Biofumigation Potential of *Brassica* Soil Amendments in Douglas-fir Seedlings

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Abstract: *Fusarium* root rot is one of the major soil borne diseases of conifers. Soil fumigation with methyl bromide has been the most effective control method. Because of safety and environmental concerns, methyl bromide use is increasingly restricted. However, the conifer seedling industry continues to use methyl bromide under a critical use exemption due to a lack of effective alternatives. A field study was conducted to examine the effects of *Brassica* seed meals and green manures on management of selected soil borne pathogens. The study consisted of five treatments: *Brassica juncea* seedmeal (BJSM), *Brassica carinata* seedmeal (BCSM), *Brassica juncea* green manure (BJGM), methyl bromide/ chloropicrin fumigation (MBC) and control, with four replications in randomized complete block design. The treatments were incorporated into soil in fall (September 2011) and Douglas-fir (*Pseudotsuga menziesii*) seedlings were transplanted into plots in spring (May 2012). Pathogen populations, nitrogen mineralization rate, and dehydrogenase enzyme activity in soil were assessed at pre-transplant, post-transplant, and seedling harvest. The initial pathogen count was not significantly different among treatments. At transplant time, *Fusarium* spp. were significantly lower in BJGM (146 CFU g^{-1}) than MBC (290 CFU g^{-1}), and control (357 CFU g^{-1}); whereas *Trichoderma* were significantly higher in MBC (5716 CFU g^{-1}) followed by BJGM (3031 CFU g^{-1}), and control (1763 CFU g^{-1}). Dehydrogenase enzyme activity was highest in BJGM (0.81 µg TPF g^{-1} hr^{-1}) followed by BJSM (0.62 µg TPF g^{-1} hr^{-1}). Similarly, the mineralizable nitrogen was higher in BJGM (5.8 and 6.8 µg NH4 g^{-1} during 7- and 28-day incubation respectively) followed by BJSM (4.0 and 5.8 µg NH4 g^{-1}). These preliminary results suggest that *B. juncea* green manure has a suppressive effect on soil borne pathogens, and both green manure and seed meals have positive impacts on soil quality.

Key Words: *Fusarium* root rot, *Brassica* green manure, *Brassica* seed meals, methyl bromide, soil health

Introduction

Fusarium spp. cause many diseases in conifer seedlings (Hamm and Hansen 1989; Brownell and Schneider 1983), some of the most intense being damping-off, blight and root rot (James 1986; Landis 1989; Hamm and others 1990). The conifer seedling nursery industry currently is facing enormous pressures from fungal diseases. The Nursery Technology Cooperative (NTC 2009) stated that the pathogens causing the most significant threats to bareroot nurseries are *Fusarium, Pythium,* and *Cylindrocarpon*. *Fusarium* root rot is one of the most common soil borne diseases of conifer seedlings. This disease is a serious problem on many different species of conifers and generally occurs wherever bare root nursery stock is produced. In addition to causing significant losses in the nursery, the survival and growth of out-planted seedling can also be adversely affected, resulting in significant replanting costs. *Fusarium* and other soil-borne pathogens must be managed for profitable nursery production, and more cost-effective management options are needed.

Fusarium species 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). Fusarium root rot of conifer seedlings caused by several species of *Fusarium* is the most serious disease in Pacific Northwest bare-root nurseries, causing severe crop and economic losses annually. *Fusarium* spp. are ubiquitous in most container and bareroot nurseries, where they occur on healthy and diseased conifer seedlings, especially Douglas-fir (*Pseudotsuga menziesii*), western white pine (*Pinus monticola*) and ponderosa pine (*Pinus ponderosa*) (James and others 1997). The major fungal pathogen was previously identified as *F. oxysporum* based on morphology (Bloomberg 1981). However, among morphologically identified *F. oxysporum* isolates from Douglas-fir seedlings and soil, Stewart and others (2006) found that disease symptoms were caused only by *F. commune*, a genetically distinct recently named species (Skovgaard and others 2003).

