Influence of brassicaceous soil amendments on potentially beneficial and pathogenic soil microorganisms and seedling growth in Douglas-fir nurseries

Bodh R. Paudel^{*}, Lynne Carpenter-Boggs, and Stewart Higgins

Department of Crop and Soil Sciences, Washington State University, PO Box 646420, Pullman,
WA 99164, USA

* Corresponding author

Bodh R. Paudel

Email: bodh.paudel@wsu.edu

Abstract

Fusarium, *Cylindrocarpon* and *Pythium spp.* are the major soil-borne pathogens of conifer seedlings. Soil fumigation with methyl bromide and chloropicrin has been the most effective method for reducing the population density and disease pressure of these organisms. Due to safety and environmental concerns, use of methyl bromide as a pre-plant soil fumigant has been

abolished in the majority of cropping systems. However, the conifer seedling industry continues to use methyl bromide under a quarantine pre-shipment exemption due to a lack of effective alternatives. Toward identifying alternatives to methyl bromide for management of soil microbial populations, a three-year field study was conducted in northwest USA. The objective of this study was to examine the effects of brassica seed meals and green manures on potentially pathogenic and beneficial microorganisms, soil health, and seedling growth in conifer nursery fields. The study treatments were *Brassica juncea* seedmeal, *B. carinata* seedmeal, *Sinapis alba* seedmeal, *B. juncea* green manure, methyl bromide/chloropicrin fumigation, and a non-treated control, with four replications in a randomized complete block design. The treatments were incorporated into soil in autumn or early spring, and Douglas-fir (*Pseudotsuga menziesii*) seedlings were transplanted into plots in late spring. Population densities of *Fusarium*, *Cylindrocarpon*, *Pythium*, actinomycetes, and *Trichoderma*; mineralizable nitrogen; and dehydrogenase enzyme activity in soil were assessed at pre-transplant, post-transplant, and seedling harvest. The pre-treatment soil pathogen count was similar among study plots. At transplant time, *Fusarium* spp. densities in soil were generally similar among most brassica treatments but fumigated plots generally had less *Fusarium*. Treatment with *S. alba*, however, increased soil densities of *Fusarium* spp. In 2012, *Fusarium* spp. density was significantly lower after *B. juncea* green manure incorporation [1.8 log CFU (colony forming units)] than after chemical fumigation (2.4 log CFU) or in the untreated control (2.6 log CFU); whereas the soil density of potentially antagonist *Trichoderma* spp. was significantly greater in fumigated plots (3.7 log CFU) followed by *B. juncea* green manure (3.4 log CFU) and lowest in control (3.2 log CFU). Fumigation produced the largest seedlings but *B. juncea* green manure also produced significantly larger seedlings than control. Dehydrogenase activity, an indicator of soil microbial activity, was greatest with *B. juncea* green manure and

lowest in fumigated soil. Mineralizable nitrogen in soil followed the same trend. These results suggest that *B. juncea* green manure may have a suppressive effect on soil-borne pathogens, and maintain or improve soil and seedling health.

Key words: Brassica green manure, soil health, brassica seed meals, methyl bromide, biofumigation, fusarium root rot

1. Introduction

Industrialized agriculture has reduced organic matter incorporation into soil, increased soil organic matter decomposition, and reduced plant diversity. Consequently, soil physical and biological quality have declined, and soil fertility and productivity decreased. Certain soil-borne pathogens thrive in these conditions (Bailey and Lazarovits, 2003; Bonanomi *et al.*, 2007; Hoitink and Boehm, 1999), which have been managed partly through inputs and practices that further degrade the soil resource. Due to negative human and environmental consequences of synthetic pesticides and intensive cultivation, there is renewed interest in the use of organic amendments such as green manure, brassica seedmeal, and compost to manage soil-borne pathogens (Litterick *et al.*, 2004; Noble and Coventry, 2005) and simultaneously improve soil fertility (Lazarovits, 2001).

In the western states of USA (Idaho, California, Oregon, Montana, and Washington), about 200 million conifer seedlings are produced annually for forest and conservation purposes (Weiland *et al.*, 2011; Weiland *et al.*, 2013). Washington's conifer seedling nursery industry and working forests contributed 4.5 billion dollars to the Washington economy in 2013 (<http://data.workingforests.org/>). This industry is facing pressures from soil-borne fungal pathogens, paired with regulations that increasingly restrict the use of reliable control measures. Some of the most problematic soil-borne pathogens include members of the *Fusarium*, *Pythium*,

and *Cylindrocarpon* (Hamm *et al.*, 1990; NTC, 2009; Stewart *et al.*, 2012). In addition to causing significant losses in the nursery, these pathogens can also reduce survival and growth of out-planted seedlings, resulting in significant replanting costs. These soil-borne pathogens must be managed and seedling health protected for profitable nursery and forest production, and more cost-effective environmentally acceptable management options are needed.

Fusarium spp. are found worldwide in soil and decaying plant debris (Moss and Smith, 1984). About half of the 40 species in the genus are parasitic on higher plants causing root rot, vascular wilts, and storage rots (Booth 1984; Price, 1984). Soil fumigation has been a common practice in forest seedling nurseries, most frequently using a combination of methyl bromide (MB) with chloropicrin for management of soil-borne disease agents (Karpouzas *et al.*, 2005; Smith and Fraedrich, 1993; Wang *et al.*, 2005;). This chemical formulation is considerably more effective than other fumigant and known non-fumigant alternatives. However, MB was listed as an ozone depleting substance by the Montreal Protocol in 1992 and its production was largely discontinued by 1995 (Bell *et al.*, 1996; Karpouzas *et al.*, 2005). Producers of a few crops continue to use MB under a critical use exemption including California strawberry growers for pre-plant use and forest nursery growers for quarantine pre-shipment exemption (Environmental Protection Agency, 2014). There is increasing restriction of the crops, areas, and conditions allowed for MB use even with the critical use exemption. Consequently, finding effective legal alternatives to MB use has become crucial.

