Green manure effects on soilborne pathogens

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Abstract - Green manures from cover crops incorporated into nursery soils stimulated growth of pathogenic soil fungi. Bare fallow treatments reduced populations of soil pathogens to levels comparable to standard chemical fumigation. Seedling disease and quality were adversely affected by higher levels of pathogens in unfumigated cover cropped treatments, but were comparable in unfumigated bare fallow and standard chemical fumigation treatments.

INTRODUCTION

Cover crops have been traditionally used during non production rotations in nurseries for a variety of reasons (see paper by Robin Rose, this volume). One of the commonly cited benefits of using cover crops in the cultural sequence is the addition of organic matter to soils. Cover crops are most often incorporated by plowing under at maturity, either in the fall or spring, as a green manure. The addition of cover crop green manures, however, causes increases in populations of soil borne fungal pathogens (Hansen et al. 1990, Hamm and Hansen 1990). Incorporation of cover crop residues provides a nutrient resource that stimulates growth and reproduction of soil fungi such as *Fusarium* and *Pythium spp.* which can be opportunistic pathogens. Most nurseries that operationally employ a cover crop green manure in their rotation also routinely employ soil fumigation after incorporation of the cover crop and before sowing production seed to reduce populations of pathogenic fungi and other nursery pests, e.g. pathogenic nematodes, insects, and weeds (Landis and Campbell 1988).

It should be noted that all of these aforementioned cultural problems corrected by soil fumigation may be contributed to or aggravated by the practice of incorporating cover crops as green manures. The need for routine soil fumigation may be reduced or eliminated entirely by eliminating cover cropping from the cultural sequence. By keeping nursery beds fallow for one season between production cycles, the nutrient resources of the soil become depleted, and populations of soil pathogens decline correspondingly. Hansen et al. (1990) reported that population levels of *Fusarium* and *Pythium spp.* from fallow, unfumigated plots and cover cropped, fumigated plots were comparable by the spring following fall fumigation.

Rapeseed *(Brassica spp.)* has been suggested as a potential cover crop that may reduce the levels of various nursery pests. The basis for this suggestion is that species of *Brassica* contain as natural secondary metabolites glucosinalates, a family of thioglucoside molecules found in members of the Cruciferae. When the glucose is cleaved off in a chemical reaction catalyzed by the enzyme myrosinase, which also occurs naturally in the *Brassica* plants, a variety of chemical products are released, depending on the structure of the particular glucosinalate species (Figure 1). Among the products released by this reaction are isothiocyanates (Hoglund et al. 1991, Appelqvist and Josefsson 1967, Fenwick et al 1983, VanEtten and Tookey 1983). Many isothiocyanates have potent antimicrobial activity, and methyl isothiocyanate is the primary active ingredient of several widely used commercial soil fumigants (Landis and Campbell 1990). The occurrence of glucosinalates and myrosinase in *Brassica* plants may help confer resistance to certain pathogens (Rawlinson 1979, Mithen and Lewis 1986, Greenhalgh and Mitchell 1976, Holley and Jones 1985, Walker et al. 1937).

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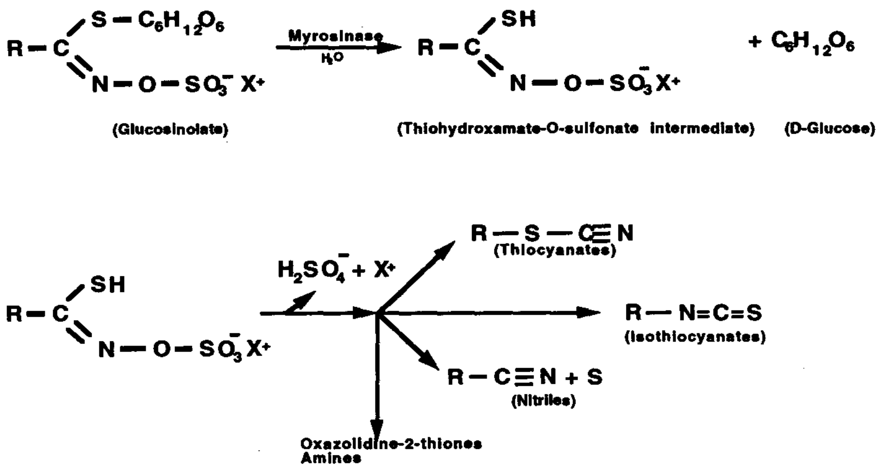

Figure 1. Pathway and products of myrosinase-catalyzed hydrolysis of glucosinalates. Based on Pessina et al. 1990.