Application of soil fumigants like methyl bromide (MeBr) and metam sodium before transplanting seedlings has been the basis for the control of soil-borne pests in developed countries. Soil fumigation is commonly used in bare-root forest nurseries to manage soilborne pests including fungal pathogens, nematodes, weeds, and insects. Formulations of methyl bromide and chloropicrin were the most commonly used fumigant treatments in US forest nurseries (Smith and Fraedrich 1993). Methyl Bromide has been the most extensively used commercial chemical because it is considerably more effective. However, methyl bromide was listed as one of the ozone depleting substance by the Montreal Protocol in 1992 and its production was discontinued by 1995 (Prather and others 1984; Bell and others 1996). This became a major concern to farmers in countries including US where it is used for the production of economically important crops including forest seedlings. However, growers are continuing use of MeBr under critical use exemption (Byrd and others 2006). Consequently, finding alternatives to MeBr use has become crucial.

Biofumigation is the beneficial use of *Brassica* green manures that release isothiocyanates chemically similar to methyl isothiocyanate, the active agent from the synthetic fumigant (Kirkegaard and others 1993; Matthiessen and Kirkegaard 2006; Omirou and others 2011). The exploitation of maximum biofumigation potential has been a key research goal. The factors affecting the release of isothiocyanates into soil have been intensively researched. This understanding has led to some commercial adoption of biofumigation, which when applied to appropriate production systems, can have efficacy and offer cost savings. More research is needed to confirm the efficacy of biofumigation in Douglas-fir seedlings. A field study was conducted to examine the effects of *Brassica* seed meals and green manures on management of selected soil borne pathogens. The major objective of this was to determine effects of soil amendment with green manure or seed meal of *Brassicaceous* spp. on soil and root pathogen levels and beneficial organisms like *Trichoderma*. The *Trichoderma* spp. are beneficial fungal populations that can naturally act as biological control agents for several soil-borne pathogens (Kucuk and Kivanc 2003) via various mechanisms like competition with the pathogen for nutrients and direct parasitism, antibiosis, induced resistance, and production of cell wall degrading enzymes (Verma and others 2007; Alabouvette and others 2009). We hypothesized that one or more *Brassica* seed meals or green manure will reduce *Fusarium* on seedling root and soil compared to untreated soils and will also enhance soil health and beneficial organisms.

Materials and Methods

Study Area and Treatments

The study was conducted in IFA Nurseries, Toledo Washington. The study consisted of five treatments:

- *Brassica juncea seedmeal (BJSM),*
- *Brassica carinata seedmeal (BCSM),*
- *Brassica juncea green manure (BJGM),*
- *methyl bromide/chloropicrin fumigation (MBC, 67% methyl bromide and 33% chloropicrin) (positive control) and*
- *no treatment (negative control)*

The study was carried out with four replications in randomized complete block design (RCBD). The *Brassica* seed meals at 4.94 tons ha^{-1} (4409 lbs $acre^{-1}$), *Brassica juncea* green manure at 11.2 kg seeds ha^{-1} (10 lbs $acre^{-1}$) and MBC at 392.2 kg ha^{-1} (350 lbs $acre^{-1}$) were incorporated into soil in fall (September 2011) followed by tarping with plastic until next spring. Douglas-fir (*Pseudotsuga menziesii*) seedlings were transplanted into plots in spring (May 2012). Pathogen populations, nitrogen mineralization rate, and dehydrogenase enzyme activity in soil were assessed.

Sampling

Soil samplings were conducted four times (pre-treatment September 2011, pre-transplant May 2012, post-transplant July 2012, and harvest January 2013) during the study period. Ten sub-samples were taken from 0-15 cm (0-5.9 in) in each plot using core sampler and were mixed well in a zip-lock bag. Aseptic procedure was used to avoid cross contamination between treatments. The sampling bags were sealed and transported to the laboratory in a cooler. All samples were maintained at field moist condition and were stored at 4 ^oC (39.2 ^oF) until analyzed.

Laboratory Analyses

Soil pathogens were analyzed using 2.5 g (0.088 oz) soil and following standard laboratory procedure of soil dilution plating. *Pythium* were grown and enumerated in V8 agar medium (Stevens 1974) and *Fusarium* along with *Trichoderma*, actinomycetes were grown on Komoda's medium (Komoda 1976).

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) g^{-1} soil hr^{-1}.

Mineralizable nitrogen was quantified in 7- and 28-day incubation at 40 °C (104 °F) and ammonium quantification (Waring and Bremner 1964).