The use of brassica green manures has been a traditional practice around the world for management of soil quality, and more recently has gained interest for management of soil-borne pathogens. The term ‘biofumigation’ has been coined as “the beneficial use of brassica crop residues that release isothiocyanates similar to methyl isothiocyanate” (Kirkegaard *et al.*, 1993;

Matthiessen and Kirkegaard 2006; Omirouet *et al.*, 2011). Biofumigation with *Brassica* spp. and other mustard species has been successful for soil-borne pathogen management in some production systems (Larkin and Griffin, 2006). The exploitation of maximum biofumigation potential has been a key research goal (Galletti *et al.*, 2008; Handiseniet *et al.*, 2013; Mazzola *et al.*, 2009). A study by Galletti *et al.* (2008) showed potential of *Brassica carinata* seed meal on suppression of *Pythium ultimum* in sugar beet. Another study found that *Brassica napus* seed meal could suppress apple root infection by *Rhizoctonia* spp. (Mazzola *et al.*, 2009). Similarly, a study by Handiseniet *et al.* (2013) revealed that brassica seed meals were relatively successful in suppressing *Rhizocniasolani* in winter wheat. The factors affecting the release of isothiocyanates into soil have been intensively researched. Research has resulted in some commercial implementation of biofumigation, however the effectiveness of the various organic amendments on the wide range of potential crops and pathogens is not consistent (Mazzola *et al.*, 2001; Pérez-Piqueres *et al.*, 2006; Tilston *et al.*, 2005). These studies suggested that wide range of host and patho-systems need to be tested to confirm effectiveness of organic soil amendments. Farmers are skeptical of the pathogen suppression and soil health benefits of organic amendments due to inconsistency and slow accumulation of the results. More research is needed to assess the efficacy of specific brassica soil amendments in a wider range of crops and settings.

Our field study was conducted to examine the effects of brassica seed meals and green manures on selected soil-borne microorganisms and soil quality in Douglas-fir (*Pseudotsugamenziesii*) seedling nurseries. The major objective of the study was to determine effects of soil amendment with green manure or seed meal of plants in Brassicaceae on densities of soil and root microorganisms such as *Fusarium*, *Cylindrocarpon*, and *Trichoderma*. The *Trichoderma* are beneficial fungi in soil which can naturally act as biological control agents for

soil-borne pathogens (Kucuk and Kivanc, 2003). These beneficial organisms antagonize other pathogens via competition, antibiosis, and direct parasitism (Alabouvette et al., 2009). The secondary objectives of this research were to analyze tree morphology and overall soil quality as affected by brassica soil amendments. We hypothesized that one or more brassica seed meals or green manure would affect the density of *Fusarium*, *Trichoderma*, and *Cylindrocarpon* in soil and Douglas-fir seedling roots, enhance soil health, and improve seedling growth compared to untreated soils.

2. Materials and methods

2.1. Study area and treatments

The study was conducted at conifer nurseries that have requested anonymity, nursery A in 2009 and 2010 and nursery B in 2012, both in western Washington State. The soil type in nursery A was classified as Cagey loamy sand (mixed, mesic, Aquic Xeropsamment) and that of nursery B was Puyallup fine sandy loam (sandy skeletal mixed mesic fluventic Haploxeroll). The mean annual low and high temperatures at both study sites were 4 and 16 °C.

Each field experiment was conducted in a randomized complete block design with four replications. Each replicate plot was 4.57 m by 9.14 m. The 2009 study consisted of seven treatments which include MB/chloropicrin fumigation, 392 kg ha⁻¹ (67% MB and 33% chloropicrin)(MBC); *Sinapis alba* cv. 'IdaGold' seedmeal at 4484 kg ha⁻¹ (SaSM) and 2242 kg ha⁻¹ (SaSM-I); *Brassica juncea* cv. 'Pacific Gold' seedmeal at 4484 kg ha⁻¹ (BjSM) and 2242 kg ha⁻¹ (BjSM-I); *B. carinata* seedmeal (commercial product, 'Biofence') at 2242 kg ha⁻¹ (BcSM-I) and untreated control (Control).

For the 2010 and 2012 studies, a subset of the more effective 2009 treatments and application rates were used. The 2010 and 2012 studies consisted of five treatments which included MBC 392 kg ha⁻¹, BjSM4484 kg ha⁻¹, BcSM4484 kg ha⁻¹, *B. juncea* green manure cv. ‘Caliente 199’ (BjGM), and untreated control (Control). The BjGM was harvested from a field near the research plot seeded at 11.2 kg seed ha⁻¹ and fresh green mass was incorporated into research plots. The dry biomass was approximately 5800 kg ha⁻¹. Treatments were incorporated with a rototiller to a depth of approximately 15 cm followed by tarping until spring with a polyethylene tarp.

The treatments were incorporated into soil in early spring or autumn, and Douglas-fir (*Pseudotsugamenziesii*) seedlings were transplanted into plots in late spring. Seedlings were transplanted into the field as 1 year old greenhouse stock at a transplanting rate of 65 seedlings m⁻², grown in the field through the summer and fall, and harvested for cold storage and sale during the winter. In 2009, treatments were incorporated in April 15; seedlings were transplanted May 25-29; 40 days after treatment application (DAT), and harvested December 6-10 (235 DAT). In the 2010 study, treatments were incorporated in August 27, 2009, with seedlings transplanted April 19, 2010 (235 DAT) and harvested in November 15, 2010 (445 DAT). In the 2012 study, the treatments were incorporated September 21, 2011 seedlings transplanted May 30, 2012 (251 DAT) and harvested in January 7, 2013 (475 DAT). Seedlings were harvested as intact seedlings with root systems using an undercutting lifter. Plants were separated at the crown into root and shoot portions, weighed as fresh biomass. Caliper was measured at root collar.

In 2009 and 2010, all plots received pre-transplant fertilizer (custom blend consisting of 2% N, 16% P and 16% K) 560.4 kg ha⁻¹ along with 89.6 kg ha⁻¹ of CaSO₄. All plots received 6 additional applications of Ca(NO₃)₂ at the rate of 168.1 kg ha⁻¹ at 2 to 3 week intervals and a final

application of $\text{Ca}(\text{NO}_3)_2$ at the rate of 56 kg ha^{-1} as pre-harvest. This total of 193 kg N ha^{-1} , $89.6 \text{ kg P ha}^{-1}$, $89.6 \text{ kg K ha}^{-1}$, $21.1 \text{ kg S ha}^{-1}$, and $277.4 \text{ kg Ca ha}^{-1}$ is the standard rate of fertilizer for nursery A.