Brassica residues used as soil amendments have been reported to reduce diseases caused by *Fusarium oxysporum* f. sp. *conglutinans* ("cabbage yellows") (Ramirez-Villapudua and Munnecke 1987) and *Aphanomyces* root rot of pea (Papavizas 1966, Chan and Close 1987, Muelchen et al. 1990). Brassica cover crops mown down at maturity and incorporated into soils as green manures therefore might reduce the populations of soilborne pathogens through the release of fungicidal isothiocyanates in the same way as some commercial fumigants.

During the past several years, a collaborative research project at Oregon State University has investigated the potential for using soil amendments, rapeseed cover crops, and bare fallowing in bareroot forest nurseries as alternatives to routine chemical soil fumigation. Populations of soilborne pathogenic fungi, incidence and severity of seedling disease, and seedling quality were assessed in nursery plots with Brassica cover crops, sawdust, and/or rapeseed meal soil amendments and compared with bare fallow and standard nursery cover crop-soil fumigation procedures in nurseries in Oregon and Washington. This paper reports some of our results to date.

METHODS

The study was located in a production field in the Bend Pine Nursery, Bend, Oregon. A full description of the climate and soil characteristics of this nursery can be found in the USDA Forest Service Pacific Northwest Region Nursery Pest Management Final Environmental Impact Statement (Anonymous 1989). The block in which the study plots were located had been fumigated with methyl bromide/ chloropicrin in the fall of 1989 and the study was installed and cover crop sown in April 1990. The study block was comprised of eight contiguous beds in a 50 ft x 400 ft block which was divided into 24 plots, 18 x 50 ft. Four replicates of 6 treatments were established in the block: Bare fallow, *Brassica hirta* cv. 'Humus' cover crop (with and without sawdust amendment), Russian pea cover crop with methyl bromide fumigation, bare fallow with rapeseed meal (cv. Dwarf Essex) added at 6000 kg/ acre, *Brassica hirta* cv. 'Humus' cover crop amended with rapeseed meal (cv. Dwarf Essex) at 6000 kg/ acre.

Soil samples were collected at four intervals during the cultural cycle: at the time of sowing the cover crop (May 1990), at cover crop maturity (Aug 1990), following fumigation or cover crop incorporation (October 1990), and prior to conifer seed sowing (May 1991). Soil samples were processed as described by Hansen et al. 1991 for estimation of *Fusarium* and *Pythium* populations.

Brassica biomass was determined from collections taken at cover crop maturity from two randomly located subplots, 1 sq. meter each, for each replicate for each treatment. Subsamples of mature *Brassica* plants were also collected for glucosinalate analysis.

Seedling density and mortality were assessed at 2-week intervals in two, 0.5 x 4 ft. fixed plots per replicate for each treatment, commencing at 4 weeks following sowing. At the end of the first growing season, seedling height and caliper were determined from four, 0.5 x 4 ft. plots per replicate plot for each treatment. Root biomass was determined from 25 seedlings dug from each replicate for each treatment. At lifting, the numbers of shippable seedlings were compared for samples from each replicate plot for the bare fallow and standard methyl bromide fumigated treatments.

Fusarium and *Pythium* populations, seedling density, mortality, height, caliper, and root biomass were analyzed by analysis of variance procedures using Statgraphics and SAS statistics software. Count data were square root transformed and proportional data were log transformed as recommended by Sabin and Stafford (1990) prior to analysis. Multiple comparisons were computed using the method of Scheffe (Sokal and Rohlf 1981).

