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FUNGAL DISEASE SUPPRESSION IN SOIL AMENDED WITH COVER CROPS AND OTHER ORGANIC INPUTS

By

Kanchan Uday Date

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ABSTRACT

FUNGAL DISEASE SUPPRESSION IN SOIL AMENDED WITH COVER CROPS AND OTHER ORGANIC INPUTS

By

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Effect of different soil amendments on suppression of *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* was assessed. The two main factors evaluated in growth suppression were cover crop species (*Brassica juncea* L. or *Secale cereale* L.) and tissue type (root, shoot or root + shoot). In the first field based experiment, comparison was done between effect of mustard and rye cover crop residues on potato root and tuber health. In the field, treatment with mustard residues of any tissue type and the combination of and rye roots + shoots tissues most effective in controlling *R. solani* infection of potato roots. A greenhouse container experiment to determine the effect of different amounts of mustard root and shoot residue on disease infection showed that the highest amount of shoot input provided the maximum disease suppression. Similar results were observed in the laboratory and greenhouse bioassays where maximum benefit of residue was observed at 24 h. As time passed, the fungal growth inhibiting effect decreased. In the second field study, the effect of different amounts of carbon input on overall health of potato tubers and roots was assessed. It was observed that treatment with highest quality and quantity of carbon yielded maximum disease suppression benefits. Potato tuber yields were significantly enhanced by the accumulative effect of rye cover crop + poultry manure, compared to a bare control.

Dedicated to my grandmother, parents, sister and husband.

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Introduction

Vegetables are grown on almost 1.5 million hectares in the US with a total value of over US$ 9 billion (USDA, 1998). Unfortunately there are many factors including several soil borne pests and diseases of vegetables that are known to negatively and substantially affect both the yield and the quality of vegetables (Abawi et al., 2000). Crop losses caused by plant pests and diseases run into millions of dollars worldwide. Soil organic matter consists of living, partially to fully decomposed organic materials and is an important indicator of soil health.

Soil health can be considered a subset of ecosystem health. A healthy ecosystem is characterized by integrity of nutrient cycles and energy flows, stability, and resilience to disturbance or stress (O'Neill et al., 1986). Thus, soil health may be associated with biological diversity and stability. Plant and animal disease outbreaks can be considered as indicators of instability and poor ecosystem health. Therefore, there is likely also a link between soil organic matter, the ability of the biological community to suppress plant pathogens, the population density of plant pathogens in soil, and ultimately disease incidence and severity (van Bruggen and Grunwald, 1996). Organic matter is one of the most important components of soil (Magdoff, 1992). Many biological, physical, and chemical properties of soils are a function of total soil organic matter. Organic matter confers many benefits to ecosystems including increasing nutrient availability to plants, providing a favorable physical condition for plant growth, increasing soil buffering capacity, stimulating root development, increasing biological diversity, and facilitating a number of global cycles such as carbon and nitrogen. Current farming practices fail to

replenish the organic matter in the soil after harvest and constitute a very important problem.

Soil organic matter serves as a primary indicator of soil quality and health for both scientists and farmers (Romig et al., 1995). For centuries, farmers have consciously and unwittingly manipulated the ecology of soil by addition or depletion of organic matter (Bailey et al., 2003). Production changes in the last 50 years have been detrimental to soil health and water quality, thereby increasing plant diseases and other pest problems, all within a relatively short period of time (Pimental et al., 1991). Growers generally respond to the threat of soil borne diseases with generous application of pesticides. The wide use of pesticides in agriculture has resulted in grower concern about the environmental impacts and there are also concerns about becoming dependent on one pesticide

In view of these facts, efforts are now aimed at promoting more sustainable methods of agriculture. In this study, we focus on two cover crops (Cereal rye and Oriental mustard) to help improve quality and yield of the subsequent crop (Potato). Cereal is a widely used winter cover crop by Michigan growers. Cereal rye was chosen keeping in mind that it produces large quantities of biomass consequently adding more organic matter to soil. Oriental mustard was selected for its inherent biofumigant properties.

Taxonomy and External Morphology of Potato

The potato (*Solanum tuberosum* L.) belongs to the family Solanaceae, with about 90 genera and 2,800 species. Though the family is found throughout the world, it is especially concentrated in the tropical regions of Latin America (Cornell, 1962). The

potato and all its wild relatives belong to the genus Solanum, which consists of about 2,000 species. The section Tuberarium (Cornell, 1962), also known as section Petota (D'Arcy, 1972) classified within this genus includes the tuber-bearing members, of which the cultivated potato is best known.

The cultivated potato is herbaceous and ranges from 0.5-1.0 meter in height. The leaves are alternate and irregularly pinnately compound. The inflorescence consists of several flowers which are pentamerous, actinomorphic, perfect, and have sympetalous corollas. The fruits are bicarpellate berries and can be absent in many cultivars (Burbank, 1921). Tubers are formed underground from stolons, from which adventitious roots are developed into a fibrous mass (Burton, 1969).

History and Importance of Potato

The potato originated in South America, specifically in the Andean highlands of Bolivia, Ecuador and Peru. It was first domesticated almost 6,000 years ago in the area around Lake Titicaca (Burton, 1948). They were an important source of starch and carbohydrates and could be stored and transported easily. The Spanish explorers of the 1500's were the first Europeans to come in contact with the potato. Native Andean potatoes are cultivated even today and display an extraordinary diversity of taste and texture.

Today the potato is the world's fourth most important food crop and by far the most important vegetable. Potatoes are cultivated worldwide on acreage of 17.9 million hectares. Potatoes are mainly grown commercially in the United States as row crops in monoculture. Tubers of the selected cultivar are cut into seed pieces, planted, treated with fertilizer, pesticide, and mechanically harvested (Burton, 1969). Each year about 7

percent of tubers are used as seed; about 48 percent are processed; and about 30 percent are used as fresh vegetable. In volume and sales dollars, potatoes are Michigan's leading produce commodity.

Studies have estimated losses due to plant disease in the four major crops (rice, wheat, potato and maize) to be between 10 and 16% of potential production which translates into about. 64 x 10^9 US$ over the years 1988-90. Despite its global dominance, the potato suffers tremendous losses due to disease that translate into billions of dollars in wasted resources and lost sales both in the US and abroad every year.

Impact of *Rhizoctonia solani* on potatoes

Soil borne pests and diseases are a serious problem to a potato grower. They are difficult to detect as they exist out of sight and are difficult to measure. Low populations causing extensive damage (e.g. soil insects), their microscopic size, (e.g. fungal pathogens, nematodes) and because of resting stages that differ from the active stage (e.g. fungal pathogens), detection of these pathogens is challenging (Matthiessen et al., University of Idaho, Moscow, Idaho, USA). Growers generally respond to the threat of soil borne diseases with generous application of pesticides such as fumigants e.g. Vapam. All economically important plants are damaged by one or several diseases that can greatly limit the yield potential and the quality of the harvested produce (Agrios 1988). Rhizoctonia disease of potato tubers and roots, often referred to as black scurf is caused by the fungus *Rhizoctonia solani* Kühn (Tsror et al., 2001). It is distributed worldwide, has physiological strains, has a wide host range, and causes different symptoms on the same host depending on the time of infection (Lewis et al., 2001). *R.solani* infects potato sprouts during the emergence period. Symptoms include delayed emergence, stem canker

and lesion expansion and girdling can seriously debilitate young plants. *R. solani* attacks tubers, underground stems, and stolons of potato plants. Early season stress caused by *R. solani* can lead to decrease in stem number, decrease in tuber set, reduction in tuber size and reduced tuber quality.

In temperate production areas, losses due to *R. solani* are sporadic and occur only when weather is cold and wet in the weeks following planting. In northern areas, where growers often must plant in cold soils, *R. solani* is a more consistent problem. Poor stands, stunted plants, reduced tuber number and size, and misshapen tubers are characteristic of the Rhizoctonia disease. *R. solani* commonly survives on seed tubers but can also survive and grow on alternate crops. The fungus exists on potato in three distinct phases. One is parasitic on the potato plant and the other two phases are not pathogenic as they exist on the potato and provide inoculum capable of later infection. These two phases are: (1) Black sclerotia present principally on tubers, which serve to carry the fungus over the winter and on which no spores are produced; and (2) White superficial mycelium present on the lower stems which represents the asexual stage of the fungus. When R. solani reproduces asexually by means of mycelia and sclerotia, it is called *Thanatephorous cucumeris*. Even with the use of seed treatment fungicides and fumigants, crop loss due to damping-off ranges from 5-10% in the US alone, which still accounts for a loss of millions of dollars (Gilpatrick, 1979). It is a common occurrence in most potato producing areas of the world. The saprophytic survival capability of *R. solani* in debris of specific crops (Specht and Leach, 1987) and the pathogenicity of specific strains of *R. solani* selected for by various preceding crops (Johnston et al., 1994) also influence the composition of soil populations pathogenic to potatoes and consequently

disease severity in succeeding potato crops. The importance of the *Rhizoctonia* disease complex of potatoes has long been debated. It is now considered a major cause of crop losses in Maine and other potato-producing areas (Johnston et al., 1994).

Soil fumigants should reach pathogens in all physical and biological niches in the soil. As a result, however, soil fumigants often lead to the eradication of beneficial organisms, and may bring about a negative shift in the biological equilibrium. This creates a microbial vacuum, which may lead to the population of pathogens increasing and causing even more damage than those originally targeted for control (Gamliel et al., 2000). Microbial diversity in soil is normally assessed as species or genetic diversity rather than structural and functional diversity. However, these last two measures of diversity may be more relevant to soil health (Visser and Parkinson, 1992). Soils, especially those with a low microbial population are more vulnerable to reinvasion of pathogens following fumigation. Thus, non-chemical methods of effectively controlling soilborne diseases are needed. In view of these observations, a number of strategies have been proposed to combat these potato diseases.

Increasing the Soil Organic Matter

Soil organic matter (SOM) is known to affect soil aeration, structure, drainage, moisture holding capacity, nutrient availability, and microbial ecology (Davey, 1996). SOM is important for enhancing water holding capacity of soils; supplies nutrients, which are crucial for crop production; for protection against erosion; and helps support a healthy and diverse set of microscopic plants and animals (The State of the Nations Ecosystem, 2002 report). The amount of carbon (C) input into the soil from crop residues generally increases SOM (Peterson et al., 1998; Hendrix et al., 1998). SOM stabilizes soil

pH, which plays a central role in nutrient supply and availability for plant uptake (Campbell et al., 1996). Lack of residues can result in low SOM. In a study by Nyakatawa et al., 2001, winter rye cover cropping rapidly increased surface SOM. This increase was attributed to the large quantities of residues produced by the winter rye cover crop. Short term benefits of increased surface SOM such as improved soil water conservation, seedling establishment, crop growth, and yield were clearly visible in this study.

Organic matter mediated disease suppression

Suppression of soil-borne diseases can be achieved by addition of organic residues in cropping systems. There are reports of organic amendment-mediated soil disease suppression since as early as the nineteenth century. In a study by Tepper, 1892, manure applications were used to control take-all of wheat. Root rot of cotton caused by *Phymatotrichum omnivorum* was reduced when the soil was amended with manure (Pammel, 1980). Considerable progress has been made in the utilization of organic materials as soil amendments for the control of plant-parasitic nematodes (Singh and Sitaramaiah, 1970; Muller and Gooch, 1982; Akhtar, 1993). Composting can be used as an effective and desirable soil organic amendment (Hoitink, 1986; Dick and McCoy, 1993). In addition to increasing organic matter of the soil, amending with composts also increases soil microbial populations (Pera et al., 1983; Perucci, 1990), which improves the soil quality. The suppressive activity of compost towards plant pathogens has been well documented with the majority of success shown in containerized systems (Hoitink, 1986; Nelson and Craft, 1992). In cropping systems amended with organic matter, suppression occurs because of activation of the indigenous soil microbial community.

Improving soil quality using cover crops

A cover crop is a crop grown as a complementary plant to a cash crop to benefit the soil in many ways: it can in some circumstances reduce erosion, weed pressure, insects, plant parasitic nematodes, other pest problems, and can improve soil quality (Mutch and Martin, 2000). The Soil Science Society of America (1987) defined cover crops as those used as green manures incorporated into the soil while green or at maturity, for soil improvement and protection.

Effective nitrogen scavenging fall-sown cover crops should exhibit rapid germination, aggressive and extensive rooting systems (Sainju et al., 1998), good winter hardiness, and early spring regrowth (Weinert et al., 2002). Winter cover crops can improve N cycling and reduce the amount of N below the root zone as shown in a study of potato based rotations (Weinert et al., 2002). Winter cover crops reduce the potential for nitrate (NO_3) leaching by (i) absorbing and storing N in plant tissue during leaching prone winter months during soil water recharge and (ii) absorbing and transpiring water, lessening water percolation (Weinert et al., 2002). Cereal and brassica crops display these characteristics and are well suited for winter cover cropping (Wagger and Mengel, 1988; Brinsfield and Staver, 1991). These cover crops can accumulate up to 150 kg N ha^{-1} (Hoyt and Mikkelsen, 1991; Shennan, 1992; Ditsch et al., 1993), with rooting systems reaching depths of between 80 and 150 cm (Frye et al., 1985; Sarrantonio, 1992).

Green manures and cover crops have been shown to differ significantly in their suppression of root rot severity and damage to plant growth, as indicated by greenhouse studies. (Abawi et al., 2000). In addition, differential effects of various cover crops on the severity of root rot and yield have been observed under field conditions (Abawi et al.,

2000). A number of cover crops and green manures can be effective in suppressing nematode populations and infections (Halbrendt, 1996; Mojtahedi et al., 1991; Mojtahedi et al., 1993). Organic amendments, such as incorporation of green plant residues in the field or compost added to potting mix, can suppress the activity of *R. solani* (Gorodecki and Hadar, 1990; Papavizas and Davey, 1960; Tuitert et al., 1998).