In 2012, all plots received a pre-transplant fertilizer of 280.2 kg ha^{-1} of CaSO_4 , 280.2 kg ha^{-1} of K_2SO_4 , 16.8 kg ha^{-1} of boron, 28 kg ha^{-1} of MgSO_4 , and 7.8 kg ha^{-1} of ZnSO_4 . In addition each plot received a starter fertilizer blend of 224.2 kg ha^{-1} (containing 0.21% N, 1.35% P and 1.28% of K) 2 weeks after transplanting. All plots received 3 applications of $\text{Ca}(\text{NO}_3)_2$ at 168.1 kg ha^{-1} and 3 additional applications of $\text{Ca}(\text{NO}_3)_2$ at 56 kg ha^{-1} in 2 weeks interval. This total of $115.3 \text{ kg N ha}^{-1}$, 3 kg P ha^{-1} , $128.6 \text{ kg K ha}^{-1}$, $126.5 \text{ kg S ha}^{-1}$, $246.7 \text{ kg Ca ha}^{-1}$, $16.8 \text{ kg B ha}^{-1}$, $3.2 \text{ kg Zn ha}^{-1}$ is the standard rate of fertilizer for nursery B.

2.2. Soil and plant sampling

Soil was sampled at pre-treatment, pre-transplant, post-transplant or pre-harvest, and at harvest during each study period. Ten sub-samples were taken from 0-15 cm in each plot using a core sampler (3.8 cm diameter) and mixed well. Aseptic procedure was used to avoid cross contamination between treatments. All samples were stored at 4°C until analyzed and the analysis was completed in 30 days.

Densities of soil-borne microorganisms, nitrogen mineralization rate and dehydrogenase enzyme activity in soil were assessed frequently at pre-treatment, pre-transplant, mid-season, and harvest. The plant samples were taken three times (pre-transplant, mid-season and final harvest) and root colonization percentage as well as tree biomass and morphology were analyzed.

2.3. Soil and plant analyses

Target soil microorganisms were assessed using 2.5 g soil following standard laboratory procedure of soil dilution plating. The media used were selective media for specific soil organisms. *Pythium* spp. and *Trichoderma* spp. were grown and enumerated in V8 agar medium (Stevens, 1974). *Fusarium* spp. and *Cylindrocarpon* spp. along with actinomycetes were grown on Komada's medium (Komada, 1976). Plating assessments were completed in triplicate.

For root study, 20 seedlings were sampled from each plot and roots were cut at the collar. Roots were bulked together from each plot separately and ten root systems were randomly selected from which 8 random 1-cm root tips were cut off with sterile scalpel. Then root sections from each sample were washed with 0.1% NaClO followed by rinsing with deionized water twice. Root colonization by organisms was analyzed with 25 (for *Pythium* and *Trichoderma*) or 50 (for *Fusarium* and *Cylindrocarpon*) 1-cm long root segments from each sample and expressed as percentage of roots colonized. We followed standard procedure by Mitchell and Kannwischer-Mitchell, 1992.

Dehydrogenase enzyme activity was assayed with triphenyl tetrazolium chloride reduction (Tabatabai, 1994). The results were quantified colorimetrically, standardized with standard curve and expressed as triphenyl formazan (TPF) $\text{g soil}^{-1} \text{ hr}^{-1}$. Mineralizable N was quantified in 7- and 28-day incubations at 40° C with NH_4 quantification (Waring and Bremner, 1964).

2.4. Statistical analyses

Data were analyzed using a randomized complete block design in statistical analysis software SAS version 9.3 (SAS, 2008) using Proc Mixed. Data collected multiple times within a season were analyzed as repeated measures to determine treatment effect and treatment X time

interactions for each parameter. The microorganism colony forming units (CFUs) were log-transformed for analysis and presentation. Pairwise comparisons of treatment means were based on protected LSD using the pdiff option in SAS. Differences were declared significant at the five percent level ($P \leq 0.05$).

3. Results

3.1. Results in 2009

MBC tended to have the lowest soil and root densities of *Fusarium*, *Trichoderma* and *Cylindrocarpon* in 2009 although not always significantly lower than other treatments (Fig. 1, Table 1). At transplanting, about one month after treatment application, density of *Fusarium* in soil was higher with *S. alba* seed meal treatments than in control or other treatments (Fig. 1). The other seed meals failed to significantly reduce *Fusarium* in the soil to levels below untreated control plots although BcSM-I and BjSM were also similar to MBC. Fumigation with MBC significantly reduced soil densities of the *Trichoderma*, while all other treatments maintained a similar level. At transplanting, there was no significant effect of treatments on *Pythium* and *Cylindrocarpon* densities in soil (data not shown).

At seedling harvest, *Fusarium* root colonization percentage of Douglas-fir seedlings was substantially higher in the SaSM, regardless of application rate, than in the other treatments (Table 1). Root colonization by *Fusarium* in MBC plots was not significantly lower than any treatment except SaSM. *Cylindrocarpon* root colonization at harvest was significantly lower with MBC than all other treatments (Table 1). *Cylindrocarpon* root colonization in all brassica seed meal plots was similar to the untreated control plots (Table 1). Root colonization with the potentially beneficial *Trichoderma* was significantly higher with the SaSM than with all other

treatments (Table 1). Root colonization by *Pythium* did not differ among treatments (data not shown).

Plots treated with MBC produced trees with above-ground fresh mass and root: shoot ratio that were significantly and substantially larger than the other treatments (Fig.2). However, all seed meal treatments except BjSM-l produced larger trees than the control (Fig. 2). All treatments except SaSM-l produced trees with more root mass than the control (Fig. 2).

3.2.Results in 2010

There was significant temporal variation of *Fusarium* density in soil. Peak densities occurred at different sampling dates for different treatments, but overall were lowest at 276 days after treatment (DAT)(Table 2). MBC had the lowest *Fusarium* densities at all samplings, and Brassica treatments generally had higher *Fusarium* than MBC except on 172 and 276 DAT. At 172 DAT, BjGM had significantly higher *Fusarium* compared to all other treatments. At 203 DAT (pre-transplant), the *Fusarium* density was significantly lower in MBC than in the brassica treatments. At 276 DAT, there were no significant differences among treatments. At harvest (445DAT), the *Fusarium* density was significantly lower in MBC compared to other treatments (Table 2).