RESULTS

Fusarium and *Pythium* populations

Initial *Fusarium* populations were low, generally below 2000 propagules per gram, and relatively uniform throughout the study area. In cover cropped treatments, populations either increased slightly from initial levels, or remained near initial levels at cover crop maturity. *Fusarium* populations increased to well above initial levels after mowing and incorporation of *Brassica* cover crops. *Fusarium* numbers then decreased somewhat by the spring pre-sow sampling, but remained much higher than initial levels. In bare fallow treatments *Fusarium* populations declined from initial levels throughout the growing season until the following spring before sowing the conifer seed. Chemical fumigation in the Fall reduced *Fusarium* populations to below detection in the two fumigated treatments, but by spring, populations had begun to recover. *Fusarium* populations for all treatments were statistically homogeneous for the initial sampling and mature cover crop sampling periods. By the spring pre-sow period, two distinct statistical groups were evident, bare fallow and chemical (methyl bromide) fumigation comprised one equivalent statistical group, the two *Brassica* cover crop treatments comprised the second group.

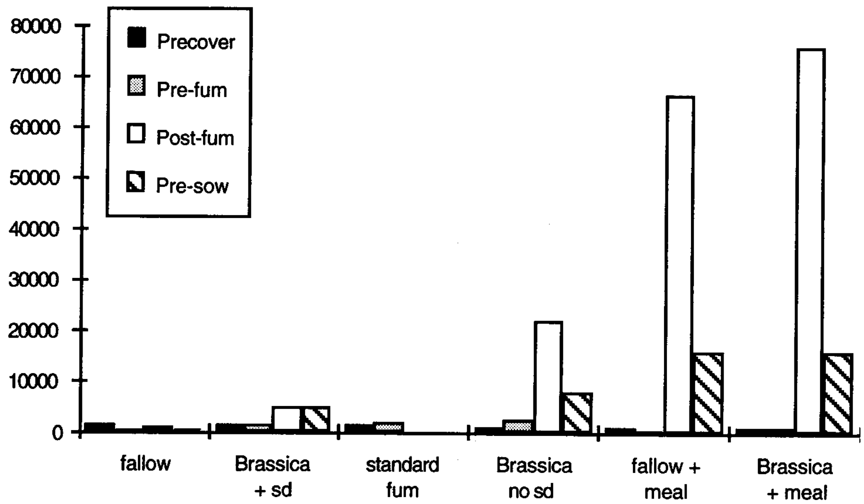

Figure 2. Averaged Fusadum levels (propagules per gram) in soil sample from four replicates of each of six treatments collected at four sampling periods in 1991 - 1992. Brassica + sd is sawdust amended treatment (all treatments had sawdust applied except Brassica no sd).

Pythium populations followed a pattern of response to treatments similar to *Fusarium* (Figure 3). Initial populations were relatively low and uniform throughout the study area and either increased slightly or remained near initial levels through the growing season to cover crop maturity. At cover crop maturity, *Pythium* levels were much greater than for the bare fallow or either *Brassica* cover crop treatments, apparently an effect of the pea cover crop. After cover crop incorporation, sharp increases in *Pythium* numbers were found. In contrast to *Fusarium,* the *Pythium* populations did not decrease between the fall and spring samples, but continued to increase, so that by the time of spring sowing the populations in cover cropped treatments were much higher than the initial levels of the previous spring. In bare fallow plots, *Pythium* populations remained near initial levels during the growing season, with populations decreasing by the spring pre-sowing sample to below 60 ppg. *Pythium* populations at pre sow sampling were significantly lower than populations in *Brassica* cover cropped plots. *Pythium is* more sensitive to fumigation than *Fusarium,* and fumigation resulted in a reduction of *Pythium* populations to below detection which persisted through the spring presow sampling. As for Fusarium, the presow *Pythium* levels were statistically equivalent for the bare fallow and chemical fumigation treatments, and different from the *Brassica* cover crop treatments.