Statistical Analyses

Data were analyzed using randomized complete block design in statistical analysis software SAS version 9.3 (SAS 2008) using Proc GLM procedure. Data collected in each sampling were analyzed separately to determine treatment effect for each parameter. Pairwise comparisons of treatment means were conducted using Tukey's procedure and differences were declared significant at five percent level of significance ($p \leq 0.05$).

Results

Soil Pathogens Counts

The initial pre-treatment counts of *Fusarium* ranged from 1298 to 1659 colony forming units (CFUs) g^{-1} dry soil and *Trichoderma* ranged from 5615 to 7472 CFUs g^{-1} among treatments. Although there is numerical variation as expected, those were not statistically significant (data not shown). The following spring after treatment, *Fusarium* counts were significantly lower in BJGM treatment (146 CFU g^{-1} soil) followed by MBC (290 CFU g^{-1} soil) (figure 1) and *Trichoderma* counts were significantly higher in MBC and BJGM compared to control (figure 2).

At post-transplant time, the *Fusarium* population was significantly lower in BJGM and MBC treatments compared to control (figure 1). At harvest time, the *Fusarium* populations increased in number in all treatments but were significantly lower in the soil amendment treatments (BJGM, BJSM, and BCSM) compared to control (figure 1).

Similarly, the *Trichoderma* were highly variable at post-transplant time, and harvest (figure 2). The *Trichoderma* were significantly higher in MBC and BJGM compared to control at summer. However, there were not significant variation among soil amendments and control at harvest (figure 2).

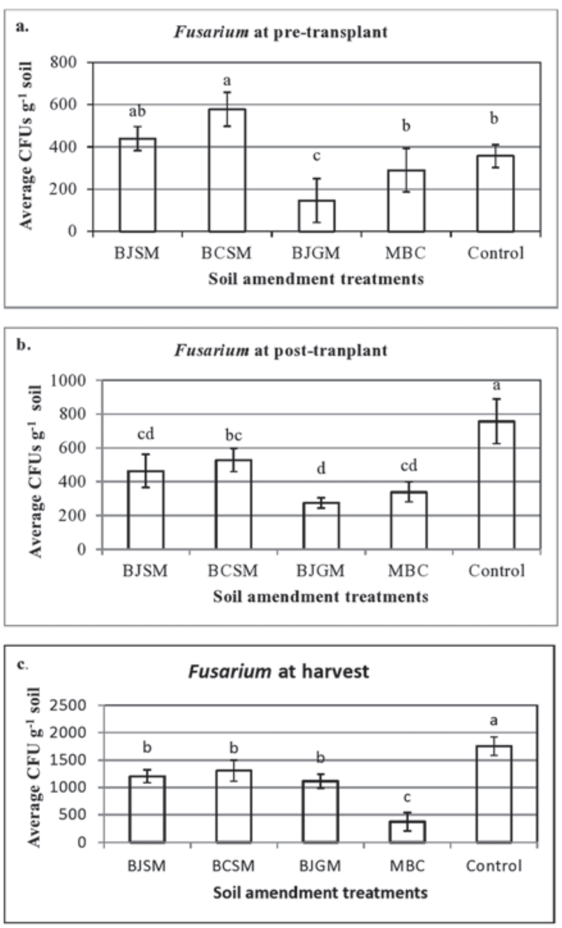

Figure 1. Bulk soil populations of *Fusarium spp.*in five study treatments, *Brassica juncea* seed meal (BJSM), *Brassica carinata* seed meal (BCSM), *Brassica juncea* green manure (BJGM), methyl bromide/chloropicrin fumigation (MBC) and control at pre-transplant, May 2012 (a.), post-transplant, July 2012 (b.), and seedling harvest, January 2013 (c.). Treatments were applied to soil the prior September 2011.

The *Pythium* populations were also significantly suppressed by MBC at all post-treatment samplings but there were not any significant difference among other treatments (data not shown). A high variability was observed in actinomycetes populations among study treatments (data not shown).

Soil Quality Assessment

Soil dehydrogenase activity, an indicator of total microbial oxidizing activity, was similar among treatments at pre-transplant time (table 1). At post-transplant, BJGM had significantly greater activity (0.81 µg TPF g^{-1} hr^{-1}) compared to MBC (0.57 µg TPF g^{-1} hr^{-1}) (table 1). At harvest all *Brassica* treatments had significantly greater activity compared to MBC, and BJGM was also greater than control.