Trichoderma densities in soil overall showed little effect of time or treatment (Table 2). At the first month of the experiment, the soil density of *Trichoderma* was significantly lower in BjSM compared to other treatments. Similar trends continued at 172 DAT (pre-transplant) when BjSM was similar to MBC but significantly lower than the other treatments. There were no significant treatment differences at 203 DAT (pre-transplant), 276 DAT (post-transplant) and

445 DAT (harvest) (Table 2). The soil density of *Trichoderma* in BjSM at 30 and 172 DAT were similar to one another but significantly lower compared to those at 203, 276 and 445 DAT.

There was no significant treatment by time interaction for *Cylindrocarpon* soil densities (Table 2). *Cylindrocarpon* was not detected at 172 or 203 DAT, but by harvest (445 DAT) soil densities were again similar to the density at 30 DAT. *Pythium* was detected in soils at 30 DAT in BjGM and BcSM, and at 172 and 203 DAT in BjGM only, and was not detected in other times or treatments. The soil density of *Pythium* was significantly higher in BjGM compared to other treatments at 30, 172, and 203 DAT. At no time was *Pythium* detected in either BjSM or MBC plots, but it was not detected in the control plots either (Table 2).

Actinomycete density in soil varied with treatment and time. MBC treatment led to undetectably low actinomycete populations at 30, 172, and 203 DAT. At 276 DAT density in MBC was still lower than all other treatments. The soil density of actinomycetes at 30 DAT was highest in BjSM and BcSM compared to BjGM, and MBC but BcSM was similar to control (Table 2). The actinomycetes in MBC were undetectable for over 200 DAT. At 172 DAT, the soil density of actinomycetes was significantly higher in BcSM than MBC but other treatments had similar densities of actinomycete to BcSM. At 203 DAT, BcSM and BjSM had higher soil density of actinomycete than all other treatments. At 276 DAT, the soil density of actinomycetes was significantly lower in MBC compared to all other treatments. Only at harvest, 445 DAT, were densities high and similar in all treatments.

At seedling harvest, *Cylindrocarpon* colonization of roots, tree biomass, height, and caliper differed among treatments (Table 3). *Fusarium* and *Trichoderma* colonization of roots was similar among treatments. *Cylindrocarpon* colonization was lowest in MBC and among the highest in BjGM, but neither brassica seedmeal differed significantly from the control (Table 3).

The tallest trees were harvested from plots treated with MBC, but these trees were not significantly taller than those from the BjGM plots (Table 3). The brassica seed meals produced trees similar in height to the control. Trees from MBC and BjGM also had the largest caliper and greatest above-ground fresh biomass (Table 3). Tree root fresh weight was not significantly affected by fumigant or brassica treatments (Table 3).

3.3. Results in 2012

Homogeneity of the study field was confirmed by assessing background soil densities of microbial groups. The pre-treatment soil densities of *Fusarium* and *Trichoderma* were similar among all plots (data not shown). Post-treatment soil analyses focused on the tree seedling growth period. At 243 DAT, when seedlings were transplanted, soil density of *Fusarium* was significantly lower in BjGM treatment compared to other treatments (Table 4). At 306 DAT, the control had significantly higher *Fusarium* density compared to others. *Fusarium* density was lowest in BjGM and MBC, and intermediate in BcSM and BjSM. At harvest (475 DAT), the control had the highest and MBC had the lowest soil density of *Fusarium* and there were no differences among brassica treatments (Table 4). *Fusarium* density in soil tended to increase as growing season progressed.

There was no significant interaction between treatment and time for soil density of *Trichoderma* or *Pythium* although the main effects of treatment and time were both significant. The *Trichoderma* density was highest in MBC, and lowest in the control which was similar to BjSM (Table 4). The BjGM plots had significantly higher *Trichoderma* density than control and BjSM but were similar to BcSM (Table 4). There were no significant effects of treatment on soil densities of *Cylindrocarpon* (Table 4). MBC significantly suppressed *Pythium*

compared to all other treatments and there were no differences among brassica treatments and the control. The soil densities of *Pythium* were lowest at harvest. At 243 and 306 DAT, the soil densities of actinomycetes were significantly lower in MBC than other treatments. At harvest, MBC had significantly higher actinomycetes than other treatments except BjGM. The control and BcSM had the lowest densities of actinomycetes at harvest (Table 4.)

Soil dehydrogenase activity, an indicator of total microbial oxidizing activity, was similar among treatments at 243 days (Fig. 3). At 306 days, BjGM had significantly greater activity compared to MBC, BcSM, and BjSM, while activity in control soils was intermediate and similar to all treatments (Fig. 3). At harvest, all brassica treatments had significantly greater activity than MBC and control. Similarly, mineralizable nitrogen in soil at the time of seedling transplanting was significantly greater in BjGM compared to MBC and there were no differences among remaining treatments ($P \leq 0.05$). After both 7- and 28-day incubation of soil samples, the mineralizable nitrogen trend was BjGM > Control > MBC (Fig 4).

4. Discussion

All the brassica treatments either improved or maintain soil microbial activity and fertility compared to MBC. Fumigation reduced densities of *Fusarium* spp. in soil, which were generally similar among most brassica treatments. Treatment with *S. alba*, however, increased soil densities of *Fusarium* spp. In 2012, *Fusarium* spp. density was significantly lower in BjGM than MBC and untreated control whereas the soil density of potentially antagonist *Trichoderma* spp. was significantly greater in MBC which was followed by BjGM and lowest in control. Fumigation produced significantly larger seedlings than other treatments but most brassica green manure plots produced significantly larger seedlings than control plots. Dehydrogenase activity,

an indicator of soil microbial activity, was greatest with BjGM and lowest in fumigated soil. Taken together, these results suggest that BjGM may have a suppressive effect on soil-borne pathogens, and maintain or improve soil and seedling health.