The increased levels of *Pythium* and *Fusarium* following incorporation of the *Brassica* cover crop compared to lower levels in chemically fumigated and fallow treatments, indicates that

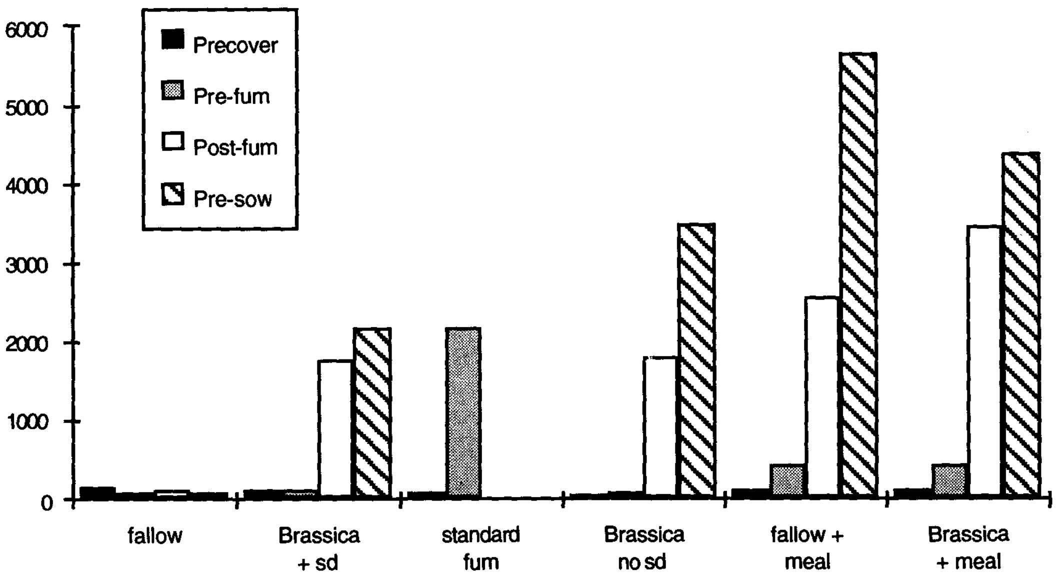

Figure 3. Averaged Pythium levels (propagules per gram) in soil samples from four replicates of each of six treatments collected at four sampling periods during 1991 - 1992.

fungitoxic byproducts of *Brassica* decomposition did not produce the expected reductions in soil fungal populations. Either these fungitoxic products were not produced in sufficient concentrations to reduce fungal populations, or additional growth and reproduction of the soil fungi on cover crop residues compensated for any population reduction. Based on biomass estimates of the *Brassica* cover crops and on glucosinalate concentrations in the mature plants, the amount of methylisothiocyanate produced on cover cropped plots would be equivalent to between one-third to one-half a commercial application of Basamid/Dazomet, assuming a 1:1 stoichiometry of conversion of glucosinalates to isothiocyanates.

Rapeseed meal

Additional treatments were designed to test the effect of supplementing glucosinalate-myrosinase concentrations in cover crop with rapeseed meal. Rapeseed meal is a waste product from oil extraction from the rapeseed and is higher in glucosinalate content than the vegetation residue. Based on glucosinalate analysis of the rapeseed meal, applications were made to plots approximately equivalent to a commercial Basamid/Dazornet application. Treatments were Brassica cover crop supplemented with rapeseed meal, bare fallow with rapeseed meal, and ryegrass cover crop with methyl bromide fumigation.

Immediately following incorporation of the rapeseed meal, *Fusarium* and *Pythium* populations increased sharply (Figures 2, 3). *Fusarium* populations in the *Brassica* with meal treatment were several times higher than those in the *Brassica* cover crop treatment, and more than 60 times higher than in the bare fallow treatment. *Fusarium* populations subsequently decreased during the winter, but residual populations in rapeseed meal supplemented treatments at the spring pre sow sample were still twice that of *Brassica* alone and 40 times that in the bare fallow treatment. *Pythium* populations continued to increase during the winter, so that by the spring pre-sow sampling *Pythium* populations on rapeseed meal supplemented plots were twice as large as those on *Brassica* alone and about 80 times greater than on bare fallow. Clearly the desired reductions of fungal populations did not result from increased quantities of *Brassica* residues, even those higher in glucosinalate content.