Rye has been effectively used as a winter cover crop as it provides sufficient ground cover to prevent wind erosion. Besides being convenient to grow, rye has a profusely branched root system which prevents soil compaction in soils that are annually tilled. The extensive root system also enables it to scavenge nutrients efficiently from the soil profile. The growth habit of common rye is very competitive. In a study by Creamer et al., 1997, rye comprised of at least 80% of the above ground biomass just before desiccation. In a study by Ranells et al., 1997, rye monoculture recovered 39% of the labeled ^{15}N fertilizer as compared to 19% by the rye-crimson clover biculture and 4% by the crimson clover monoculture. This study confirmed the superior ability of rye, in monoculture and biculture, to scavenge residual soil N compared with crimson clover.

Impact of crop residue on *Rhizoctonia solani*

Plant residues left of or near the soil surface may contribute to suppression of soilborne pathogens in minimum tillage systems. Disease suppression can be induced by organic and fertilizer amendments (Baker and Chet, 1982) or practices such as mulching which stimulates aggressive competition among soil inhabitants in the root zone (Baker and Cook, 1974; Boosalis et al., 1981; Tu and Findlay, 1986). Organic amendments, such as the incorporation of green plant residues in the field or compost added to potting mix, have been shown to suppress the activity of *R. solani.* (Gorodecki and Hadar, 1990;

Papavizas and Davey, 1960; Tuitert et al., 1998). This suppression has been correlated with increased antagonistic soil microbial activity (Tuitert et al., 1998), and sterilization of composts or suppressive field soils has eliminated the suppressive effects (Wiseman et al., 1996; Gorodecki and Hadar, 1990; Kobayashi and Ko, 1985).

Sustainability of farming systems using short term rotations has become a major concern in potato production. Research with potato cropping systems has shown that the frequency and host range of crops in a potato rotation influences potato soilborne disease incidence and development (Hide and Read, 1991; Honeycutt et al., 1996; Specht and Leach, 1987). Continuous potato production schemes have been shown to result in 58% of plants developing stem lesions caused by *R. solani*, as compared to 12–22% of stems from potatoes grown with other rotation crops (Honeycutt et al., 1996). Longer rotations (out of potatoes) are associated with reduced soil inoculum levels of *R. solani* as compared to short rotations (Scholte, 1987); the preceding crop and its relative ability to harbor potato pathogens will also affect the incidence of potato diseases in the succeeding crops. Thus, when buckwheat is grown prior to potatoes, greater soil population densities of *R. solani* and increased stem canker in potatoes developed (Specht and Leach, 1987). However, oat–potato (Frank and Murphy, 1977), annual ryegrass–potato (Johnston et al., date; Specht and Leach, 1987) or clover–potato (Johnston et al., 1994) sequences were found to reduce both *R. solani* inoculum levels in soil and subsequent disease development in the potato crop.

In soil, the success or failure in the struggle for survival by *R. solani* is affected by physical and biological factors. Along with quantity, type and availability of energy materials, volatiles released as decomposition products from plants considerable affect

survival of the pathogen. Plant residues which are low in their C:N ratio when decomposed in soil result in the release of volatiles which causes pigmentation of *R. solani* in culture and reduces survival and saprophytic activity of the pathogen in soil (Lewis and Papavizas, 1974).

Decreasing disease incidence using Biofumigation

Despite the wide use of pesticides in agriculture, growers are now beginning to become concerned about environmental impacts and there are also concerns about becoming dependent on one pesticide. 'Biofumigation', using plants that produce toxic compounds to exert control over pests and diseases in soil, offers a biological alternative to the use of chemical pesticides. Since the late 19th century, there have been reports of plant extracts showing biocidal activity. Biofumigation refers to the use of plants as rotation crops that contain biologically active compounds or green manures that suppress soil-borne pests and diseases in agricultural production systems. Biofumigation offers a biological alternative for producing fumigant-like chemicals in the soil, providing an option for suppressing soil-borne pests and diseases, and helping promote soil health. This technique exploits a plant defensive enzymatic system which is common to some plant families such as Brassicaceae, Capparidaceae, Moringaceae etc. Over the past decade, several authors have reported that many compounds extracted from wild, cultivated, and medicinal plants show fungitoxic activity (Gourinath and Manoharachari, 1991; Rahalison et al., 1993; Yegen et al., 1992).

Glucosinolates are a class of naturally occurring ionic compounds found in plants usually as the potassium or sodium salt. They have been the subject of much attention because of their involvement in biofumigation. In biofumigation, plant based

glucosinolates are hydrolyzed in field soil to form toxic products like isothiocyanates, thiocyanates, nitriles, oxazolidinethiones, and ionic thiocyanate (Brown and Morra, 1997). These degradation products may exert a suppressive or controlling effect on a wide range of soil-borne plant pathogens including *R. solani* (Lewis and Papavizas, 1974).

Biocidal effect of crucifers

Crucifer tissues have long been known to produce biocidal effects following their addition to the soil (Angus et al., 1994). Crucifers such as oriental mustard emit volatiles that inhibit growth of undesirable soil fungi. Field experiments during the early 1990's demonstrated that wheat crops grew more vigorously following Brassica break crops such as canola and Indian mustard than other break crops such as linseed or oats (Angus et al. 1991, Kirkegaard et al. 1994). Glucosinolate-containing plants in the Brassicaceae family represent a potential source of allelochemical control for a variety of soil-borne pests (Fahey et al., 2001). Walker et al., (1937) observed antifungal activity of mustard oils; Hooker et al., (1943) confirmed the antifungal activity of cruciferous plant extracts containing allyl and phenethyl isothiocyanates (ITC). Glucosinolates are precursors of ITC's which are detrimental to many soil-borne organisms. ITC are released when plants are physically broken up, and during the break down of residues in the soil. Compounds from the Cruciferae are used as potentially valid alternatives to controlling soil-borne pathogens (Luisa et al., 1997). This observed pest suppression is assumed to result from ITC and possibly other allelochemicals produced from glucosinolates within the tissues (Morra et al., 2002). Incorporation of Brassica tissues into the soil reduces the fungal population in the soil. Suppression of *Pythium ultimum* population in soil observed

during the first weeks after incorporation into soil could prevent the rapid increase of *P. ultimum*, thus reducing the period in which this pathogen can cause damping-off or root rot of seedlings and young plants (Lazzeri et al., 2001). Oriental mustard (*Brassica juncea* L.), known commercially as Pacific Gold, gave the best combination of traits: medium height and biomass production, high glucosinolate level, and late flowering (Bryant, 2003). The oriental mustard soil incorporation treatment, produced 18 % more marketable fruit yield than non-amended control plots (Harvey et al., 2001). Different plant species in the Brassicaceae family have different levels of glucosinolate. Plant tissues exhibit varied responses on fungal colonies depending on the amount of glucosinolate present in them. A study by Harding et al., (2001) showed that most Brassica plant materials suppressed fungal growth, but the response varied between plant parts and fungal species. For example *B. juncea* meal completely inhibited growth of all fungi whereas leaf tissue inhibited fungi between 20 – 50%. Studies have demonstrated the ability of *B. juncea* to reduce incidence of *R. solani*. In principle, the utilization of ITC of glucosinolates may be a good compromise between fungitoxic activity toward some phytopathogenic fungi and environmental protection.

Objectives

The overall objective of this study was to improve quality of potato roots and tubers and reduce disease incidence using cover crops and other soil amendments.

The specific objectives were;

(1) To study the biocidal effect of *Brassica juncea* root v/s shoot on the incidence of *Rhizoctonia solani* on potato tubers and roots.

(2) To evaluate the dose response of *Brassica juncea* (oriental mustard) on *R. solani*

(3) Noting the overall effect of soil amended with *Secale cereale* (cereal rye) on potato tuber yield, quality and root rot disease.

(4) To see influence of soil with different organic amendments on disease.

Overall hypothesis are; (1) Organic amendments reduce disease severity of potato plants compared to a bare fallow without organic inputs. (2) Organic amendments will enhance potato health as much as a fumigated control. (3) Organic amendment benefits will be related to the properties of the cover crop incorporated and also to the quantity of carbon added. (4) The combination of poultry manure plus rye has the most diverse substrates and will be associated with the greatest health benefits compared to either applied alone.

Root-health hypothesis are; (1) Organic amendments enhance root health in potato plants compared to a bare fallow without organic inputs. (2) Organic amendments will enhance root health as much as fumigated control. (3) Diversity of substrate will determine the root health benefit.

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Chapter 2: Effect of Oriental Mustard (*Brassica juncea* L.) residues on potato (*Solanum tuberosum* L.) fungal diseases.

ABSTRACT

Cover crops have the potential to significantly suppress *Rhizoctonia solani* Kuhn, a common potato fungal pathogen. There is limited knowledge of the processes involved in cover crop residue interaction with soil-borne diseases. To study this interaction, a field experiment was conducted in parallel with a container study to determine effect of cover crop tissues on fungal incidence. In the field experiment, cover crops were incorporated according to the experimental design and the table stock variety Onaway was planted 2 weeks later. The seeding rate of rye was 100 kg/ha while that of mustard was 23 kg/ha. Plants were destructively harvested and roots were recovered to assess disease presence using WinRhizo^{TM} to quantify dark versus white root tissue. Soil from the same field was collected for a large-volume container study This study evaluated oriental mustard tissues (root, shoot and root plus shoot) with two rates of biomass application. Incorporation of oriental mustard (*Brassica juncea*) and rye (*Secale cereale*) proved beneficial to the preceding potato (*Solanum tuberosum*) crop by reducing *Rhizoctonia solani* root and tuber symptoms. In the field study, rye roots plus shoots and mustard roots plus shoots reduced *R. solani* infection by > 50% when compared to a fumigated treatment. Rye and mustard roots and shoots on their own were less effective. Neither treatment had any significant effect on the yield. In the container study the shoots with the higher rate were the most effective at enhancing root health and suppressed *R. solani* infection by 75%. Treatment with mustard roots and shoots applied together at the

higher rate reduced symptoms of *R. solani* by 43%, however the tubers with the least black scurf were associated with this treatment that included roots and shoots.

INTRODUCTION

Cover crops can be considered the backbone of any sustainable annual cropping system. A cover crop is a crop grown as a complementary plant to a cash crop to benefit the soil in many ways: it can reduce erosion, weed pressure, insects, and plant parasitic nematodes, soil fungal diseases, thus improving soil quality (Mutch and Martin 2000). A cover crop can be used as a green manure. The Soil Science Society of America (1987) has defined green manures as plant materials incorporated into the soil while green, flowering or at maturity, for soil improvement and protection.

Advantages of incorporating cover crops into soil have been seen in many studies (Weinert et al., 2002). It has been hypothesized that cover crops contribute to suppression of soil diseases by increasing the soil active organic matter content, microbial biomass and thereby the microbial activity, which in turn supports competition that may suppress pathogenic organisms (Kuo et al., 1997, Mendes et al., 1999, Workneh et al., 1993). Green manures and cover crops have been shown to differ significantly in their suppression of root rot severity and damage to plant growth, as indicated by greenhouse studies (Abawi et al., 2000). In addition, differential effects of various cover crops on the severity of root rot and yield have been observed under field conditions (Abawi et al., 2000).

There are reports of organic amendment-mediated soil disease suppression since as early as the nineteenth century. Root rot of cotton caused by *Phymatotrichum omnivorum* was reduced when the soil was amended with manure (Pammel, 1980).

A number of cover crops and green manures can be effective in suppressing nematode populations and infections (Mojtahedi et al.,1991; 1993; Halbrendt, 1996).

Considerable progress has been made in the utilization of organic materials as soil amendments for the control of plant-parasitic nematodes but soil borne disease suppression is less well understood (Singh and Sitaramaiah, 1970; Muller and Gooch, 1982; Akhtar, 1993).

A rapid increase in soil microorganisms occurs after a young, relatively lush green manure crop is incorporated into the soil; this can include beneficial and parasitic organisms. The soil microbes multiply to attack the freshly incorporated plant material. During microbial breakdown, nutrients held within the plant tissues are released and made available to the following crop. The suppressive activity of soil amendments towards plant pathogens has been well documented in particular for containerized systems (Hoitink, 1986 and Nelson and Craft, 1992). In cropping systems amended with organic matter, it has been hypothesized that suppression occurs because of activation of the indigenous soil microbial community.

Despite the wide use of pesticides in agriculture, growers are now beginning to become concerned about environmental impacts. In addition, there are concerns about becoming dependent on one or a narrow range of chemical formulations. If species or varieties of cover crops could be used to destroy plant pathogen propagules, thereby decreasing intensity of plant diseases, this would be a most sustainable alternative to pesticides (Kazmar., 1995; Reddy and Patrick, 1989; Sequeira, 1962; Shetty, 2000). Reducing the crop damage caused by soilborne fungal pathogens through soil amendments with brassica plants have received considerable research attention recently. Biofumigation, defined as the incorporation of biomass into soil, results in the release of toxic volatiles that reduce soil pests. Other benefits of biofumigation may include:

improved soil texture, increased water holding capacity, and improved soil microbial community structure, if the biofumigant treatment includes enough organic matter.