Development of alternatives for management of seedling health and soil-borne pathogens in Douglas-fir seedlings is a daunting task considering the complexity of pathogenicity of target pathogens and high variability of resident soil biology. However, development of effective non-chemical methods for control of root rot and damping off of conifers will be of significant value. This and other studies (Cohen *et al.*, 2005; Subbarao *et al.*, 1999) have studied management of the soil biota and associated diseases by use of organic soil amendments such as composts, brassicas, and green manures. A review by Baysal-Gurel *et al.* (2012) revealed that organic matter amendments could be useful to manage soil-borne diseases caused by various pathogens such as *Fusarium* spp. (Klein *et al.*, 2011), *Pythium* spp. (McKellar and Nelson, 2003; Veeken *et al.*, 2005), and *Rhizoctonia solani* (Diabet *et al.*, 2003). During our initial trial, the brassica seed meals, especially *B. carinata* and *B. juncea* at the high application rate, showed promise in reducing soil *Fusarium* densities and increasing *Trichoderma* densities. The *S. alba* seed meal was dropped in further experiments as it was ineffective.

In 2010, MBC produced the largest seedlings but *B. juncea* green manure also produced significantly larger seedlings than control. However, root colonization of *Fusarium* was not significantly different among brassica treatments compared to MBC or control. In 2012, BjGM significantly suppressed early season soil densities of *Fusarium* compared with other treatments including MBC. Several studies have observed reductions in soil-borne pathogens after brassica plant tissue incorporation into soil (Cohen *et al.*, 2005; Mazzola *et al.*, 2001; Mazzola and

Mullinix, 2005; Pinkerton *et al.*, 2000; Smolinska, 2000; Subbarao *et al.*, 1999), including reduced *Fusarium* pathogens (Relevante and Cumagun, 2013).

There are examples of research where brassica amendments have been successful for soil-borne pathogen control (Cohen *et al.*, 2005; Subbarao *et al.*, 1999) as well as examples of failure (Blok *et al.*, 2000; Zasada *et al.*, 2003). In a few similar studies, the *Fusarium* and *Pythium* soil densities in brassica-amended soils were even significantly higher than those in control (Njoroge *et al.*, 2008). Hence, the effect of biofumigation has not been consistent, possibly due to many factors. Efficient isothiocyanate production from brassicas is dependent on the species and variety, amount of tissue incorporated, thoroughness of tissue cell disruption, and other factors (Morra and Kirkegaard, 2002). There may be additional biological mechanisms governing pathogen suppression by brassicas besides glucosinolate hydrolysis to isothiocyanates (Blok *et al.*, 2000; Cohen *et al.*, 2005; Mazzola, 2007).

It is important to monitor both plant pathogens and beneficial organisms to better characterize the effect of soil amendments. In this study, the decrease in *Fusarium* density was highly correlated with an increase in *Trichoderma* density ($R = -0.536$, $P < 0.0001$) in MBC and BjGM. Beneficial soil microorganisms play an important part in maintaining soil health, nutrient cycling and sustainability of ecosystems (Ahemad and Kibert, 2014; Parab *et al.*, 2015). It has been reported that after soil amendments with *B.juncea* and *B.napus*, the soil densities of both fungi and bacteria including fluorescent pseudomonads increased compared with nonamended soils (Smolinska, 2000). However the soil densities of pseudomonads do not always change following brassica amendments (Scott and Knudsen, 1999). This suggests that the species and variety of brassica, genotype of crop (Mazzola and Gu, 2002), soil type, climate, and other

conditions may influence the effect of amendments on fluorescent pseudomonads and other beneficial and pathogenic organisms.

When there was an effect of treatment, MBC generally provided the most effective control of soil-borne *Fusarium* and *Pythium*. MBC had no effect on *Cylindrocarpon* density in soil, but it provided the best control of root colonization of *Cylindrocarpon* (Table 5). In 2012, MBC encouraged *Trichoderma* soil density but it reduced the actinomycete density early in both 2010 and 2012 growing seasons. The N mineralization rates were among the lowest in MBC plots (Fig. 4, Table 5). Soil dehydrogenase activity, which represents overall soil oxidation activity, was reduced by MBC compared to brassica treatments (Fig. 3, Table 5). The dehydrogenase enzyme activities were similar between MBC and control initially but at harvest, control also had better activities than MBC. Together, these data show that MBC reduced multiple measures of soil microbial populations and activities, with positive benefits to plant growth but negative effects on soil functions.

BjGM effects on soil-borne *Fusarium* were not consistent, increasing during the 2010 season, but providing some of the best control during the 2012 season (Table 5). BjGM encouraged *Pythium* growth in 2010 but was similar to control in 2012. BjGM had no effect on soil-borne *Cylindrocarpon*, but encouraged root colonization by *Cylindrocarpon* relative to control. Soil colony counts of actinomycetes were generally similar in BjGM plots and control plots. For the important variables of tree size, height, caliper and shoot and root biomass, the effect of BjGM was never significantly different from MBC. BjGM was among the best treatments for dehydrogenase activity and N mineralization. Thus overall the BjGM treatment provided the most benefit to soil biological function and communities, with some benefit to plant growth.

The effects of BjGM could be due to chemical, biological, and/or physical changes in the soil. The glucosinolate content of many brassicas can directly contribute to disease suppression (Gardiner et al., 1999; Matthiessen and Kirkegaard, 2006; Peterson et al., 2001). Green manures can also promote soil biological fertility (Scotti et al., 2013; Scotti et al., 2015) including mineralization of nutrients (Elfstrand et al., 2007; Erikson et al., 2005). We could not separate the effects due to BjGM chemistry from the soil biological fertility effects of BjGM residue in this study. Soil enzyme activities and microbial community composition are important components of soil biological fertility. More overall microbial activity and available nutrients may thereby improve seedling health. These changes may, in turn, affect plant response to pathogens, effectively increasing the pathogen densities or virulence required to cause significant plant damage.

The effects of brassica seed meals were not as great as brassica green manure, which also resembles the findings of prior research (Mazzola and Gu, 2002). The two seed meals, either from *B. juncea* or *B. carinata*, generally had similar effects on response variables, the exception being that *Trichoderma* soil density tended to be higher in BcSM plots than in BjSM plots. Otherwise, the seed meals seldom had an effect that was significantly different from the untreated control plots. There are mixed results in the literature regarding the effects of brassica seed meals. A study by Mazzola *et al.* (2007) reported that control of *Pratylenchus* spp. was higher with certain seed meal mixtures compared to single seed meals. The same study showed that brassica seed meal suppressed *Rhizoctonia solani* in apple replant diseases but the efficacy varied with seed meal type and time. Similarly, brassica seed meals may be more effective in pathogens and weed control when used in combination (Mazzola and Brown, 2010). Another experiment reported the incidence of chickpea wilt by *Fusarium oxysporum* was significantly

reduced when combination of *Brassica carinata* seed meals of two different cultivars were used (Aberaet *et al.*, 2011). Yet another study by Ma *et al.* (2015) revealed that a combination of *Brassica juncea* and *Sinapis alba* seed meals exhibited good disease control of *Fusarium* wilt in chili pepper. The results observed in our experiment might be different if seed meal mixtures were used rather than single seed meals alone.