Mineralizable nitrogen at pre-transplant was significantly greater in BJGM compared to MBC and there were no difference among other treatments ($P \leq 0.1$). After both 7- and 28-day incubation of soil samples, the extractable ammonium trend was BJGM > control > MBC (table 2.)

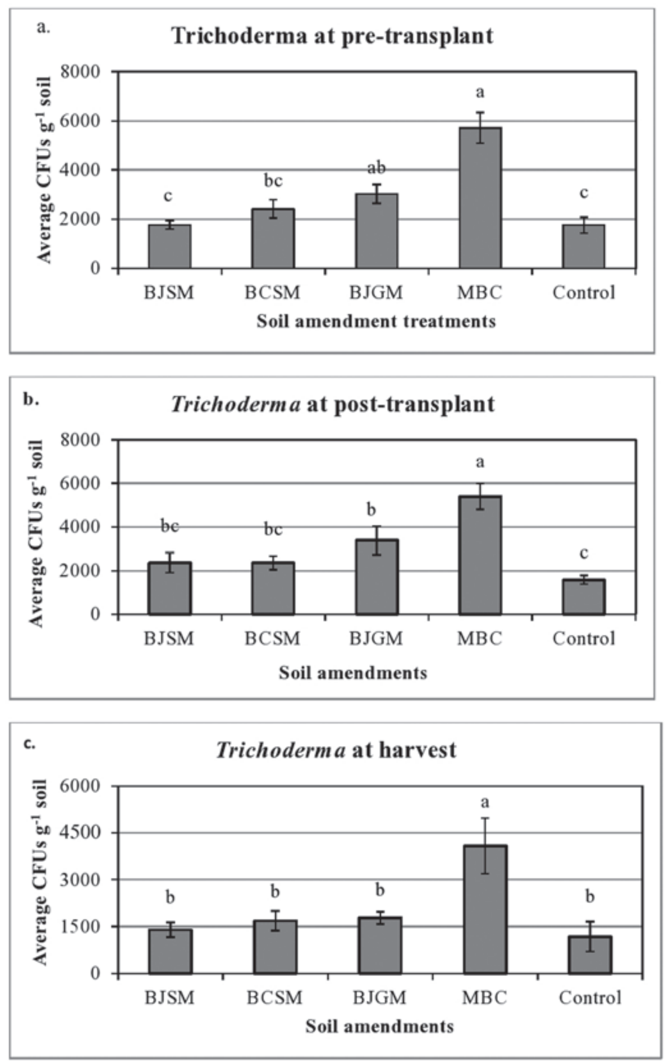

Figure 2. *Trichoderma* population in soil in five study treatments, *Brassican juncea* seed meal (BJSM), *Brassica carinata* seed meal (BCSM), *Brassica juncea* green manure (BJGM), methyl bromide/chloropicrin fumigation (MBC) and control at pre-transplant (a.), post-transplant (b.) and harvest time (c.)

Table 1. Dehydrogenase enzyme activity in bulk soil at seedling transplant as affected by study treatments *Brassican juncea* seed meal (BJSM), *Brassica carinata* seed meal (BCSM), *Brassica juncea* green manure (BJGM), methyl bromide/chloropicrin fumigation (MBC) and control.

	Dehydrogenase enzyme activity (μg TPF g^{-1} hr^{-1})		
Treatments	**Pre-transplant**	**Transplant**	**Harvest**
BCSM	0.485 a	0.570 b	0.670 ab
BJGM	0.463 a	0.810 a	0.735 a
BJSM	0.452 a	0.618 b	0.617 ab
MBC	0.384 a	0.585 b	0.310 c
Control	0.366 a	0.688 ab	0.463 bc

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

Table 2. Mineralizable nitrogen as affected by treatments at pre-transplant *Brassica juncea* seed meal (BJSM), *Brassica carinata* seed meal (BCSM), *Brassica juncea* green manure (BJGM), methyl bromide/chloropicrin fumigation (MBC) and control