Seedling mortality

Methods for detecting and quantifying *Fusarium* and *Pythium* spp. in soil do not differentiate between pathogenic and non-pathogenic strains, and thus the propagule counts for these fungi in soils are not always correlated with incidence or severity of seedling disease. It is possible that fungal strains adapted to a non-conifer host, such as *Brassica*, may be benign to conifers. If these strains are non-pathogenic to conifers, then the higher populations resulting from incorporation of cover-crop residues might even beneficially interfere with colonization of seedlings by pathogenic strains. On the other hand, strains in nursery soils may be alternately saprohytes and opportunistic pathogens; higher levels of soil infestation may then lead to

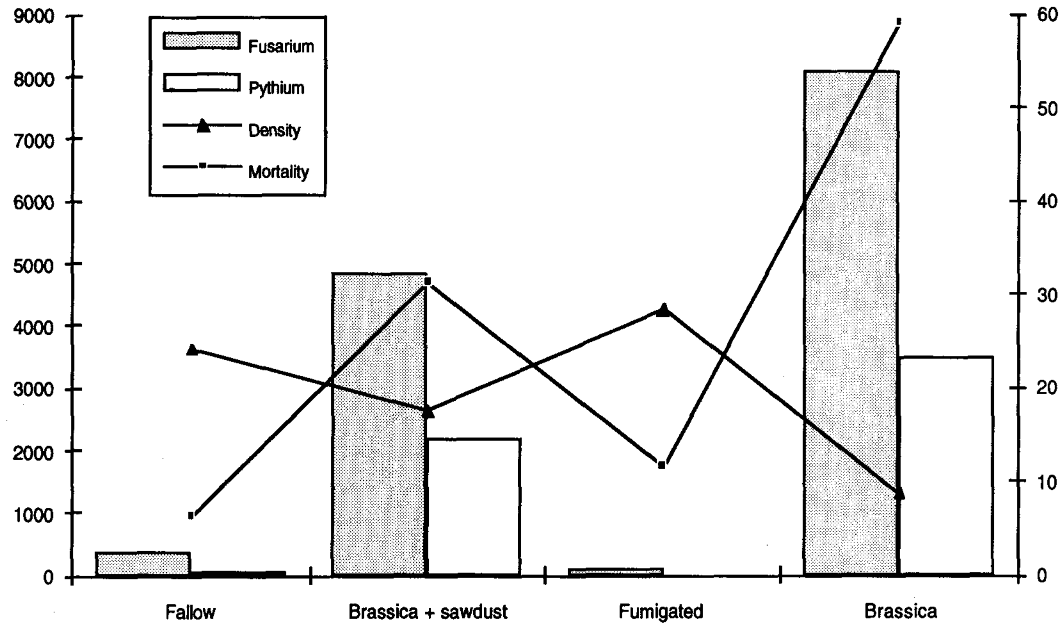

Figure 4. Seedling density (1-0) and post emergence mortality compared with pre-sow levels of Fusadum and Pythium.

increased disease potential. It is important therefore to evaluate the effects of cover crop treatments with respect to seedling disease as well as fungal populations.

Seedling density, the number of surviving germinants per sq foot, is an indirect measure of pre-emergence and early post-emergence mortality (damping off). Seedling densities were significantly different among different treatments, and these differences were inversely related to presow levels of *Fusarium* and *Pythium* spp. (Figure 4). Average seedling density was highest in the chemically fumigated plots, 28 seedlings/sq. ft., although not statistically different from the density in bare fallow plots, 24 seedlings/sq.ft. Average density was lowest in the *Brassica* without sawdust treatment, which was statistically different from the other three treatments.

Post-emergence seedling mortality, the percentage of seedlings with identifiable symptoms of *Fusarium* diseases, was also significantly different among the different treatments, and was positively correlated with presowing levels of *Fusarium* (Figure 4). Bare fallow and methyl bromide fumigation treatments were again statistically equivalent with respect to seedling mortality. Average post emergence mortality was actually lower in the bare fallow treatment (6%) than in the methyl bromide fumigation treatment (11%). Mortality was much higher in the two *Brassica* cover crop treatments (31 and 59%); these treatments were significantly different from each other as well as from the bare fallow and fumigated treatments with respect to seedling mortality. Mortality was so severe in the rapeseed meal amended treatments that data were not collected.