Numerous biotic and abiotic factors and phenomena contribute to the pathogen suppressiveness of soil amendment treatments (Bonanomi *et al.*, 2007). Biotic factors include activity of antagonistic microorganisms (Hoitink and Boehm, 1999), competition for nutrients (Bonanomi *et al.*, 2007; Lockwood, 1990) and production of antibiotic compounds (Smolinska, 2000; Tenuta and Lazarovits, 2002). In addition, organic matter with green manure and seed meals improves soil structure, water holding capacity, soil nutrients and microbial activities which in turn benefit subsequent crops and the farming system as a whole (Baysal-Gurel *et al.*, 2012; Klein *et al.*, 2011; Matthiessen and Kirkegaard, 2006; Thorup-Kristensen *et al.*, 2003). For example, Matthiessen and Kirkegaard (2006) reported that brassica green manure incorporation prevented wind erosion and improved the water infiltration in sandy soils of western USA (Gies 2004; McGuire, 2004). These improvements to crop nutrition and water relationships may also improve disease tolerance regardless of changes in soil microbial communities.

5. Conclusions

There is utmost need to find sustainable strategies for management of seedling health and soil-borne pathogens in the Douglas-fir nursery industry that are economically viable and socially acceptable. A large number of studies have been conducted on organic amendments for soil-

borne pathogen management in other crops. In this study, *B.juncea* green manure and, to a much lesser extent, seedmeals of *B. juncea* and *B.carinata* showed some potential to suppress *Fusarium* densities in soil and increase potentially beneficial organisms like *Trichoderma*. In summary, among the treatments, the seed meals BcSM and BjSM were seldom different than untreated control plots. BjGM showed mixed results regarding its effect on soil- and root-borne microbial colonization; however its effects on tree growth parameters and soil health were encouraging. The treatments used in this study did not greatly affect soil N availability but *B.juncea* green manure did support plant growth at a similar level as fumigation treatment. These results suggest focusing further research on soil and plant health promotion, as well as disease-suppressing mechanisms and plant-pathogen-antagonist relations. Research on newly registered allyl isothiocyanate biofumigants along with other chemical and non-chemical alternatives will be worthwhile in discerning biofumigant effects from other biotic and abiotic factors.

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List of figures

Figure 1. Soil *Fusarium* and *Trichoderma* densities at the time of Douglas-fir seedling transplanting in 2009 study. The study treatments were BcSM-l (*Brassica carinata* seed meal at 2242 kg ha⁻¹), BjSM (*Brassica juncea* seed meal at 4484 kg ha⁻¹), BjSM-l (*B. juncea* at 2242 kg ha⁻¹), Control (untreated control), MBC (methyl bromide/chloropicrin at 392 kg 392 kg ha⁻¹), SaSM (*Sinapis alba* seed meal at 4484 kg ha⁻¹), SaSM-l (*S. alba* seed meal at 2242 kg ha⁻¹). Different letters indicate significant difference at $P < 0.05$ within each organism, CFU= colony forming units.

Figure 2. Seedling fresh biomass at harvest in 2009 study. The study treatments were BcSM-l (*Brassica carinata* seed meal at 2242 kg ha⁻¹), BjSM (*Brassica juncea* seed meal at 4484 kg ha⁻¹), BjSM-l (*B. juncea* at 2242 kg ha⁻¹), Control (untreated control), MBC (methyl bromide/chloropicrin at 392 kg 392 kg ha⁻¹), SaSM (*Sinapis alba* seed meal at 4484 kg ha⁻¹), SaSM-l (*S. alba* seed meal at 2242 kg ha⁻¹). Different letters indicate significant difference at $P < 0.05$ within each category.

Figure 3. Dehydrogenase activity in 2012 as affected by study treatments: BcSM (*Brassica carinata* seed meal at 4484 kg ha⁻¹), BjGM (*Brassica juncea* green manure seeded at 4484 seeds kg ha⁻¹), BjSM (*Brassica juncea* seed meal at 4484 kg ha⁻¹), Control (untreated control), and MBC (methyl bromide/chloropicrin, 392 kg ha⁻¹; 67% methyl bromide, 33% chloropicrin). Data within a treatment with same upper case letter were not significantly different between 3 sampling times ($P \leq 0.05$) and data with same lower case letter at the top of the column were not significantly different between treatments ($P \leq 0.05$). (DAT= days after treatment application, transplanting was done at 251 DAT and harvested at 475 DAT; TPF= triphenyl formazan)

Figure 4. Mineralizable nitrogen at seedling transplant in 2012 as affected by study treatments. The treatments were BcSM (*Brassica carinata* seed meal at 4484 kg ha⁻¹), BjGM (*Brassica juncea* green manure seeded at 4484 seeds kg ha⁻¹), BjSM (*Brassica juncea* seed meal at 4484 kg ha⁻¹), Control (untreated control), and MBC (methyl bromide/chloropicrin, 392 kg ha⁻¹; 67% methyl bromide, 33% chloropicrin). Different letters indicate significant difference at $P < 0.05$ between treatments within each incubation period.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1. Root colonizationpercentage at harvest in soil left untreated, fumigated with methyl bromide/chloropicrin, or treated with brassica seed meals in 2009 study.

Treatment [†]	<i>Fusarium</i>	<i>Trichoderma</i>	<i>Cylindrocarpon</i>
-----Percentage of root segments colonized-----			
BcSM-l	6±3c	7±7 b	44±16 ab
BjSM-l	10±7c	14±6 b	45±15 ab
BjSM	8±6 c	9±7 b	53±15 a
Control	7±6 c	11±12 b	44±9 ab
MBC	0.5±1 c	7±8 b	7±8 c
SaSM-l	26±6 b	16.2±8 b	32±4 b
SaSM	36±9 a	32±9 a	32±19 b

Data followed by same letter within a column were not significantly different ($P \leq 0.05$)

[†]MBC = methyl bromide/chloropicrin; SaSM = *Sinapis alba* seed meal at 4484 kg ha⁻¹; SaSM-l = *S. alba* seed meal at 2242 kg ha⁻¹; BjSM = *Brassica juncea* seed meal at 4484 kg ha⁻¹, BjSM-l = *B. juncea* at 2242 kg ha⁻¹; BcSM-l = *Brassica carinata* seed meal at 2242 kg ha⁻¹; Control = untreated control.