	Net μg NH_4-N g^{-1} soil	
Treatments	**7-day incubation**	**28-day incubation**
BCSM	2.53 b	5.87 a
BJGM	5.78 a	6.82 a
BJSM	4.01 ab	5.82 a
MBC	3.80 b	3.63 b
Control	4.15 ab	5.73 a

Data followed by same letter within a column were not significantly different ($P \leq 0.1$)

Discussion

In this study and in most conifer nurseries using fumigation or green manures, treatments were applied in the fall. By the next spring, *Fusarium* populations in all treatments including control were much lower, with BJGM the lowest. Several studies have observed similar reductions in *Fusarium* after *Brassica* plant tissue incorporation (Subbarao and others 1999; Pinkerton and others 2000; Smolinska 2000; Mazzola and others 2001; Cohen and others 2005; Mazzola and Mullinix 2005). *Fusarium* populations increased over the growing season in all treatments but at harvest all treatments had lower populations than control.

There are examples of research where *Brassica* amendments have been successful for soil-borne pathogen control (Subbararao and others 1999; Cohen and others 2005) as well as examples of failure (Blok and others 2000; Zasada and others 2003). In a few similar studies, the *Fusarium* and *Pythium* populations in *Brassica* amended soils were even significantly higher than those in control (Njoroge and others 2008). Hence, the effect of biofumigation has not been consistent, possibly due to many factors. It has been reported that after soil amendments with *Brassica juncea* and *Brassica napus*, the populations of both fungi and bacteria including *Fluorescent pseudomonads* increased compared with nonamended soils (Smolinska 2000). Hence there may be additional biological mechanisms governing disease control by brassicas besides glucosinolate hydrolysis to isthiocyanates (Blok and others 2000; Takehara and others 2004; Cohen and others 2005). The populations of pseudomonads do not always change following *Brassica* amendments (Scott and Knudsen 1999). This suggests that the type of *Brassica*, even genotype of plant (Mazzola and Gu 2002), and the soil type may influence the effect on fluorescent pseudomonads and other beneficial and pathogenic organisms. It is highly important to monitor the plant pathogens as well as beneficial organisms to better characterize the effect of soil amendments.

In this study, the decrease in *Fusarium* populations was highly correlated with increase in *Triochoderma* counts (figure 1, 2). The effects of *Brassica* seed meals were not as great as *Brassica* green manure which also resembles other research in similar arenas (Mazzola and Gu 2002). *Brassica* seed meals were more effective in disease and weed control when used in combination or certain formulation (Mazzola and Brown 2010).

Green manures and other organic amendments can also provide benefits to following crops and to farming systems in general, including maintenance of soil cover and soil integrity, soil sanitization, reduced erosion, greater soil organic matter, and soil structural improvements that improve water penetration (Bailey and Lazarovits 2003; Thorup-Kristensen and others 2003). On sandy irrigated soils, the inclusion of *Brassica* green manure crops has reduced the level of wind erosion and improved the water infiltration of soils, with improvement in soil structure (Gies 2004; McGuire 2004). *Brassica* green manure crops are effective at capturing soil mineral nitrogen, and when incorporated into the soil can provide a source of organic nitrogen that can become available to subsequent crops. These improvements to crop nutrition and water relationships may also improve disease tolerance regardless of changes in soil microbial communities.

Following the dynamics of soil organisms, the soil dehydrogenase activity, which represents overall soil oxidation activity, was enhanced by *Brassica* green manure (table 1). Also the mineralizable nitrogen was marginally higher in *Brassica* green manure treatment. These improvements may be attributed to the increased organic matter and greater activities of roots compared to un-amended soil (Myers and others 2001; Kremer and Li 2003; Mungai and others 2005). Increased foodstuffs directly from amendments and indirectly from root exudation may enhance the diversity of organisms and the ecosystem functions they perform. More overall microbial activity and available organic nitrogen may thereby improve seedling health.

Conclusions

The nature of results of *Brassica* soil amendments observed in this study support the hypothesis that *Brassica* green manure or seed meals will suppress soil pathogens and enhance beneficial organism in soil. Results hold true that the effectiveness and mechanism of disease suppression are influenced by various factors including *Brassica* species and variety, soil texture, and timing and process of incorporation. To conclude, the *Brassica juncea* green manure showed good potential to control *Fusarium spp*. in Douglas-fir seedling soil and enhance soil health.

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