Seedling height, caliper, and root biomass

Seedling size and vigor also varied with treatment. Average seedling height was greater in the fumigated (7.1 cm) and the bare fallow treatments (6.9 cm) and these were statistically homogeneous. Seedlings were significantly smaller (4.9 and 5.1 cm) in the two *Brassica* treatments. There was a similar pattern among treatments for root weight, although differences between treatments were not as large Figure 6). Average root weight was greatest for the fumigation treatment (1.2 g). Bare fallow and fumigation treatments were statistically equivalent for root biomass, however only fumigation, but not bare fallow differed significantly from the *Brassica* treatments. Seedling caliper was homogeneous for all treatments (Figure 6). Severe mortality in the rapeseed meal amended plots

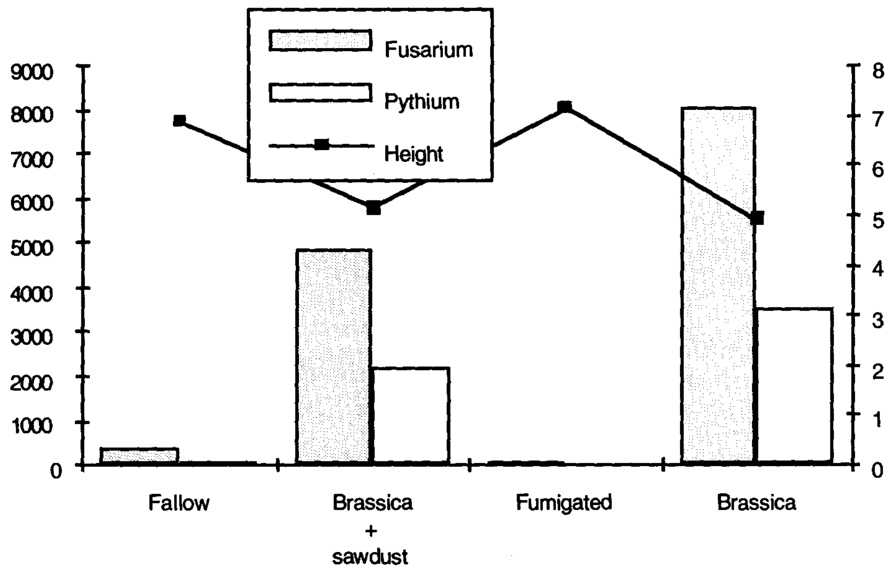

Figure 5. Average seedling height (1-0) compared with pre-sow levels of Fusarium and Pythium.

prevented data collection for those treatments.

Shippable seedlings

At lifting, the percent of shippable seedlings was determined from samples within each replicate plot for bare fallow and methyl bromide treatments according to standard nursery quality evaluation. Seedlings from the *Brassica* treatments were not assessed, inferiority of seedlings from these treatments has been described. The mean percent shippable seedlings from the bare fallow treatment was 81.3, the average for the methyl bromide treatment was 87.5; the means were not statistically different.

Effect of sawdust amendment

Fusarium and *Pythium* populations were lower in the *Brassica* with sawdust amendment than in the unamended *Brassica* treatment. These differences may partially reflect differences in *Brassica* biomass for these two treatments, which would directly influence fungal populations. Biomass of the unamended *Brassica* cover crop averaged 2.42 metric tons, about 30% greater than that of the sawdust amended treatment, 1.86 metric tons. Although additional fertilizer was added to the sawdust amended treatment to compensate for the increased microbial nitrogen consumption from sawdust decomposition, nitrogen deficiency probably accounts for the reduced biomass.

There is also however, an indication of possible suppression of disease in the sawdust amended *Brassica* treatment compared to the unamended treatment. Seedling density was lower in the no sawdust than in the sawdust amended treatment, which was not statistically different from the bare fallow treatment with respect to density. Post emergence *(Fusarium)* mortality was also higher in the unamended than in the sawdust Brassica treatment, which was statistically equivalent to the fumigated treatment with respect to *Fusarium* mortality. An earlier study of organic amendments at Bend nursery identified sawdust as a potential method for reducing *Fusarium* root disease, stimulating populations of beneficial microbes, and adding soil organic matter (Lu 1968), and other studies in northwest nurseries have reported reductions of *Fusarium* populations by sawdust additions (Hamm and Hansen 1990).