The *Brassicaceae* family is a potential source of potential biofumigation material. The family consists of approximately 375 genera and 3200 species. The Brassica genus consists of around 100 species including *Brassica juncea* commonly called "Oriental or Indian Mustard". Family members contain secondary plant metabolites, glucosinolates, which are believed to be involved in plant defense. *Brassicas*, including numerous mustard species, provide not only biomass to the soil but when incorporated they release glucosinolates, which further break down into isothiocyanates with fumigant properties similar to metham-sodium. Good controls of *Pythium ultimum*, *Fusarium oxysporum* f. sp *cumini* and *Rhizoctonia solani* have been reported by amending the soil with leaves of Brassica species (Ramirez-Villapudua and Munnecke, 1988; Charron and Sams, 1999; Marwar and Lodha, 2002).

Brassicas in many studies have also been shown to suppress fungal populations indirectly. In a field study by Subbarao et al., 1999, broccoli residues suppressed *Verticillium dahliae* wilt of cauliflower this effect however was not observed *in vitro* (Shetty et al., 2000). This could be attributed to increased microbial populations in the field study which may have created competition that suppressed *V. dahliae* microsclerotia thereby reducing infection.

Glucosinolates and their breakdown products have been the focus of many studies because of the possibility of using them as natural pesticides (Lichtenstein et al., 1964; Tsao et al., 1996). However, there is evidence that glucosinolates themselves possess limited biological activity until they are hydrolyzed (Borek et al., 1994). When tissues are

damaged, glucosinolates are enzymatically broken down by myrosinase to produce nitriles, thiocyanates, isothiocyanates and other products. The breakdown products are generally relatively small molecules, which make many of them volatile. Isothiocyanates, the predominant breakdown product, show biocidal activity on fungi (Charron,. and Sams 1999; Harvey and Sams, 2000), bacteria (Delaquis, and Mazza. 1995) and other pests. They are reported to be highly biocidal to a diverse range of organisms including soil fungi, insects and germinating seeds (Brown and Morra 1997; Kirkegaard et al., 1994). Walker (1997) demonstrated that residues of Brassica spp. reduced soil populations of citrus nematode, *Tylenchulus semipenetrans* with humus rape resulting in 81% reduction of larvae. Soil amended with Brassica leaf tissues can exhibit highly nematicidal effects. Leaf tissues of Brassicas have been noted to have higher glucosinolate levels than root tissue which may explain 56-95% reduction in *Pratyelnchus neglectus* by Brassica shoots and only 0-48% reduction by roots (Potter et al., 1998). In soil, reduction of *Pythium ultimum* and *Sclerotium rolfsii* by dry cabbage leaf amendment together with solarization was correlated to release of isothiocyanates and aldehydes (Gamliel and Stapleton, 1993).

Oriental mustard has been effectively used as a biofumigant. Anti-fungal compounds have been isolated from yellow mustard seeds (Neumann et al., 1996). Amending soil with mustard and providing heat causes differential effect on soil fungi. This effect was investigated by Stapleton and Duncan (1998). Heating of soil amended with Brassica tissues to a maximal temperature of 38°C reduced incidence of propagules of *Pythium ultimum*, *Sclerotium rolfsii* and *Meloidogyne incognita*. When cruciferous soil amendments were combined with the sublethal heating regime, nematode galling was reduced by 95-100%, and recovery of active fungi was reduced by 85-100%.

The concentration of glucosinolate is variable in different parts of the mustard plant. The majority of studies involving biofumigation using mustard as a cover crop utilize the shoots as controls against fungal diseases. This may be due to higher amounts of glucosinolates present in shoots than in roots. It also appears that research has not substantially investigated root tissue impact. Previous studies have shown that shoots of *Brassica oleracea*, *Brassica nigra* and *Brassica juncea* contain high concentrations of sinigrin (a precursor of isothiocyanate) as compared to roots (Sang et al., 1984; Kirkegaard 1998 and Sarwar 1998). However there has been no research we know of that investigates effect of field grown effect of mustard root versus mustard shoot on soil fungal disease, which was why we initiated this study.

Rhizoctonia solani, *Pythium ultimum*, *Fusarium solani* and other soilborne diseases have emerged as important problems for potato growers in recent years. *R. solani* infection of potato sprouts during the emergence period can delay emergence and seriously debilitate young plants. It can reduce plant growth and tuber production, and cause lesions on tubers, seriously affecting tuber quality and marketability. Once the pathogen becomes established in an area, it will persist indefinitely. Organic amendments, such as the incorporation of green plant residues in the field or compost added to potting mix, have been shown to suppress the activity of *R. solani*. Gorodecki and Hadar, 1990, in a study found that barn (cow) manure significantly suppresses *R. solani* incidence. Compost from two commercial composting facilities was investigated to see effect on *R. solani* in potting mixtures. It was noted that both the composts suppressed growth of *R. solani* in potting mixtures provided the composts were long-matured (Tuitert et al., 1998). Papavizas and Davey, 1960 studied effect of corn (*Zea*

mays) residues on *R. solani* and found that incidence of the pathogen was reduced when soil was amended with corn residues.

P. ultimum is generally a problem in soils that remain wet for very long periods of time or soils that harbor stagnant water. It is a very fast growing saprophytic soil fungus. *Pythium* spp complete their life cycle within 44 h of inoculation but the populations decline within 30 days as investigated in a field study by Hancock, 1981. They are not good competitors in soil. If the soil has already been colonized by other microorganisms, populations of *Pythium* have been shown to be reduced (Barton, 1961). Because of this effect, if the cover crop used can increase the soil microbial populations, a reduced *Pythium* infection may be possible. Similarly a study by Park, 1958, reported that *Fusarium* spp are also not good competitors in soil.

Longer rotations (out of potatoes) generally tend to reduce soil inoculum levels of fungi more than short rotations (Scholte, 1987). Soils, especially those with a low microbial population are more vulnerable to reinvasion of pathogens following fumigation. It is possible that the fumigants used in cropping systems reduce populations of soil beneficial microbes as well. Because of this non-chemical method of effectively controlling soilborne diseases are needed.

The objectives of this study were: 1) To evaluate the effect of mustard and rye cover crop residues on potato root and tuber health. 2) To determine if mustard root or shoot is more effective in controlling disease incidence on potato roots and tuber. 3) To determine if quantity of mustard root, shoot or root and shoot combined residues are is important to disease suppressive activity.

MATERIALS AND METHODS

MUSTARD FIELD EXPERIMENT

Plot information

The field experiment was conducted at Sandhill research plot near Michigan State University in East Lansing, Michigan in 2004. The plot was 53*107 m in area. The soil was well drained and sandy type. The top soil had been removed 15 years ago and sandy subsoil remained. It was a grass fallow until continuous potatoes 3 years ago was initiated. This management of continuous potatoes was a strategy representative of field potato production conditions and susceptible to potato pathogens. Cover crops were established in 2002 and then in 2003 for this experiment keeping in mind the experimental design described below.

Cover crop details

The two cover crops studied were rye (*Secale cereale* L.) and oriental mustard (*Brassica juncea* L.). The variety of mustard used was "Pacific Gold". This variety was selected for its medium height, high biomass production, high glucosinolate level, and late flowering properties. The seeding rate of rye was 100 kg/ha while that of mustard was 23 kg/ha. The amount of shoot biomass incorporated into the soil was calculated by using a standard 0.5 x 0.5 m quadrat. The quadrat was randomly thrown to a spot in the plot, the shoots were cut and the fresh and dry weight was calculated. For the root biomass, six soil cores per quadrat at 0-8 inches depth were taken. The soil was composited and wet sieved using 6/64 in mesh. The roots were collected with tweezers. The roots and shoots were placed in an 80°C oven and dry weights were determined. The biomass by dry weight was determined to be 1834 (±153) kg/ha for mustard shoot, 370 (±

96) kg/ha for mustard root, 2809 (±185) kg/ha for rye shoot, and 589 (± 132) kg/ha for rye root.

Inoculation of Plots

After cover crops were established and before the cover crops were incorporated into the soil, all the plots were inoculated with 3 potato fungal pathogens (14th June 2004). *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* isolates were obtained from the Department of Plant Pathology at Michigan State University. These three pathogenic organisms were cultured on potato dextrose media and then inoculated on millet seed. The amount of inoculum needed for the field was determined on a weight basis. The number of spores on 1 g of millet seed was determined. The millet seed was taken in a beaker, mixed with 5ml water and stirred for 5 mins. The solution was filtered through cheese cloth. 40 micro liters of this filtered solution was loaded on a hemacytometer. Spores were counted on the hemacytometer grid and were diluted in water for a final spore count of 1600 spores/ml for (*F. solani)* was 1600 spores/ml, 2000 oospores/ml for P. ultimum and as *R. solani*, does not produce asexual spores or conidia the inoculum was added at the rate of 490 g millet seed/plot (875 sq ft). Around 25 kg/ha millet seed was used for inoculating the field for each fungus and for Pythium. The millet seed was spread evenly on the entire plot. The cover crops were moved and disked in 7 days after inoculum was applied so that the fungal spores had time to establish and grow in the soil.

Incorporation of cover crop residues

The cover crops were moved based on treatments in each plot (Table 2.1). In treatments with shoot only, the shoots of an entire plot were razed using a forage

harvester and were then moved to the needed plot. The shoots were spread evenly using rakes and were then disked in. The plots from where the shoots were moved were the plots which had roots only as treatments. The shoots were moved to the shoot only plot.

Treatments

The treatments were based on previous preliminary experiments. For this study since we were looking at effect of root versus shoot of cover crops, the treatments were root, shoot and root plus shoot of rye and mustard respectively. The control was a winter fallow plot with no treatment at all.

Table 2.1. Mustard Field Experiment. Treatments used in the experiment.

Trt No.	Treatment
1	Mustard Roots + Shoots
2	Mustard Roots
3	Mustard Shoots
4	Fumigated
5	Rye Roots + Shoots
6	Rye Roots
7	Rye Shoots

Potato variety and fertilization

The variety of potato used was "Onaway". Seed pieces (52 g) were planted on 30th June 2004 at 34 in distance within rows and 12 in between rows. Nitrogen fertilizer was applied in splits of 100 kg N /ha at planting, 50 N kg/ha at hilling and 50 N kg/ha at tuberization for a final rate of 200 kg N/ha (Snapp et al., 2002). Potassium (0-0-60) and

phosphorous (19-17-0) were applied at 200 K_2O and 35 P_2O_5 kg/ha respectively before planting. The plot was irrigated using a traveler irrigation system.

Potato measurements

Soil Characterization - Soil organic C was determined by the Walkley-Black method (Allison *et al.*, 1965)., and total N was by the Kjeldahl method; the total N was extracted by digestion in H_2SO_4 in a bloc digester, then the N content of the digest was determined on a Technicon Auto-analyser. Ammonium nitrites and nitrates (NH_4^+ and NO_3^-) were extracted with KCl (2N) (Bremner 1982) and measured on a Technicon Auto-analyser.

Soil Biology - Twice over the season, a series of soil dilution bioassays were done to study effect of the treatments on soil fungi. Soil was sampled 7 days and 40 days after cover crops were incorporated. 10 g soil was placed in a sterile flask with 90 ml water and stirred on a mechanical shaker at 160 rpm for 30 mins. 10 ml of suspension was withdrawn while in motion and 90 ml sterile water was added to it. This was shaken for 1 min and another 10 ml was withdrawn. A dilution of 10^{-4} was obtained. 1 ml of this suspension was spread over the surface of PDA media and spread using a flamed L-shaped rod. The plated were incubated for 3-4 days and at the end of the 4^{th} day, fungal colonies were isolated. The fungi obtained from these tests were isolated and cultured on selective media to ascertain the kind of fungal species.

Microbial biomass analysis was done once mid-season. Soil microbial respiration (CO2) was used to measure biological activity potential, (Kumar and Goh, 2000). A quantity of 100 grams of soil, collected in the field after soil had been mixed with different organic residues, was incubated in plastic containers (500 ml) at 25^0C for 20

days. A solution of glucose (glucose-demineralized water ratio 1:10) was added in each soil sample to adjust soil moisture to 85% of the field capacity. Vials containing 10 mL NaOH (1N) were introduced to trap evolved CO_2 during incubation. The samples were incubated for 20 days at what temeprature. After 10 and 20 days, vials containing NaOH were sampled to measure the CO_2 content. Soils were also sampled at each period to measure mineralized N.

The amount of accumulated CO_2 was determined by titration of excess NaOH after precipitation of carbonates by barium chloride ($BaCl_2$). The titration was made by automatic titrimeter with hydrochloric acid (HCl 0.25N). (Anderson 1982). Mineralized N was extracted with 2 M KCl solution (sol-solution ratio 1:4) (Bremner 1982), and measured using a Technicon Autoanalyzer. Mineralized C and N were expressed in mg kg^{-1}.

Root monitoring - For determining % of root infection, the potato roots were washed with distilled water and analyzed using the WhinRHIZO™ program (Figure 2.1). Fungi were reisolated from the infected roots using selective media. For this, the root was washed thoroughly with sterile water. The infected portion of the root was cut using a scalpel. The cut section was washed with water and then dipped in ethanol. Before transferring the section on media, it was washed with water again to leave no trace of ethanol. The root section was placed in a fungal selective media and the petri plate was incubated for 4-5 days. The tubers were scored visually for infection on a scale of 0-3. The scale was based on % of infection. 0 was 0-25% infection, 1 was 25-50% infection, 2 was 50-75% infection and 3 was 75-100% infection (Figure 2.4). The dry weights of the roots and tubers were obtained.