Table 2. Least squared means of the log₁₀ of colonyforming units (Log(1 + CFU g soil⁻¹)) for microorganisms in soil collected at nursery A, Washington during the 2010 conifer growing season.

Treatment [†]		Day after treatment application				
		30	172	203	276	445
		Log (1 + CFU g soil ⁻¹)				
<i>Fusarium</i>	BcSM	A 2.7±0.1a	B 1.4±1.4 b	AB 2.1±1.2 a	C 0±0 a	AB 2.2±0.4a
	BjGM	A 2.8±0.5a	A 3±0.4 a	AB 1.9±1.1 a	B 1.3±1.3a	AB 2.1±0.3a
	BjSM	B 1.4±1ab	B 1.4±1.4 b	B 1.9±1.1a	B 1.3±1.4a	A 3.3±0.4 a
	Control	B 0.7±1 b	AB 1.4±1b	B 0.6±1ab	B 0.7±1.2a	A 2.5±0.1 a
	MBC	A 0 b	A 0 b	A 0b	A 0a	A 0.6± b
<i>Trichoderma</i>	BcSM	A 3±0.3 a	A 2.8±0.4a	A 3±0.5a	A 2.8±0.3a	A 2.9±0.1 a
	BjGM	A 3.3±0.2a	A 3.2±0.2a	A 3.1±0.2a	A 2.9±0.3a	A 2.9±0.2 a
	BjSM	B 0.8±1 b	B 0.6±1b	A 2.6±0.4a	A 2.9±0.4a	A 2.9±0.3 a
	Control	A 3.2±0.3a	A 3±0.3a	A 3±0.2a	A 3.1±0.2a	A 2.9±0.2 a
	MBC	A 2.9±0.4a	A 1.6±1ab	A 2.2±1.3a	A 2.2±1.3a	A 2.2±1.3 a
<i>Cylindrocarpon</i> [‡]	BcSM	1.4±0.8	0	0	0.6±0.9	0.7±0.6
	BjGM	0.3±0.6	0	0	0±0	1.3±0.8
	BjSM	0.6±0.9	0	0	0.6±0.9	0.8±0.9
	Control	1.2±0.7	0	0	0.6±0.9	1.1±0.9
	MBC	0	0	0	0	0.3±0.6
	Average	AB 0.7	CD 0	D 0	BC 0.3	A 0.9
<i>Pythium</i>	BcSM	A 0.6±1 b	A 0 b	A 0b	A 0 a	A 0 a
	BjGM	A 2.8±0.3a	B 2±1.2a	B 1.4±1.4a	C 0 a	C 0 a
	BjSM	A 0 b	A 0 b	A 0 b	A 0 a	A 0 a
	Control	A 0 b	A 0 b	A 0 b	A 0 a	A 0 a
	MBC	A 0 b	A 0 b	A 0 b	A 0 a	A 0 a
Actinomycetes	BcSM	A 4.3±1 ab	A 3.2±2a	A 3.6±2.1a	A 3.9±0.9a	A 4.2±0.7 a
	BjGM	B 0.7±1cd	B 1±1.8ab	B 0±0 b	AB 2.8±0.2a	A 3.5±0.3 a
	BjSM	A 4.8±0.7a	B 2.2±2ab	A 4.4±0.7a	AB 4.1±0.6a	AB 4.1±0.5a
	Control	B 2.4±1bc	C 0.9±1.6ab	C 1.2±2.1b	B 2.5±1.7a	A 3.7±0.4 a
	MBC	B 0 d	B 0b	B 0 b	B 0.6±0.9b	A 3.4±0.6 a

[†]Soil had been left untreated (control) or treated with *Brassica carinata* seed meal (BcSM; 4484 kg ha⁻¹), *Brassica juncea* seed meal (BjSM; 4484 kg ha⁻¹), *B. juncea* green manure (BjGM at 11.2 kg seeds ha⁻¹) or methyl bromide/chloropicrin (MBC; 392 kg ha⁻¹, 67% methyl bromide, 33% chloropicrin). Seedling transplanting was done at 235 DAT (days after treatment application) and harvested at 445 DAT. Means within a row and preceded by the same upper case letter are not significantly different ($P \leq 0.05$). Means within a column and associated with a given microbial group and followed by the same lower case letter are not significantly different ($P \leq 0.05$).

[‡]Only the main effect of time was significant. Time X treatment means were presented for consistency.

15 Table 3. Douglas-fir seedlingsize and root colonization at Nursery A, WA at the end of 2010
 16 growing season.

Treatment [†]	<i>Fusarium</i>	<i>Trichoderma</i>	<i>Cylindroc arpon</i>	Height	Caliper	Shoot fresh biomass	Root fresh biomass
	-----%-----			--cm--	--mm--	--g--	--g--
BcSM	55±11	9±3	31±12b	41±2bc	7.9±0.4b	33±3bc	24±4
BjGM	56±15	2±4	61±15a	45±3ab	8.3±0.2ab	41±6ab	27±2
BjSM	59±22	6±4	35±18ab	41±3bc	7.6±0.5b	33±6bc	23±5
Control	38±8	5±4	33±7b	40±2c	7.6±0.4b	30±4c	25±2
MBC	28±26	6±4	12±7c	46±1a	8.6±0.6a	43±5a	29±4

17
 18 [†]BcSM = *Brassica carinata* seed meal at 4484 kg ha⁻¹; BjGM= *Brassica juncea* green manure
 19 seeded at 11.2 kg seeds ha⁻¹; BjSM = *Brassica juncea* seed meal at 4484 kg ha⁻¹; Control =
 20 untreated control; MBC = methyl bromide/chloropicrin, 392 kg ha⁻¹ (67% methyl bromide, 33%
 21 chloropicrin). Data followed by same letter within a column were not significantly different
 22 (P≤0.05).