CONCLUSIONS

Brassica cover crops did not result in reduced populations of soil fungi, instead fungal populations increased in cover crop treatments and with additional *Brassica* residues (rapeseed meal). Cover crops incorporated as green manures are likely to result in increased levels of opportunistic pathogens, a situation that has routinely been corrected by chemical fumigation. Bare fallowing, on the other hand may afford a reduction of soil borne pathogens equivalent or comparable to that obtained by periodic fumigation, and enable seedling mortality and quality to be maintained with reduced use of chemical microbicides. Additional benefits of non-fumigation should be the establishment of more stable communities of soil microbes that should be less vulnerable to periodic invasion and colonization by opportunistic pathogens such as *Fusarium* spp. The establishment of stable soil microbiota, with effective competitors and antagonists to *Fusarium* spp.

should be the future objective for reduction of disease losses in conifer nurseries.

With the removal of the soil fumigant methyl bromide pending, and with costs associated with chemical fumigation increasing, nurseries are naturally anxious to find alternatives for control of nursery pests. The results of our research suggest that bare fallowing should be considered as an alternative to chemical fumigation. Bare fallow and chemical fumigation were statistically equivalent with respect to the levels of potentially pathogenic soil fungi recovered, in the levels of seedling mortality, and with respect to seedling quality after the first growing season. We do not advocate bare fallowing as a substitution for soil fumigation where extreme persistent pest problems exist, but feel that as a management option bare fallowing has been underutilized. From the standpoint of disease management, our experiments show bare fallowing to be the only non-chemical strategy to achieve levels of control comparable to those obtained from chemical fumigation. It is emphasized that scrupulous control of weeds must be maintained in order for bare fallowing to be effective in controlling pathogen populations. It seems likely that improved control of soil borne pathogens can be achieved under bare fallowing through a combination of organic soil amendments, such as sawdust, to promote microbial antagonists of pathogens, periodic cultivation to expose fungal spores, weed seeds and germinants to desiccation, management of fertilization and irrigation, and judicious use of chemical pesticides. Potential advantages of bare fallowing compared to other disease management options include:

1) nurseries have the capability to implement this practice immediately
2) bare fallowing in combination with periodic cultivation may also help control weed problems
3) beneficial soil microbes may build populations in soils not routinely fumigated
4) of the alternatives, bare fallowing seems to hold the most promise for providing a long term reduction in pathogen populations
5) costs will probably be lower than for routine chemical fumigation.
6) alternative fumigants to methyl bromide may have improved efficacy when used in combination with bare fallow.

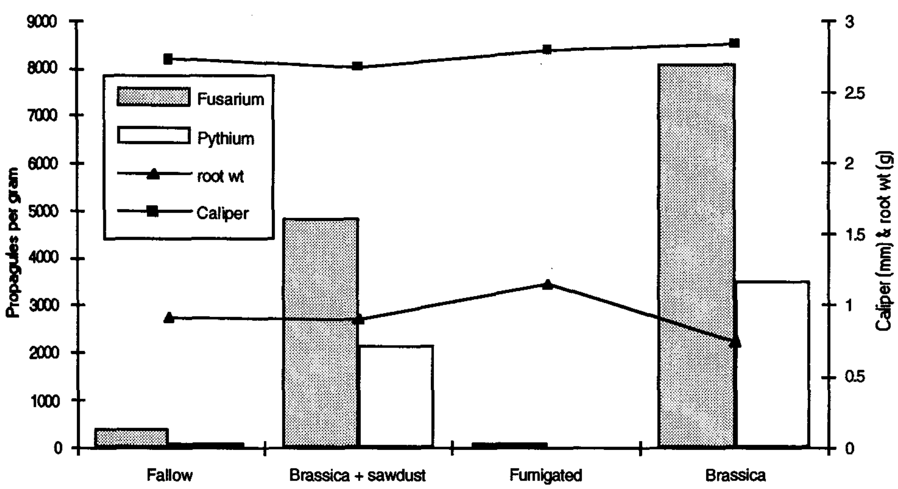

Figure 6. Average seedling root biomass and caliper compared to pre-sow levels of Fusarium and Pythium.

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