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

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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* seed meal, *B. carinata* seed meal, *Sinapis alba* seed meal, *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.

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 seed meal, and compost to manage soil-borne pathogens (Litterick et al., 2004;

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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; Omirou 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; Handiseni 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 Handiseni et al. (2013) revealed that brassica seed meals were relatively successful in suppressing *Rhizoctonia solani* 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 (*Pseudotsuga menziesii)* 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' seed meal at 4484 kg ha^{-1}(SaSM) and 2242 kg ha^{-1} (SaSM-l); *Brassica juncea* cv. 'Pacific Gold' seed meal at 4484 kg ha^{-1} (BjSM) and 2242 kg ha^{-1} (BjSM-l); *B. carinata* seed meal (commercial product, 'Biofence') at 2242 kg ha^{-1} (BcSM-l) 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}, BjSM 4484 kg ha^{-1}, BcSM 4484 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 (*Pseudotsuga menziesii*) 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,