Table 4. Soil microorganisms as affected by study treatments during 2012 experiment at Nursery B, Washington (log₁₀ of colony forming units (log (1+CFU g soil⁻¹)).

Treatments [†]		Days after treatment application			
		243	306	475	Average
		Log (1 + CFU g soil ⁻¹)			
<i>Fusarium</i>	BcSM	AB 2.8±0.1 a	B 2.7±0.1 b	A 3.1±0.1 ab	
	BjGM	C 1.8±0.1 b	B 2.4±0.1 c	A 3.1±0.1 b	
	BjSM	B 2.6±0.1 a	B 2.7±0.1 b	A 3.1±0.1 b	
	Control	B 2.6±0.1 a	B 2.9±0.1 a	A 3.2±0.1 a	
	MBC	A 2.4±0.2 a	A 2.5±0.1 c	A 2.5±0.2 c	
<i>Trichoderma</i> [‡]	BcSM	3.4±0.1	3.4±0.03	3.2±0.1	3.3 bc
	BjGM	3.5±0.1	3.5±0.05	3.3±0.1	3.4 b
	BjSM	3.3±0.1	3.4±0.1	3.2±0.1	3.2 cd
	Control	3.3±0.1	3.2±0.06	3.1±0.2	3.2 d
	MBC	3.8±0.1	3.7±0.07	3.6±0.1	3.7 a
	Average	A 3.4	A 3.4	B 3.3	
<i>Cylindrocarpon</i> ^δ	BcSM	0	0.5±1.1	0.7±1.2	
	BjGM	0.5±0.9	0.6±1.2	1.8±1.1	
	BjSM	0.5±0.9	0.6±1.1	0±0	
	Control	0.5±0.9	0.5±1	1.3±1.3	
	MBC	0	0.6±1.2	0.6±1	
<i>Pythium</i> [‡]	BcSM	1.7±0.1	1.7±0.2	0.4±0.7	1.3 a
	BjGM	1.5±0.1	1.8±0.1	0.8±0.3	1.4 a
	BjSM	1.7±0.1	1.6±0.3	0.7±0.5	1.3 a
	Control	1.6±0.1	1.8±0.2	1±0.6	1.5 a
	MBC	0.3±0.4	0.8±0.1	0.6±0.6	0.5 b
	Average	A 1.4	A 1.5	B 0.7	
Actinomycetes	BcSM	AB 3.4±0.1 a	A 3.2±0.1 a	A 3.6±0.1 bc	
	BjGM	AB 3.4±0.1 a	B 3.4±0.1 a	A 3.8±0.1 ab	
	BjSM	AB 3.6±0.1 a	B 3.4±0.1 a	A 3.7±0.1 b	
	Control	A 3.3±0.1 a	A 3.4±0.1 a	A 3.5±0.1 c	
	MBC	C 1.1±1 b	B 2.2±0.1 b	A 3.9±0.1 a	

[†]Soil amendments were *Brassica carinata* seed meal (BcSM; 4484 kg ha⁻¹), *B. juncea* green manure (BjGM 11.2 kg seeds ha⁻¹), *B. juncea* seed meal (BjSM; 4484 kg ha⁻¹), untreated control, and methyl bromide/chloropicrin (MBC; 392 kg ha⁻¹, 67% methyl bromide, 33% chloropicrin). Seedling transplanting was done at 251 DAT (days after treatment application) and harvested at 374 DAT. Data within a row preceded by same upper case letter were not significantly different (P≤0.05). The data within a column, associated with a given organism and followed by same lower case letter were not significantly different (P≤0.05).

[‡]Only the main effect of time and treatment were significant. Time x treatment means were presented for consistency.

^δNeither treatment nor time was significant. Time x treatment means were presented for consistency.

37 Table 5. Summary of soil treatment effects reported in Tables 2-4 and Figures 3-4 for studies in Douglas-fir nurseries in the 2010 and
 38 2012 growing seasons of nursery A and nursery B, Washington, respectively. Comparisons are to negative control unless noted
 39 otherwise.

Source table, figures, and growing season	Response variable	MBC [†] effect	BjGM [‡] effect	BcSM and BjSM ^δ effect
Table 2; 2010	Soil <i>Fusarium</i> Soil <i>Trichoderma</i>	Reduced. Similar to control	Increased early Similar to control	Generally similar to control BcSM similar to control BjSM reduced early
Table 3; 2010	Soil <i>Cylindrocarpon</i>	No effect of treatment	No effect of treatment	No effect of treatment
	Soil <i>Pythium</i>	Similar to control	Increased	Similar to control
	Soil actinomycetes	Reduced	Similar to control	Increased or similar to control
	Root <i>Fusarium</i>	No effect of treatment	No effect of treatment	No effect of treatment
	Root <i>Trichoderma</i>	No effect of treatment	No effect of treatment	No effect of treatment
	Root <i>Cylindrocarpon</i>	Reduced	Increased	Similar to control
	Tree height	Increased	Increased	Similar to control
	Tree caliper	Increased	Similar to control and MBC	Similar to control
	Shoot fresh biomass	Increased	Increased	Similar to control
Table 4; 2012	Root fresh biomass	No effect of treatment	No effect of treatment	No effect of treatment
	Soil <i>Fusarium</i>	Reduced late season	Reduced	Generally reduced
	Soil <i>Trichoderma</i>	Increased	Increased	BcSM increased BjSM similar to control
Fig. 3 & 4; 2012	Soil <i>Cylindrocarpon</i>	No effect of treatment	No effect of treatment	No effect of treatment
	Soil <i>Pythium</i>	Reduced	Similar to control	Similar to control
	Soil actinomycetes	Reduced early, increased at harvest	Similar to control, increased at harvest	Generally similar to control
	Dehydrogenase activity	Similar to control, reduced at harvest	Similar to control, increased at harvest	Similar to control, increased at harvest
	N mineralization	Reduced	Similar to control, greater than MBC	Similar to control

40 [†]MBC = methyl bromide/chloropicrin, 392 kg ha⁻¹ (67% methyl bromide, 33% chloropicrin).

41 [‡]BjGM = *Brassica juncea* green manure seeded at 11.2 kg ha⁻¹;

42 ^δBcSM = *Brassica carinata* seed meal at 4484 kg ha⁻¹; BjSM = *Brassica juncea* seed meal at 4484 kg ha⁻¹