Yield tuber monitoring - Destructive harvest measurements were taken twice over the entire season. The first measurement was taken one month after planting and the second two months after planting. At harvest, the potato vines were killed using two herbicides Matrix^{TM} at 0.98 L/ha and Poast^{TM} at 1.23 L/ha + 0.9 L crop oil concentrate. The last measurement was done at the final harvest which was on 11th October 2004. Yield measurements were taken at final harvest along with tuber infection and size classification. The tubers were sorted based on their diameter into A (2.5 in), B (1.87 in), over sized (> 3.25 in) and pick outs (deformed tubers).

Analysis for detection of *Pratylenchus penetrans* and *Verticillium dahliae*

Processing and analysing the soil samples for *P. penetrans* and *V. dahliae* was done by the Entomology Department at Michigan State University. The amount of soil used for the analysis was 100 cc for *P. penetrans* and 10 g for *V. dahliae*. The method used for extraction was sieving and decanting (Barker, 1985).

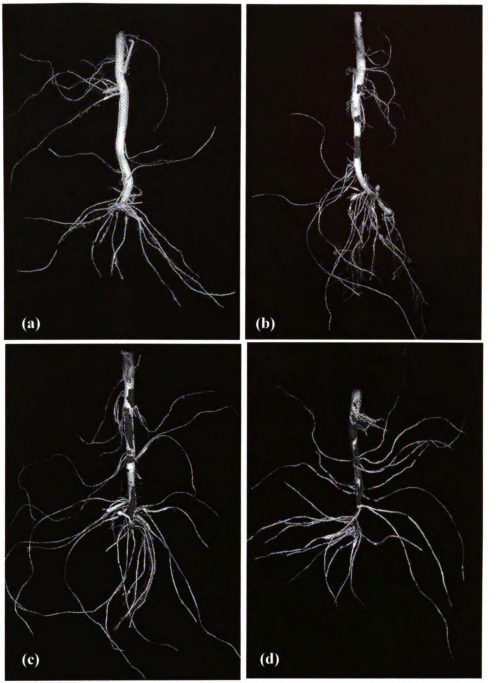

Figure 2.1. Mustard Field Experiment. Scale for root infection used by WinRHIZO™. a: 0-25% infection, b: 25-50% infection, c: 50-75% infection, d: 75-100% infection.

Experimental design and Statistical Analysis

The experimental design was a randomized complete block with four replications. The data was checked for normality and there was no need to transform it. All data were analyzed by two way ANOVA to test the effect of the cover crop (Rye or Mustard) and tissue type (Root, Shoot or Root+Shoot). In cases where significance was observed, least square means was used to determine the effect of an individual treatment. Planned contrasts were considered using to differentiate significance of each factor. The SAS glm procedure was used for analyses and an alpha value of 0.05 was maintained.

GREENHOUSE CONTAINER STUDY

Treatments

A study was conducted in a container experiment to determine the effect of different quantities of mustard residues and tissue type on common potato pathogens. The tissues that were studied were mustard shoots, mustard roots and mustard roots plus shoots. For each type, two levels were maintained depending on the dry weight of incorporated tissue (Table 2.2). The weight of the tissue to be added was determined on the basis of area of the container in comparison with the weight of dry matter obtained in field conditions. For mustard shoots, the two levels were fresh weight of shoots equivalent to (1) 40 g dry weight and (2) 160 g dry weight. For mustard roots, the two levels were fresh weight of roots equivalent to (1) 17 g dry weight and (2) 70 g dry weight. For mustard roots+shoots, the two levels were fresh weight of root and shoot combined equivalent to (1) 57 g dry weight and (2) 230 g dry weight. The tissues were mixed into the soil at a depth of 0 – 20 cm with a trowel. After incorporation of the tissues, a waiting period of 10 days was observed. During this period, the containers were covered with "Saran wrap" to avoid loss of volatile glucosinolates. The experimental set-up is shown in Figure 2.2 and 2.3. The mustard tissues used for this study were obtained by growing it in the greenhouse at controlled conditions.

Table 2.2. Greenhouse container study. Treatments used in the study.

Trt No.	Treatment
1	Mustard Root Lvl 1
2	Mustard Shoot Lvl 1
3	Mustard Root + Shoot Lvl 1
4	Mustard Root Lvl 2
5	Mustard Shoot Lvl 2
6	Mustard Root + Shoot Lvl 2
7	Bare Soil

Mustard Growing conditions

The variety of mustard that was used is commonly called 'Pacific Gold'. Plants were grown in 43 x 33 x 15 cm plastic tubs. Seeds were sown at twice the field seeding rate (22.5 kg/ha) to get more biomass. The soil used was a mixture of sandy soil + 40% perlite. The soil was collected from a research trial field (Sandhill) in East Lansing, Michigan. The soil was steamed at 70°C for one hour, and then sieved with a 5 mm sieve before mixing with perlite. Plants were grown in the greenhouse at Michigan State University at 28°C. The light was maintained at 16 h daylight period using halogen lights. The fertilizer used was- Osmocote™, which is a slow release plant food with 14-14-14 N, P, K. All plants were watered uniformly. Both roots and shoots of the plants were harvested just before flowering. They were washed with distilled water and cut into small pieces. A compound sample from a large number (10-15 plants) of plants was used in the bioassay. The tissue samples were used immediately after harvest.

Greenhouse conditions

The study was done in a greenhouse at Michigan State University. The daylight was maintained at 16 h days using halogen lights and the temperature in the greenhouse was constant at 70°F throughout the study.

Experimental setup

The containers had a volume of 75L with a height of 58.4 cm and a diameter of 48.3 cm. The soil was a sandy texture and was collected form top soil of the plot at "Sandhill" site adjacent to the experimental field at Michigan State University. Soil was collected at 0-25 cm depth. The soil was steamed at 82°C for 3 h to make sure that it was free of pathogens. It was then sieved through a 6 mm mesh sieve and mixed with 40% perlite to prevent compaction. Before the containers were filled with soil, 2 minirhizotron tubes were placed criss-cross to each other with their base touching the base of the container. The container was then filled with soil to the top.

Inoculation

Each of the containers was inoculated with the potato fungi to be studied. In this experiment we studied *Rhizoctonia solani* Kuhn., *Fusarium solani* L., and *Pythium ultimum* L. The three isolates were obtained from the department of Plant Pathology at Michigan State University. Each isolate was grown on potato dextrose agar media till the desired spore count level was obtained. For this study the spore count was set at 1600 spores/ml for *F. solani*, 2000 spores/ml for *P. ultimum* and for *R. solani*, the inoculum level was 55 g dry inoculum/sq m. After inoculation of soil, it was incorporated with mustard tissues at desired quantities

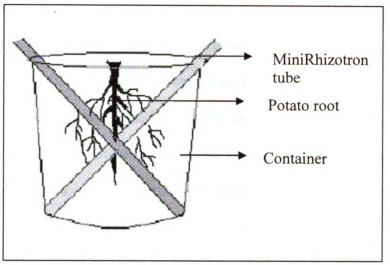

Figure 2.2. Experimental setup for greenhouse container study.

Figure 2.3 Greenhouse container experiment. Experimental set-up in greenhouse showing containers with minirhizotron tubes.

Potato plant growth conditions

The potato cultivar used was "Onaway". The seed tubers were surface sterilized with 5% Clorox bleach and washed thoroughly with distilled water to avoid any cross contamination. Seed pieces were planted 8 cm deep. Two seed pieces were planted per container and the more vigourous plant was left in the container. The plants were watered as needed based on soil moisture content measured by tensiometers. To maintain soil moisture, soil tensiometers were installed at 15 cm depth. Fertilizer was applied at field rates. Nitrogen fertilizer was applied in 3 splits at 200 N kg/ha as recommended. 100 N kg/ha was applied at planting, 50 N kg/ha at hilling and 50 N kg/ha at tuberization (Snapp et al., 2002). Potassium (0-0-60) and phosphorous (19-17-0) were applied at 200 K_2O and 35 P_2O_5 kg/ha respectively before planting.

Monitoring root and tuber disease

Root disease was monitored by taking picture of roots using the Mini Rhizotron camera in tubes. Pictures were taken nine times over the entire growth season at seven day intervals. Each time, pictures were taken at 12 depths along each tube. Pictures were analyzed using BTC-ICAP™ software to observe infection (browning) of roots over time inside the soil. At the end of the plant growth, plants were harvested and roots were analyzed for infection. The roots were washed with distilled water and were stored for a week before analysis in a solution of 5% ethanol. They were analyzed using the WinRHIZO™ program. The tubers were scored visually for infection on a scale of 0-3. The scale was based on % of infection. 0 was 0-25% infection, 1 was 25-50% infection, 2 was 50-75% infection and 3 was 75-100% infection (Figure 2.4).

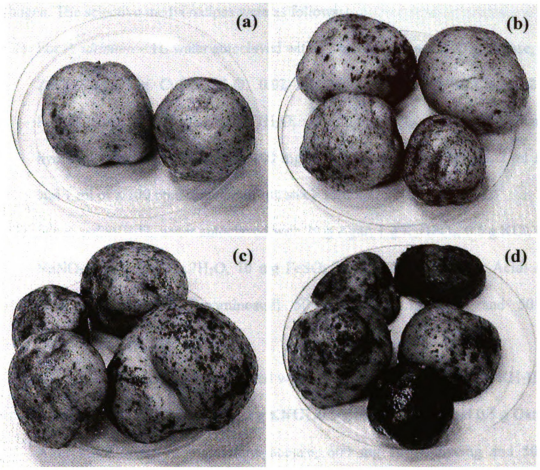

Figure 2.4. Greenhouse container study. Scale used for rating tubers on basis of % infection. a: 0 (0-25%), b: 1 (25-50%), c: 2 (50-75%), d: 3 (75-100%)

Reisolating fungi from roots

Since there were three fungal species used for this study it was important to determine how many or which of the three pathogens had infected the roots. For this, the infected portion of roots was cut using a scalpel. This root section was washed in water and ethanol and cultured on fungal selective media. Since the three pathogens used were

P. ultimum, *R. solani* and *F. solani*, the selective media used was specific for each pathogen. The selective media recipes were as follows:

1) For *P. ultimum* – 1L water autoclaved with 17 g cornmeal agar, 20 g sucrose, 1mg $ZnCl_2$, 0.02 mg $CuSO_4.5H_2O$, 0.02 mg MoO_3, 0.02 mg $MnCl_2$, 0.02 mg $FeSO_4.7H_2O$, 10 mg $MgSO_4.7H_2O$, 10 mg $CaCl_2$, 100 mg Thiamine hydrochloride, 10 mg Benomyl, 100 mg PCNB, 10 mg Rose Bengal, 2 ml RAN and 1 ml of a 100 ppm dimethomorph stock solution.
2) For *R. solani* – 1L water autoclaved with 20 g Agar, 1 g K_2HPO_4, 0.5 g KCl, 0.2 g $NaNO_2$, 0.5 g $MgSO_4.7H_2O$, 10 mg $FeSO_4.7H_2O$ and 0.4 g Gallic Acid. After autoclaving 90 mg Fenaminosulf, 50 mg Chloramphenicol and 50 mg Streptomycin was added to it.
3) For *F. solani* – 1L water autoclaved with 20 g agar, 20 g sucrose, 1 g KH_2HPO_4, 0.5 g KCl, 0.375 g Quintozene, 2 g KNO_3, 0.5 g $MgSO_4.7H_2O$ and 0.5 g Oxagall. After autoclaving 70 mg dodine acetate, 600 mg streptomycing and 50 mg chlortetracycline HCl was added.

Growth of colonies was monitored on the media for a period of 10 days and the species that grew was identified.

Experimental design and Statistical Analysis

The experimental design was a completely randomized block design with 3 replications. A two way ANOVA was used to determine the significance of the study. The first factor was the type of mustard tissue used (root, shoot or root+shoot). The second factor was the rate at which the tissue was incorporated (Rate1 or Rate2). Planned contrasts (root v/s shoot and rate 1v/s rate 2) were used to determine the effect of one

treatment against another and least square means using a one way ANOVA was used to determine the best treatment. SAS was used for all the above statistical analyses and an alpha value of 0.05 was maintained.

RESULTS

MUSTARD FIELD EXPERIMENT

Fungal infection of potato roots:

The components of the analysis of variance for % area of diseased root is presented in Table 2.3. For the 1st and the 2nd harvest, the cover crop (mustard or rye), the tissue (root, shoot or root + shoot) was significant. While the interaction between cover crop and tissue was significant only for the 1st harvest. This can be attributed to a decreased effect of glucosinolates later in the season. Throughout the growing season, the treatment with roots + shoots was consistently associated with the lowest fungal infection on roots. Addition of roots and shoots together may have increased the soil microbial organisms which indirectly created competition for the fungal pathogens. This is consistent with the findings of Kuo et al., 1997, Mendes et al., 1999 and Workneh et al., 1993. Although the SIR results did not conclusively show increased microbial respiration in the treatments with root and shoot together, it is possible that addition of the tissues together increased the microbial biomass around the potato rhizosphere. Analysis with the least square means show that the % of disease infection was lowest in mustard roots + shoots (Figure 2.5). The treatment with rye including both the roots and shoots gave almost as good results as the mustard treatment. This can be related to the amount of biomass that was incorporated in the soil. The rye root and shoot biomass getting disked in the soil was greater than the mustard. It is not yet determined, but this disease reduction in the rye treatment could also be associated with the allelopathic effect of rye. Mustard roots and mustard shoots by themselves gave consistent results in both the 1st and the 2nd harvest.

Earlier in the season, planned contrasts for the effect of mustard versus rye determined that there was a significant difference between the two cover crops (Table 2.4.). As time progressed, this contrast between the two decreased and they showed almost the same effects. This effect could be due to greater glucosinolates levels in the mustard treatment when residues were freshly incorporated. Glucosinolates being volatile, it maybe possible that the effects later subsided.

