen, G. H., and Yorston, J. 1991. Soil disinfection and monoammonium phosphate fertilization increase precocity of apples on replant problem soils. *J. Am. Soc. Hortic. Sci.* 116:651-654.
- Oka, Y., and Pivonia, S. 2003. Effect of a nitrification inhibitor on nematicidal activity of organic and inorganic ammonia-releasing compounds against the root-knot nematode *Meloidogyne javanica*. *Nematology* 5:505-513.
- Ramette, A. 2007. Multivariate analyses in microbial ecology. *FEMS Microbiol. Ecol.* 62:142-160.
- Rodríguez-Kábana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *J. Nematol.* 18:129-135.
- Rudolph, R. E., Sams, C., Steiner, R., Thomas, S. H., Walker, S., and Uchanski, M. E. 2015. Biofumigation performance of four *Brassica* crops in a green chile pepper (*Capsicum annuum*) rotation system in southern New Mexico. *HortScience* 50:247-253.
- Rumberger, A., Merwin, I. A., and Thies, J. E. 2007. Microbial community development in the rhizosphere of apple trees at a replant disease site. *Soil Biol. Biochem.* 39:1645-1654.
- Rumberger, A., Yao, S., Merwin, I. A., Nelson, E. B., and Thies, J. E. 2004. Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil. *Plant Soil* 264:247-260.
- Sarwar, M., Kirkegaard, J. A., Wong, P. T. W., and Desmarchelier, J. M. 1998. Biofumigation potential of brassicas. *Plant Soil* 201:103-112.
- Shipton, P. J. 1977. Monoculture and soilborne plant pathogens. *Annu. Rev. Phytopathol.* 15:387-407.
- Slykhuis, J. T., and Li, T. S. C. 1985. Responses of apple seedlings to biocides and phosphate fertilizers in orchard soils in British Columbia. *Can. J. Plant Pathol.* 7:294-301.
- Smolinska, U., and Horbowicz, M. 1999. Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. *J. Phytopathol.* 147:119-124.
- St. Laurent, A., Merwin, I. A., Fazio, G., Thies, J. E., and Brown, M. G. 2010. Rootstock genotype influenced apple replant disease and root-zone microbial community composition in an orchard soil. *Plant Soil* 337: 259-272.
- Tewoldemedhin, Y. Y., Mazzola, M., Botha, W. J., Spies, C. F. J., and McLeod, A. 2011. Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. *Eur. J. Plant Pathol.* 130:215-229.
- Traquair, J. A. 1984. Etiology and control of orchard replant problems: a review. *Can. J. Plant Pathol.* 6:54-62.
- van Schoor, L., Denman, S., and Cook, N. C. 2009. Characterisation of apple replant disease under South African conditions and potential biological management strategies. *Sci. Hortic. (Amsterdam)* 119:153-162.
- Weerakoon, M. D., Reardon, L. C., Paulitz, T. C., Izzo, A. D., and Mazzola, M. 2012. Long-term suppression of *Pythium abapprosorium* induced by *Brassica juncea* seed meal amendment is biologically mediated. *Soil Biol. Biochem.* 51:44-52.
- Yao, S., Merwin, I. A., Abawi, G. S., and Thies, J. E. 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. *Soil Biol. Biochem.* 38:587-599.
- Yates, S. R., Gan, J., Papiernik, S. K., Dungan, R., and Wang, D. 2002. Reducing fumigant emissions after soil application. *Phytopathology* 92: 1344-1348.
- Yu, Q., Tsao, R., Chiba, M., and Potter, J. 2005. Selective nematicidal activity of allyl isothiocyanate. *J. Food Agric. Environ.* 3:218-221.
- Zhu, Y., Fazio, G., and Mazzola, M. 2014. Elucidating the molecular responses of apple rootstock resistant to ARD pathogens: challenges and opportunities for development of genomics-assisted breeding tools. *Hortic. Res.* 43:1-8.