Table 2.3. Mustard Field Experiment. Analysis of variance for % diseased root area.

ANOVA	**Pr > F**		
Source of variation	**df**	**% diseased area**	
		1st harvest	2nd harvest
Cover Crop	1	<0.0001	0.0026
Tissue	2	<0.0001	<0.0001
CoverCrop X Tissue	2	<0.0484	0.2396 NS

NS: Not Significant at P<0.05

Table 2.4. Mustard field experiment. Planned contrasts for % of diseased root.

	Pr > |t|	
Parameter	**1st Harvest**	**2nd Harvest**
Mustard v/s Rye	0.0033	0.2943 NS
Shoots v/s Roots	0.0653	0.3176 NS

NS: Not Significant at P<0.05

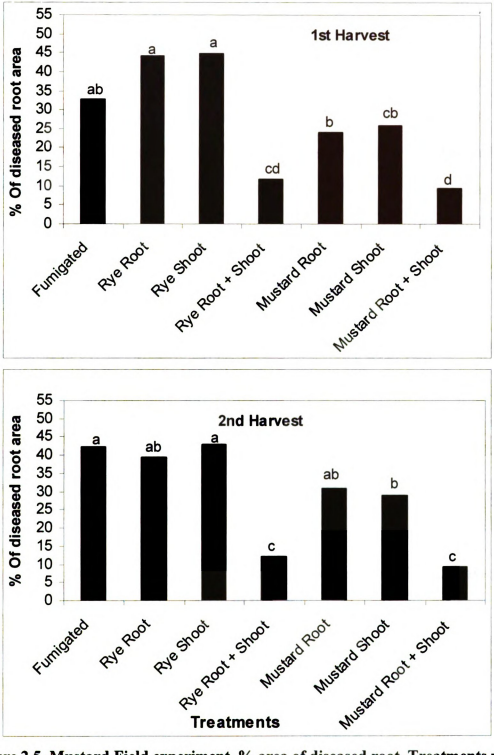

Figure 2.5. Mustard Field experiment. % area of diseased root. Treatments with different letters are statistically different at P<0.05.

Dry weight of roots, specific gravity and yield of tubers:

Dry weights of roots were not significantly different in either the treatments or the harvests. The specific gravity for all the treatments was almost the same (figure 2.6, table 2.7). The treatments did not significantly alter the tuber yields (table 2.7). Although the overall yields for all treatments were lower than expected (figure 2.7.) The analysis of variance for dry weight of root is given in table 2.5. ANOVA for specific gravity and yield are given in table 2.6.

Table 2.5. Mustard Field Experiment. Analysis of variance for dry weight of roots.

ANOVA Pr > F			
Source of variation	**df**	**Dry wt of root**	
		1st harvest	2nd harvest
Cover Crop	1	0.0192	0.8962 NS
Tissue	2	0.0947 NS	0.1665 NS
CoverCrop X Tissue	2	0.1696 NS	0.0888 NS

NS: Not Significant at P<0.05

Table 2.6. Mustard Field Experiment. Analysis of variance for specific gravity and yield of tubers.

ANOVA		**Pr > F**	
Source of variation	**df**	**Specific Gravity**	**Yield(g/plant)**
Cover Crop	1	0.7078 NS	0.5449 NS
Tissue	2	0.2439 NS	0.6854 NS
CoverCrop * Tissue	2	0.8226 NS	0.3659 NS

NS: Not Significant at P<0.05

Table 2.7. Mustard Field Experiment. LS Means and Standard Errors for specific gravity and yield of tubers.

LS MEANS		
	Specific Gravity	**Yield**
Fumigated	1.072 (± 0.002)	354.20 (± 32.48)
Rye Root	1.076 (± 0.003)	442.95 (± 66.38)
Rye Shoot	1.076 (± 0.001)	399.60 (± 46.81)
Rye Root + Shoot	1.073 (± 0.002)	350.00 (±41.17)
Mustard Root	1.075 (± 0.001)	369.80 (± 111.80)
Mustard Shoot	1.077 (± 0.001)	487.75 (± 46.49)
Mustard Root + Shoot	1.076 (± 0.0008)	429.55 (± 34.25)

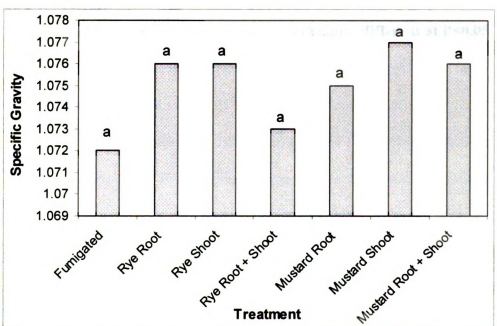

Figure 2.6. Mustard Field Experiment. Specific Gravity for different treatments. Treatments followed by the same letter are not significantly different at P<0.05

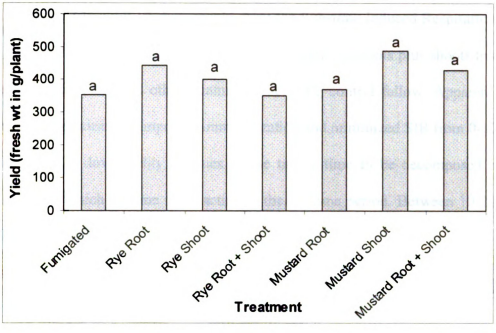

Figure 2.7. Mustard Field Experiment. Yield of fresh tubers in g / plant. Treatments followed by the same letter are not significantly different at P<0.05.

Microbial and Nematology analysis

Figures 2.9 and 2.10 show results from the Substrate Induced Respiration (SIR) at 0-10 days and 10-20 days respectively. At 0 – 10 days, rye roots plus shoots treatment had the lowest SIR than all other treatments including control fallow. Apparently, the root+shoot of rye caused a temporary immobilization and minimized SIR from 0-10 days, presumably due to low quality residues of rye taking time to be decomposed by soil microorganisms which became more active in the 2nd time period. Between 10-20 days, the SIR was highest for rye root+shoot (1.64 mg CO_2/kg/h) and lowest for rye shoots (1.43 mg CO_2/kg/h) (table 2.12). SIR overall was lower at 10-20 days.

The disease suppression effect associated with mustard tissues (Figure 2.5) can be related to microbial activity in the soil which may compete with the soil pathogenic fungal diseases. Table 2.11 presents the ANOVA for different sources of variation. Contrasts between rye versus mustard and root versus shoot are given in Table 2.13 and show no significant treatment effects.

Soils were monitored for presence of root rot nematodes (*Pratylenchus penetrans*) and colonies of *Verticillium dahliae*, which are associated with the potato disease verticillium wilt. In the first sampling on May 2004 (7 days after tissue incorporation), treatments from root+shoot residue incorporation did not show presence of *P. penetrans* (Figure 2.10). The number of nematodes increased by the second evaluation date - September 2004. It is possible that reduced number of nematodes for mustard and rye root+shoot be attributed to maximum effect of the glucosinolates and increased microbial population early in the season. While later in September, effect due to the soil amendments reduces. Although the treatments show differences in the spore

count for *P. penetrans*, the threshold level for the pathogen to cause a significant damage to crop is 150 spores / 100 cc of soil. In the given experiment the levels were very low. The carbon content was low at the experiment site. The organic carbon % for each treatment is presented in table 2.8.

Treatment with the mustard root+shoot combination consistently showed lowest colony populations of *Verticillium dahliae* in May and September 2004. The concentration of *V. dahliae* was very low in May and increased by September. Treatment with mustard root had the highest colony count in May while rye root has the highest colony count in September. At 2nd monitoring time period (September 2004), high variability was seen in the colony count for *V. dahliae*. This may have resulted in no significant differences observed in the treatment with root+shoot. Overall low levels of *V. dahliae* were seen in this study. This can be as a result of the very sandy nature of the soil at the field site.

The analysis of variance and planned contrasts for *P. penetrans* are presented in Tables 2.11 and 2.12. Analysis of variance and planned contrasts for *V. dahliae* colonies are given in table 2.13 and table 2.14 respectively.

Table 2.8. Mustard Field Experiment. Least Square Means for Organic Carbon % in the treatments.

Treatment	LS Means
Fumigated	0.61 a
Rye Roots	0.56 a
Rye Shoots	0.56 a
Rye Roots + Shoots	0.63 a
Mustard Roots	0.63 a
Mustard Shoots	0.56 a
Mustard Roots + Shoots	0.58 a

LS Means with different letters are significantly different at $P<0.05$. Least Significance difference = 0.1046

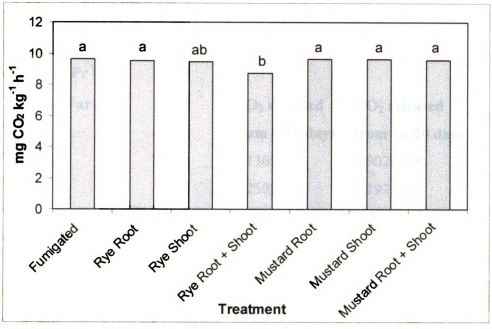

Figure 2.8. Mustard Field experiment. Substrate induced respiration in mg CO_2/kg/h between 0 – 10 days. Treatments followed by the same letter are not significantly different at P<0.05.

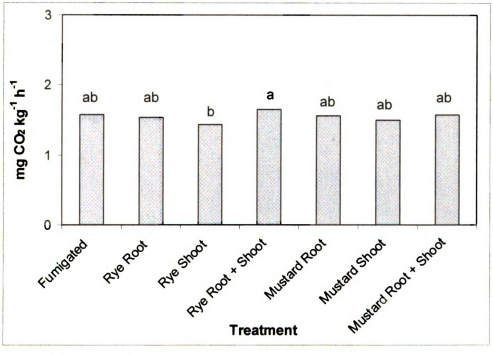

Figure 2.9. Mustard Field experiment. Substrate induced respiration in mg CO_2/kg/h between 10-20 days. Treatments followed by the same letter are not significantly different at P<0.05.

Table 2.9. Mustard Field Experiment. Analysis of variance for substrate induced respiration.

ANOVA Pr > F			
Source of Variation	**df**	**CO_2 released from 0-10 days**	**CO_2 released from 10-20 days**
Covercrop	1	0.1360 NS	0.9020 NS
Tissue	2	0.2502 NS	0.1935 NS
Covercrop * Tissue	2	0.3792 NS	0.5929 NS

NS: Not Significant at P<0.05

Table 2.10. Mustard Field Experiment. Planned contrasts for substrate induced respiration.

Pr > |t|		
Parameter	**CO_2 released from 0-10 days**	**CO_2 released from 10-20 days**
Mustard v/s Rye	0.1203 NS	0.5936 NS
Roots v/s Shoots	0.2290 NS	0.1468 NS

NS: Not Significant at P<0.05

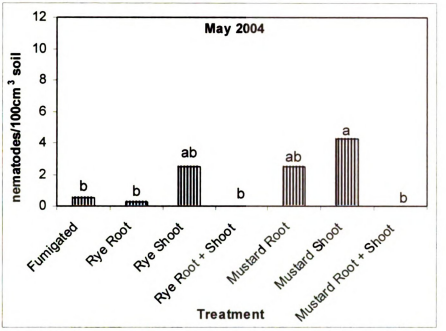

Figure 2.10. Concentration of *Pratylenchus penetrans* spores/100 cm^3 soil observed in May 2004. Treatments followed by the same letter are not significantly different at P<0.05.

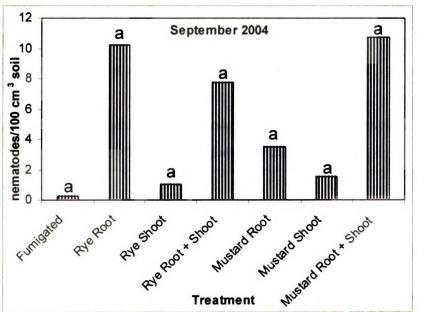

Figure 2.11. Concentration of *Pratylenchus penetrans* spores/100 cm^3 soil observed in September 2004. Treatments followed by the same letter are not significantly different at P<0.05.

Table 2.11. Mustard Field Experiment. Analysis of Variance for *Pratylenchus penetrans* in May 2004 and September 2004.

ANOVA Pr > F			
Source of variation	**df**	**May 2004**	**September 2004**
Covercrop	1	0.3097 NS	0.7566 NS
Tissue	2	0.0956 NS	0.1786 NS
Covercrop * Tissue	2	0.5731 NS	0.4997 NS

NS: Not Significant at P<0.05

Table 2.12. Components of analysis of variance and planned contrasts for spore population of *Pratylenchus penetrans* in 100 cm^3 soil in May and September 2004 in plots treated with incorporations of mustard or rye shoots only or roots only.

Pr > |t|		
Parameter	Date of evaluation	
	May2004	September
Mustard v/s Rye	0.9365 NS	0.2898 NS
Root v/s Shoot	1.0000 NS	0.2898 NS

NS: Not Significant at P<0.05

Table 2.13. Mustard Field Experiment. Analysis of Variance for *Verticillium dahliae* in May 2004 and September 2004.

ANOVA Pr > F			
Source of variation	**df**	**May 2004**	**September 2004**
Covercrop	1	0.1419 NS	0.2883 NS
Tissue	2	0.2179 NS	0.5142 NS
Covercrop * Tissue	2	0.2179 NS	0.4516 NS

NS: Not Significant at P<0.05

Table 2.14. Mustard Field Experiment. Planned contrasts for *Verticillium dahliae* in May 2004 and September 2004.

Pr > |t| Parameter	May 2004	September 2004
Mustard v/s Rye	0.0449	0.2248 NS
Root v/s Shoot	0.1228 NS	0.8627 NS

NS: Not Significant at P<0.05

GREENHOUSE CONTAINER EXPERIMENT

% Area of diseased root

% of disease infection on the roots was lower for potatoes grown after incorporation of cover crop shoots. This is consistent with the findings of Charron and Sams, 1999, who determined that a 75% decrease in *Rhizoctonia solani* growth was seen when subjected to shredded leaves of *Brassica juncea*. The distribution of glucosinolate in a mustard plant is not uniform. The amount of glucosinolate was (report number in units of measurement) in the shoots and (report number) in the roots (Figure 2.12). From figure 2.12 we can infer that the amount of glucosinolate is higher in the shoots than in the roots. This is also consistent with the findings of Sang et al., 1984; Kirkgaard 1998 and Sarwar 1998. Disease infection was lowest in the treatments with the higher rate of tissue incorporation. Reduction in disease in these treatments is attributed to the increased amount of glucosinolates being added to the soil.

The treatments with mustard roots only were not associated with decrease in infection. This may be related to two factors. 1) Roots of mustard may not be high in glucosinolate concentration, 2) Since the amount of root biomass being added into the soil was almost one third the amount of shoots being incorporated, the low rate might have had an effect on disease control.

Mustard roots + shoots treatment did not show any added benefit in this experiment as compared to mustard shoot only. Although in a field study, it would be impractical to separate the roots and shoots for incorporation. Taking this fact into consideration, treatment with mustard root + shoot could be used as an effective fungal disease control.

Contrasts between the two rates and roots v/s shoots were shown in Table 2.16. There was a significant difference measured for the tissue type that was incorporated into the soil (root, shoot or root+shoot) with $P < 0.0003$. The rate of tissue incorporation ($P < 0.6931$) and the interaction between the two ($P < 0.4523$) was found to be not significantly different (table 2.15).

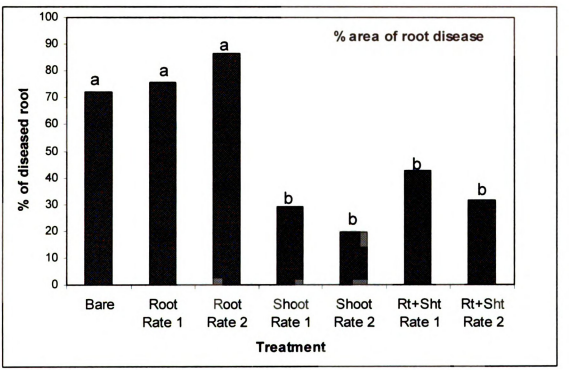

Figure 2.12. Greenhouse container study. % area of diseased root. Treatments followed by the same letter are not significantly different at P<0.05.

Table 2.15. Greenhouse container experiment. Analysis of Variance for % area of diseased root.

ANOVA Pr > F		
Source of Variation	**df**	**Pr > F**
Tissue	2	0.0003
Rate	1	0.6931 NS
Tissue * Rate	2	0.4523 NS

NS: Not Significant at P<0.05

Table 2.16. Greenhouse container study. Planned contrasts for % of diseased root.

Pr > |t|	
Parameter	
Rate 1 v/s Rate 2	0.0737 NS
Shoots v/s Roots	0.3299 NS

NS: Not significant at P<0.05

Tuber Infection Scoring

The analysis of variance is given in table 2.17. The tubers from the experiment were rated on basis of the surface infection by the fungi. They were sorted into 4 classes depending on the % of infection on each tuber. The percent of tubers with sclerotia of *R. solani* on the surface area in each of the four classes for each incorporation treatment is shown in Figure 2.13. The higher the % belonging to the 0-25 % category the better is the tuber health.

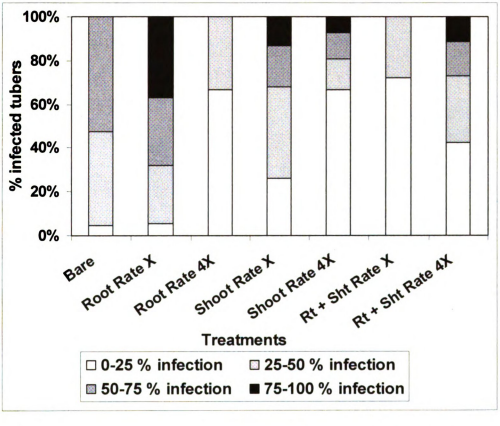

Figure 2.13. Greenhouse container study. % Area of infected tubers.

Table 2.17. Greenhouse container experiment. Analysis of Variance for tuber scoring

ANOVA Pr > F		
Source of Variation	**df**	**Pr > F**
Tissue	2	1.0000 NS
Rate	1	0.9998 NS
Tissue * Rate	2	1.0000 NS

NS: Not significant at P<0.05

Reisolation of fungi from roots and tubers

The soil was inoculated with *Pythium ultimum*, *Fusarium solani* and *Rhizoctonia solani*. But when the infected portion of roots and tubers was cultured on selective media, it was observed that the only fungal infection was that by *R. solani* (Figure 2.14.) *R. solani* was isolated from both the roots and the tubers. *P. ultimum* grows only in very wet conditions where the soil needs to be damp all the time. This was not the case in our experiment because of which we did not see any *P. ultimum* infection. Although the F. solani inoculum had a very high spore count, we still did not see any infection because of it.

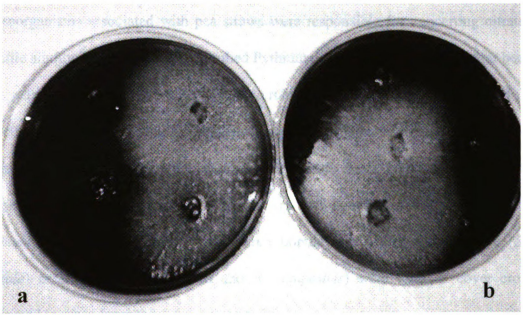

Figure 2.14. Reisolation of *Rhizoctonia solani* from, a: potato tuber and b: potato root.

DISCUSSION

Suppression of all soil-borne diseases and nematodes in the field experiment was greatest with mustard root + shoot treatment at both the first and second harvest dates in May and September. In contrast, in the container study, treatment with mustard shoots at the higher level exhibited maximum disease suppression. This can be related to growing condition differences in the field and in the greenhouse. In the field, it is possible that the roots and the shoots interacted to bring about increase in the soil microbial community which indirectly created competition for the fungi. This can also be related to some change in the soil fertility that was caused by the microbes which indirectly resulted in controlling fungal growth as shown by Bardin et al., 2004. In this container study, microorganisms associated with pea straws were responsible for concerting nitrate into volatile ammonia which in turn controlled Pythium damping-off disease in sugar beet.

In the field study, treatment with rye root+shoot also effectively controlled fungal infection. It is not clear why this treatment may result in disease suppression, but it could be related to microbial community competition or to some allelopathic effect of rye. These effects can be compared to a field study by Sumner et al., 1995 who found that *Pythium irregulare* and *Rhizoctonia solani* populations decreased when rye (*Secale cereale*) or Brassicas (*B. oleracea* and *B. campestris*) were used as cover crops as compared to when legumes were used as cover crops.

Although not significantly different with most of the other treatments, it was observed that the dry weight of roots was highest for treatment with rye root+shoot at harvest 1 and mustard root+shoot at harvest 2.

The overall *P. penetrans* and *V. dahliae* populations were very low in our field. Since the field soil was very sandy in nature and the history of potato production was short, it is not surprising that these populations were low. Interestingly in a study by Knudsen et al., 2002 who studied disease suppression soil types and found that sandy soils are more suppressive to disease than clay soils, thus texture shall be considered.

It is possible that reduced number of nematodes for mustard and rye root+shoot may be attributed to maximum effect of the glucosinolates and increased microbial population early in the season. The nematicidal property of plant residues was shown in a study by Guertal et al., 1998 who in a greenhouse study found that *Rotylenchulus reniformis* nematode populations were reduced following a rye amendment. There have been many studies demonstrating nematicidal effect of Brassicas. Potter et al. (1998) showed that soil amended with leaf tissues of Brassicas was highly nematicidal killing 56-95 % of exposed root lesion nematode, *P. penetrans*. *V. dahliae* populations were consistently lower in both May and September in soil treated with mustard root+shoot. Longer term studies in our field experiments should provide more insights.

CONCLUSION

Taken together data from the two experiments supports the use of mustard as a cover crop for controlling *Rhizoctonia solani* in the subsequent potato crop. Although mustard in this experiment did not produce high biomass, the results were clear. Treatments with mustard shoots consistently gave better results than treatments with only roots. Since glucosinolates, the compounds responsible for reduction in general soil-borne inoculum, are predominant in shoots, greater reduction in symptoms of potato tuber and root diseases in treatments with shoots is not surprising. The treatments did not significantly differ in yield but over a longer period of time, the biofumigant property of mustard is sure to have a favorable effect on the yield. The specific gravity for all the treatments was almost the same and did not show any significant pattern.

Rye, which is very easy to establish had provided some suppression of disease infection in this field experiment. This maybe due to rapid increase in the number of beneficial soil microbes, supported by the cover crop residue substrate, which creates competition for *R. solani* in early stages of its growth. This could have an effect on the overall population of *R. solani*. However bulk soil measurements of soil microbial organism activity was moderately suppressed by rye root + shoot and thus was not correlated to suppressive activity. It is possible that the competition occurs in the potato root rhizosphere, not in the bulk soil. Further work is needed to determine reason for reduction in disease when rye roots and shoots together are used as cover crops.

FUTURE WORK

Analysis of glucosinolate content in the root and shoot of mustard could provide insights regarding the superior biofumigant property of shoots over roots.

The best growth stage for incorporating mustard residues can be studied so as to obtain maximum fungal disease reduction benefit.

Effect of rye and mustard on soil microbial population immediately after incorporation should be determined, focusing on crop root rhizosphere communities rather than a bulk soil.

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Chapter 3: Bioassay to test suppression of *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium solani* by Mustard (*Brassica juncea* L.) and Rye (*Secale cereale* L.) tissues.

ABSTRACT

Bioassays are needed to assess the suppressive effects of cover crop residues on soil borne diseases. We experimented with 2 bioassays to study this. The first was a jar bioassay where we investigated the suppressive effect of 10 g mustard and rye tissues (root, shoot and root plus shoot) on *Pythium ultimum*, *Fusarium solani* and *Rhizoctonia solani*. Following this, a dose response bioassay was done to study effect of different amount of mustard and rye tissue on inhibiting growth of the 3 fungi. Here the dosage was 10 g, 5 g and 1 g. The tissues were macerated and put inside glass jars. A plug of *R. solani*, *P. ultimum* and *F. solani* was placed in the center of a 100 mm Petri dish containing fresh Potato Dextrose Agar. The Petri dish was then inverted over the mouth of the glass jar containing the above mentioned treatments and fungal growth on the PDA plate was monitored. This was expressed as % of growth on the Petri dish. The second bioassay was a tuber bioassay which was done thrice over the entire growing season based on date of cover crop incorporation. Tubers were placed in 9 * 8 * 2 in clear containers in soil collected from the individual field treatments presented in chapter 2. The tubers were scored for fungal infection and suppressive effects of the residue amended soil were monitored. Mustard residues had markedly suppressive effect on growth of all three fungi as compared to the no residue control. At 48 h, treatment with mustard shoot had no *P. ultimum* growth while the no residue control had 100 % growth on the petri dish. A similar trend was observed with no residue control showing 100 % (at 48 h) and 8.36 % (at 120 h) growth for *R. solani* and *F. solani* respectively, while

mustard shoots showed 11.3 % growth for *R. solani* and 0 % growth for *F. solani*. The mustard root+shoot treatment had significant effect in suppressing fungal growth for all 3 fungi as compared to the no residue control. In all three cases, the effect was more pronounced at 24 h than at 48 h (*P. ultimum* and *R. solani*) or 120 h (*F. solani*). For the dose response bioassay, overall 10 g mustard residues were most effective in controlling fungal expansion. For *P. ultimum*, at 48 h, 10 g mustard shoots showed 17.4 % growth as compared to 56.5 % growth shown by 1g mustard shoot. Similarly trend was seen for treatment with 10 g mustard shoot which exhibited 18 % growth while 1g mustard shoot showed 100 % growth for *R. solani* at 48 h. Overall as residue biomass was increased, the suppressive effective increased. For the tuber bioassay, the treatment with mustard root + shoot had healthiest tubers (rating 0.75) which were comparable to the fumigated control (rating 1.00). Overall effect of the cover crop and tissue interaction was significant at 7 days incorporation ($P < 0.0001$) but at 40 days, the interaction was not significant ($P < 0.4377$). When planned contrasts between different treatments were drawn, it was observed that there was no significant difference between mustard and fumigated. Mustard versus rye showed significance ($P<0.0001$) at both 7 days and 40 days incorporation.

INTRODUCTION

Soilborne plant pathogens survive in the soil for very long periods of time and cause extensive damage to many crops. For many years the most common practice for their control was fumigation either before or after cropping. Unfortunately certain fumigants possess negative attributes, such as health hazards, environmental pollution, and even potential atmospheric ozone depletion. Taking all these factors into consideration, there is now increased environmental concern which has triggered regulatory restriction on the use of soil fumigants.

The induction of disease suppression in soils has been successfully achieved using organic and fertilizer amendments (Hoitink and Fahy, 1986), or cultural practices, such as mulching, which it is believed, stimulates aggressive competition for food substrate between soil inhabitants in the rhizosphere (Baker and Cook, 1974 and Chen et al., 1987), the most biologically active fraction of the soil (Lynch 1990, and Brussaard et al., 1990). The disease suppressive activity of manures and composts can also be attributed to the production of volatile and non-volatile toxic compounds released during plant residue decomposition, or to changes in the proportion of pathogen-antagonistic microorganisms in the rhizosphere (De Brito Alvarez et al., 1995 and Chen et al., 1987).

Incorporation of green manure, rotation crop residues, or other organic materials is frequently recommended to prevent the increase of pathogens in newly cultivated soil and to produce conditions less favorable to pathogens in established crop land (Butler, 1961). In the U.S. approximately 1 billion tons of organic and inorganic agricultural recyclable by-products are generated yearly out of which 400 million tons are crop residue (Edwards and Someshwar, 2000). These residues can be effectively used for

agricultural purpose with great potential benefits. High yields for extended periods of crop cultivation in areas of China were associated with the use of organic sources of fertilizer or waste products (Kelman and Cook, 1977 and Shen, 1997). Such amendments contribute to root health by improving soil structure and reducing the negative impact of soil-borne pathogens. Davis et al., 2001, examined 100 commercial potato fields for soil characteristics, disease, and yield and found that the factors most closely related to soil quality, i.e. organic matter, organic nitrogen and increased nutrient availability, were associated with reduced Verticillium wilt and higher tuber yields.

There are many factors involved in the mechanism of disease suppression during decomposition of amendments in soil. One factor of considerable interest is the effect of volatile compounds evolved during decomposition of amendments in soil on pathogens (Angus et al., 1994). Secondary products from higher plants represent an enormous diversity of biologically active compounds that can be exploited as fumigants when plant residues are incorporated as green manure. Glucosinolates, a group of secondary metabolites are present in the seeds and vegetative tissue of many plants belonging to the family *Cruciferaceae*. Good controls of *Pythium ultimum*, *Fusarium oxysporum* f. sp *cumini* and *Rhizoctonia solani* have been seen by amending the soil with leaves of Brassica species. (Ramirez-Villapudua and Munnecke, 1988; Charron and Sams, 1999; Marwar and Lodha, 2002). In biofumigation, plant based glucosinolates are hydrolyzed in field soil to form toxic products like isothiocyanates, thiocyanates, nitriles, oxazolidinethiones, and ionic thiocyanate (Brown and Morra, 1997). Glucosinolates are usually present in the leaves of *Brassica* spp. at concentrations that can yield bioactive catabolites in amounts sufficient to prevent development or spread of certain pathogens

(Sang et al., 1984; Kirkegaard 1998 and Sarwar 1998). Studies have been reported as early as 1937 relating mustard oil and other volatile substances to plant disease resistance (Walter et al., 1937). Glucosinolates and their break-down products are known to be involved in conferring resistance to certain pests and diseases in plants (Donkin et al.,1995, Gidamoustaris and Mithen 1995, Ludwig-Muller et al., 1997). A study by Papavizas in 1966 determined that several cruciferous amendments when incorporated into soil reduced Aphanomyces root rot of peas considerably. Field experiments during the early 1990's demonstrated that wheat crops grew more vigorously following Brassica break crops such as canola and Indian mustard than other break crops such as linseed or oats (Angus et al. 1991, Kirkegaard et al. 1994). Crucifers grown as cover crops offer several advantages. They grow quickly, resist cold fall temperatures, are excellent nitrogen accumulators, the seeds are relatively inexpensive and they are winter killed. Most importantly they have been proven to be an effective control against soil diseases such as scab and rhizoctonia (Lewis and Papavizas, 1974). Oriental mustard (*Brassica juncea* L.), a variety known commercially as Pacific Gold, belongs to the family *Cruciferaceae* and provides an excellent combination of traits: medium height, high biomass production, high glucosinolate level, and late flowering (Dan Bryant, Western Farm Press, 2003). Soil-borne fungal disease suppression is more likely to be improved by using a variety high in glucosinolate. Oriental mustard is high in its glucosinolate content.

Macerated tissues of Brassica species have been shown to reduce disease incidence. Anti fungal activity associated with Brassica residues is often attributed to release of isothiocyanates from the plant tissue. Isothiocyanates are break-down products

of glucosinolates. *Brassica juncea* completely suppressed activity of *Pythium ultimum* and *Rhizoctonia solani* in a study by Charron and Sams, 1999. *Rhizoctonia solani*, in a container study, was also suppressed by cabbage volatiles (Lewis and Papavizas, 1974). In a study by Pavlica et al., 1978, volatile compounds have also been shown to reduce the number of fungal propagules. Gamliel and Stapelton (1993) determined that *Pythium ultimum* propagules were reduced by >95% when exposed to volatiles from heated cabbage amended soil.

In this chapter, we studied 2 bioassays which investigated effect of oriental mustard (for its biofumigant product) and wheeler rye (for its allelopathic property) on 3 soil fungi – *Pythium ultimum, Rhizoctonia solani* and *Fusarium solani.* In this study it was our aim to study effect of different tissues of the 2 cover crops. For this, 2 bioassays were done to primarily investigate the effect of tissues on potato (*Solanum tuberosum* L.) roots and tubers. From the 2 bioassay's we established a relation between incidence and infection of fungal diseases and effect of mustard and rye cover crops; their tissues; and amount of tissue.

In the following study, we experimented with 2 bioassays (jar bioassay and container tuber bioassay) to test the following hypotheses;

Objectives

Jar Bioassay

a) Mustard root+shoot will suppress development of all three fungi.

b) Mustard shoot only will be as effective as the mustard root+shoot in controlling disease growth.

c) Over time, effect of the treatments will wane.

d) Rye root+shoot will give noticeable results earlier which will subside over time.

e) A higher dose of mustard treatment will be more effective at restricting fungal proliferation.

Container Tuber Bioassay

a) Soil from the mustard field experiment treatments will show similar effects on tubers.

b) Tubers in sterile soil will show absolutely no fungal infection

c) Tubers placed in soil inoculated with each of the fungi will show very distinctive infection symptoms.

MATERIALS AND METHODS

JAR BIOASSAY

Plants used and growing conditions:

Oriental mustard (*Brassica juncea* L.) and Cereal rye (*Secale cereale* L.) were the two plants used in this bioassay. The variety of mustard that was used is commonly called 'Pacific Gold'. Plants were grown in 17 x13 x 6 in. plastic tubs. Seeds were sown at twice the field seeding rate (22.5 kg/ha) to get more biomass. The soil used was a mixture of sandy soil + 40% perlite. The soil was collected from a research trial field (Sandhill) in East Lansing, Michigan. The soil was steamed at 70° C for one hour, and then sieved with a 5 mm sieve before mixing with perlite. Plants were grown in the greenhouse at Michigan State University at 28°C. The light was maintained at 16 h daylight period using halogen lights. The fertilizer used was- Osmocote®, which is a slow release plant food with 14-14-14 N, P, K. All plants were watered uniformly. Both roots and shoots of the plants were harvested just before flowering. They were washed with distilled water and cut into small pieces. A compound sample from a large number (10-15 plants) of plants was used in the bioassay. The tissue samples were used immediately after harvest.

Pathogen culture:

The three fungi tested in this bioassay were *Rhizoctonia solani*, *Fusarium solani* and *Pythium ultimum*. All three were obtained from Department of Plant Pathology at Michigan State University. *R. solani* and *F. solani* were cultured on 39 g Potato dextrose agar (PDA) mixed with 1 L distilled water and autoclaved for 30 mins. 25 g/L ampicillin (Sigma-aldrich chemicals) was added to the PDA to eliminate bacterial contamination. Given its sensitivity to ampicillin, *P. ultimum* was cultured on PDA without ampicillin.

The fungi were cultured at room temperature in aseptic conditions under the hood. *R. solani* was cultured in dark while the other two were cultured in light.

Fungal response to plant:

Residues from mustard and rye root and shoot and the control were tested. The residues were chopped with scissors into 2 cm pieces. The amounts of residues used were- 10 g plant root, 10 g plant shoot, 10 g root + shoot, and control (no plant tissue). The tissues were macerated using a mortar and pestle and put inside 400 ml glass jars. A 5 mm plug of *R. solani*, *P. ultimum* and *F. solani* was removed from 10 day old cultures and placed in the center of a 100 mm Petri dish containing fresh PDA. The Petri dish was then inverted over the mouth of the glass jar containing the above mentioned treatments. The glass jar and the Petri dish were sealed at the junction using two layers of parafilm. The whole procedure was done in the hood under aseptic conditions. Fungal growth was monitored.

Dose Response Curve:

In addition to the above experiment, another experiment was done to see response of *R. solani* to different amounts of plant root and shoot. Rye and mustard were grown and processed in the same way as given above. The treatments used for this experiment were 10 g, 5 g, and 1 g of plant shoot, 10g, 5 g, and 1 g of plant root and 10 g, 5 g, and 1 g of plant shoot + root and control (no plant tissue). A 5 mm plug was removed from a 10 day old culture of *R. solani* and placed in the center of a 100 mm Petri dish containing fresh PDA. The Petri dish was then inverted over the mouth of the glass jar containing the above mentioned treatments. The glass jar and the Petri dish were sealed at the

junction using two layers of Para film. The whole procedure was done in the hood under aseptic conditions. Fungal growth was monitored.

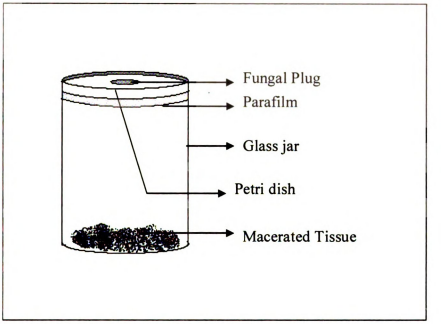


Figure 3.1. Jar bioassay. Experimental set up for bioassay

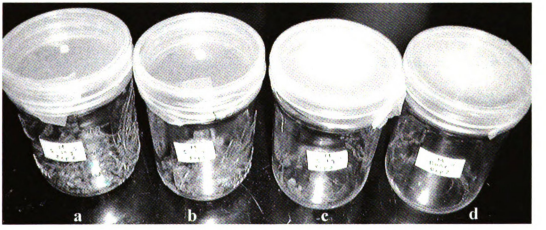


Figure 3.2. Jar bioassay. Experimental set-up for dose response of fungi to mustard tissue. Figure shows fungal growth reaction to different amounts of mustard shoots. a) 10 g mustard shoot, b) 5 g mustard shoot, c) 1 g mustard shoot, d) bare.

Recording fungal growth:

Radial mycelial growth of the fungi was recorded at 24-h intervals for 72 h for *P. ultimum* and *R. solani* as the mean of 2 perpendicular diameters. Since the growth rate of *F. solani* is lower than *P. ultimum* and *R. solani*, it was monitored at 24 h, 48 h and at 120 h. Fungal growth was expressed as a percentage of area growth on Petri dishes in control jars without plant material.

Testing biocidal activity of Mustard

The fungal plugs were removed from the Petri dishes and were transferred to fresh PDA media outside the glass jars. Fungal growth was monitored for *P. ultimum*, *R. solani* and *F. solani* for a period for 48 h.

CONTAINER BIOASSAY

Container Specifications

This experiment was done in the greenhouse. The container was a clear plastic box 9 X 8 X 2 inches in volume. This is a standard size box manufactured by Pactive-ClearView® Sell Out®. The boxes were drilled with 4 holes on the top to allow air circulation.

Soil and treatments

Soil was collected from the same plot where the mustard research trial was in progress (Sandhill). The bioassay monitoring was repeated thrice over the entire growing season from samples taken on 10th June 2004, 22nd June 2004 and 22nd July 2004 to reflect field conditions during those periods. These time periods were based on the day when the cover crops were incorporated (15th June 2004). Soil was sampled from treatment 1 to 7 which are listed in table 3.1. 6 subsamples were taken from each plot which were composited and used for the study. The soil was sampled at 0-4 in depth.

For the first 7 treatments, soil was collected directly from the corresponding plot in the field experiment. For treatment 8, soil was collected from an area outside the research plot. This soil was sterilized by autoclaving at 82 C for 30 min. This was done to ensure that the soil was completely free of all pathogens. Treatment 9 was divided into 3 sub treatments. Each sub treatment was inoculated with different fungi. The 3 fungi used in this experiment were *Pythium ultimum, Rhizoctonia solani* and *Fusarium solani.* Each fungus was used from the same batch that was used to inoculate the field. The inoculum rate used was twice that used in the field to ensure infection in those positive controls.

Table 3.1. Container tuber bioassay. Treatments used for bioassay.

NO	TREATMENTS
1	Rye Root
2	Rye Shoot
3	Rye Root + Shoot
4	Mustard Root
5	Mustard Shoot
6	Mustard Root + Shoot
7	Fumigated
8	Sterile Soil
9	Inoculated Soil (*P. ultimum, R. solani* and *F. solani*)

Tubers

The tubers used were from the same batch that was used to plant in the field. Tubers were washed with distilled water; surface sterilized with 5 % Clorox® bleach and then again washed thoroughly with distilled water. Care was taken to make sure that tubers were of equivalent size.

Procedure

The soil from the above-mentioned treatments was put inside the containers, the volume of soil being 100 cubic in. Each tuber was cut into 2 halves and 2 tubers were placed on top of the soil in the containers. The cut surface facing the soil and the soil was compacted around each half tuber to provide maximum contact between the soil and the tuber (Figure 3.3). The containers were watered everyday to maintain uniform moisture as judged by moisture condensation on top of lid and on appearance of the soil. The experiment was monitored for 10 days. Pictures were taken at the end of the 10^{th} day and

tubers were rated on the basis of degree of infection. The ratings were 0, 1, 2, 3, 4, and 5 with 0 being no infection at all and 5 being completely rotted (Figure 3.4)

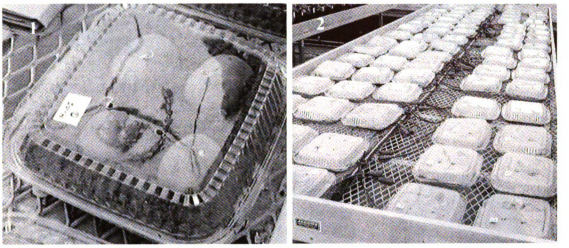


Figure 3.3. Container tuber bioassay. Experimental set-up of container tuber bioassay. (1) container used for the experiment. (2) randomized complete block design in the greenhouse.

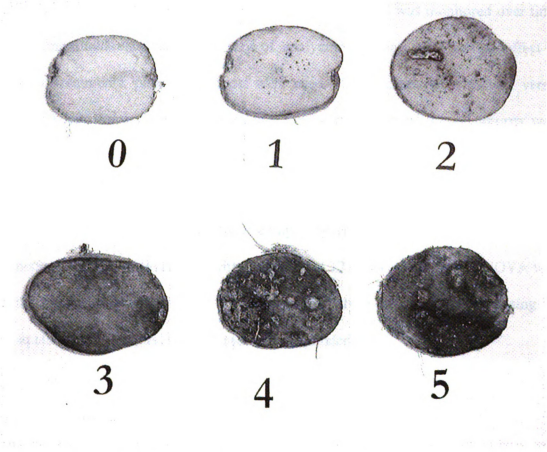


Figure 3.4. Container bioassay. Scale used for rating tubers.

Replications and Statistical analysis

Jar Bioassay

The experimental design was a completely randomized block with three replications of each combination of fungus and plant residues or control.

For dose response curve experiment: A completely randomized design with four replications of each treatment was used. A one way ANOVA was used to test growth response to different amounts of plant tissue.

ANOVA with 2 factors (covercrop and tissue type) for each fungal species was used to test growth response to plant tissue. Since the growth was monitored over time, repeated measured was used as a way of analyzing the data. To see the effect of individual treatments, planned contrasts were used. The contrasts were 1) bare versus mustard 2) bare versus rye and 3) mustard versus rye. Each of these contrasts were compared on the basis of the hours of incubation.

Container tuber bioassay

The experimental design was a completely randomized block design with 9 treatments and 3 replications each with 4 tubers and 12 observation units. ANOVA with 2 factors (cover crop and tissue type) was used to analyze the data. For calculating the least square means and planned contrasts were calculated.

RESULTS

JAR BIOASSAY

Pythium ultimum – In this experiment 10 g of each tissue and tissue combination was used to test effect on *P. ultimum*. At 24 h, mustard root+shoot and mustard shoot only, completely inhibited growth of fungi. This effect is attributed to presence of high levels of volatile glucosinolates released from cut up mustard shoot residues which suppressed fungal growth. Mustard root also restricted growth at 24 h but at 48 h, 7.5% (Table 3.2) of the petri dish was covered with *P. ultimum* indicating that fungal growth suppression effect was more temporary with root residues. This could be related to lower concentration of glucosinolates present in mustard root, which lost anti-fungal properties by the 2nd day. Rye root had a similar effect to mustard root in restricting fungus growth to 0.75% area of the Petri dish covered with mycelia (Figure 3.5). Rye root limited fungal growth to 8% at the end of 48 h (Table 3.2). In comparison, no residue controls showed *P. ultimum* growth on 16% area of the Petri dish at 24 h and 100% growth at 48 h (Figure 3.6). Treatment with rye shoot and rye root plus shoot did not have as inhibitory effects as rye roots which apparently contain allelopathic compounds. At 48 h, mustard shoot completely inhibited *P. ultimum* growth (Table 3.2) and mustard root+shoot limited fungal expansion to 5% of the petri dish (Figure 3.5). Since the treatment with mustard root+shoot had 5 g root and 5 g shoot, it is possible that lower amounts of glucosinolates could have allowed growth of P. ultimum to a certain degree. The ANOVA's are presented in table 3.2 and the planned contrasts are shown in table 3.3.

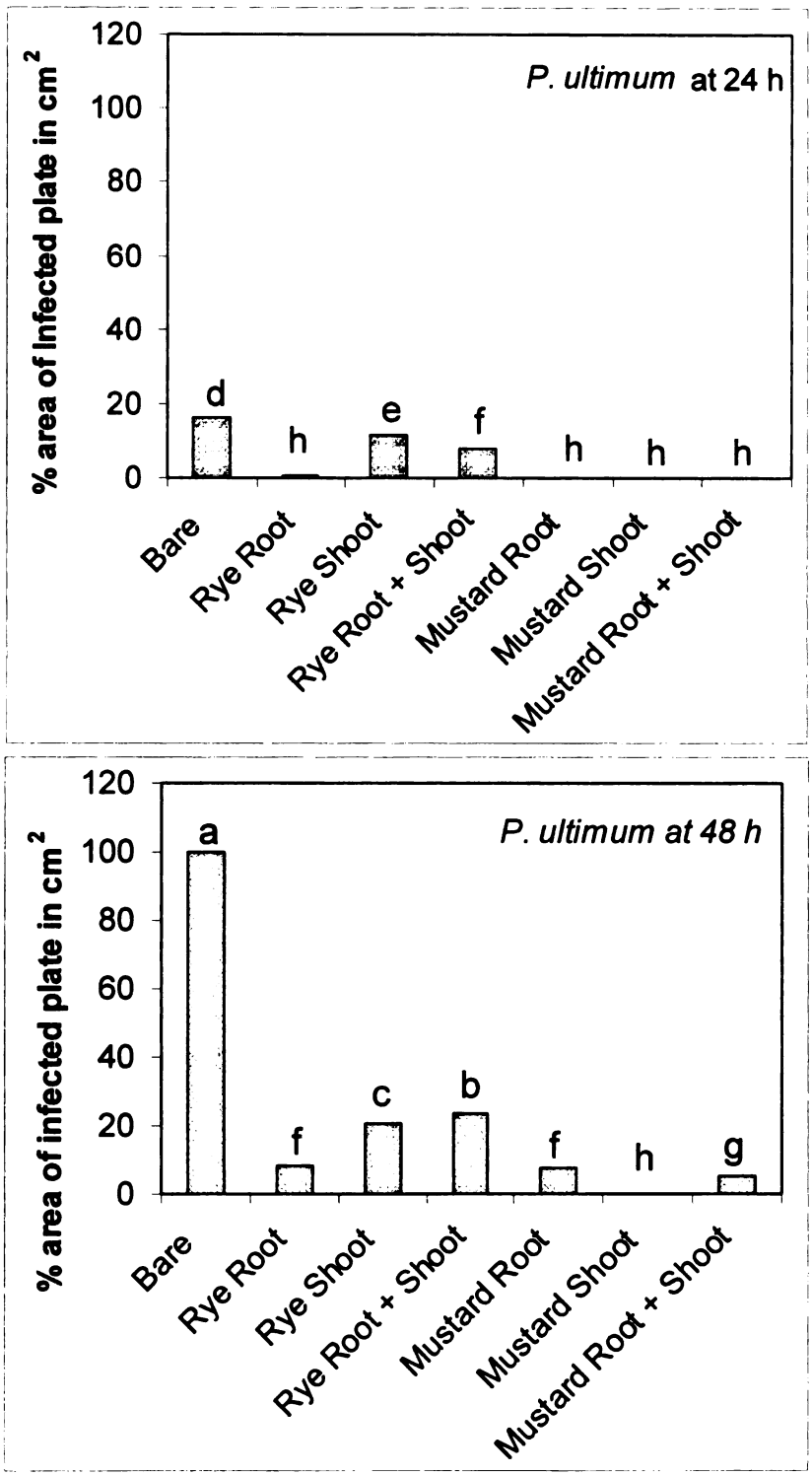


Figure 3.5. Jar bioassay. Growth observed at 24 h and 48 h for *Pythium ultimum* Fungal growth expressed as % area of growth on plate.

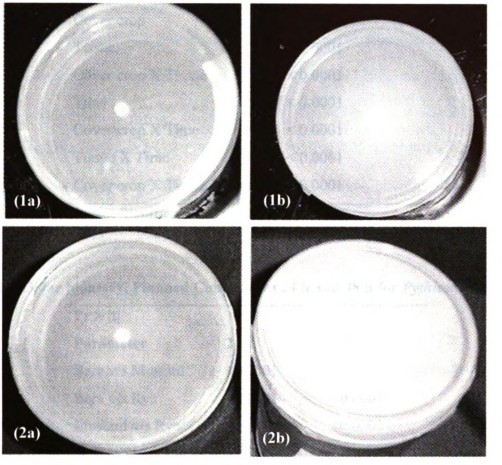


Figure 3.6. Jar bioassay. Growth of *Pythium ultimum* on Petri dish. (1a) Mustard Root+Shoot at 24 h, (1b) Bare at 24 h, (2a) Mustard Root+Shoot at 48 h, (2b) Bare at 48 h.

Table 3.2 Jar bioassay. Analysis of variance for *Pythium ultimum*.

ANOVA Pr > F	
Cover crop	< 0.0001
Tissue	< 0.0001
Cover crop X Tissue	< 0.0001
Time	< 0.0001
Cover crop X Time	< 0.0001
Tissue X Time	< 0.0001
Cover crop X Tissue X Time	< 0.0001

NS: Not Significant at P < 0.05

Table 3.3 Jar bioassay. Planned Contrasts at 24 h and 48 h for *Pythium ultimum*.

Pr > |t|		
Parameter	**24 h**	**48 h**
Bare v/s Mustard	<0.0001	<0.0001
Bare v/s Rye	<0.0001	<0.0001
Mustard v/s Rye	<0.0001	<0.0001

NS: Not Significant at P < 0.05

Fusarium solani – In this experiment 10 g of tissue was used to test effect of residues on *F. solani.* The results were observed at 24, 48 and 120 h because *F. solani* is a slow growing fungus. Mustard root and mustard shoot completely inhibited expression of *F. solani* throughout the experiment (Table 3.5), while neither of the rye treatments had a pronounced effect on inhibiting growth of *F. solani* as compared to the mustard treatment. Mustard root+shoot controlled fungal growth to a certain extent initially and succeeded in limiting its growth substantially after 120 h (Table 3.5). It was observed that no residue control showed no fungal growth at 24 h; at 48 h, the growth was 0.63 % and at 120 h fungal expansion had increased to 8.36% (Figure 3.7). The ANOVA's are presented in table 3.5, and the planned contrasts in table 3.4.

Table 3.4 Jar bioassay. Planned Contrasts at 24 h and 48 h for *Fusarium solani.*

Pr > |t|			
Parameter	**24 h**	**48 h**	**120 h**
Bare v/s Mustard	0.9534 NS	0.3090 NS	< 0.0001
Bare v/s Rye	0.4912 NS	0.8256 NS	<0.0001
Mustard v/s Rye	0.3739 NS	0.2595 NS	0.0094

NS: Not Significant at P < 0.05

Table 3.5 Jar bioassay. LS Means with Standard Errors and analysis of variance at 24 h, 48 h and 120 h for *Fusarium solani*.

	24 h	48 h	120 h
LS MEANS			
Bare	0.00 (± 0.00)	2.63 (± 0.14)	8.36 (± 0.58)
Rye Root	0.12 (± 0.04)	0.18 (± 0.02)	0.20 (± 0.07)
Rye Shoot	0.33 (± 0.33)	0.80 (± 0.80)	1.84 (± 1.84)
Rye Root + Shoot	0.72 (± 0.28)	0.84 (± 0.18)	1.34 (± 0.64)
Mustard Root	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
Mustard Shoot	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
Mustard Root + Shoot	0.10 (± 1.10)	0.14 (± 0.14)	0.18 (± 0.08)
ANOVA Pr > F			
Cover crop	0.8687 NS		
Tissue	0.0011		
Cover crop X Tissue	0.0089		
Time	<0.0001		
Cover crop X Time	0.9634 NS		
Tissue X Time	0.002		
Cover crop X Tissue X Time	0.0311		

NS: Not Significant at $P < 0.05$

Rhizoctonia solani – All treatments used 10 g of tissue in this bioassay to observe residue tissue effect. At 24 and at 48 h maximum *R. solani* growth was observed in the no residue treatment and minimum in the treatment with mustard root+shoot (Figure 3.7). Although mustard shoot had twice the amount of shoot than in treatment with mustard root+shoot, mustard root+shoot was more effective at controlling fungal growth. This effect could be related to some interaction between the roots and the shoots. At end of 48 h, control, rye root, rye shoot and rye root+shoot showed similar levels of fungal growth – 100% (Figure 3.7). All the mustard treatments were very effective in limiting growth of *R. solani*, indicating the potential to use mustard can be an effective tool in controlling *R. solani* infection in the field. The ANOVA's are presented in table 3.7 and the planned contrasts are shown in table 3.6.

Table 3.6. Jar bioassay. Planned Contrasts at 24 h and 48 h for *Rhizoctonia solani*.

Pr > |t|		
Parameter	**24 h**	**48 h**
Bare v/s Mustard	<0.0001	<0.0001
Bare v/s Rye	<0.0001	<0.0001
Mustard v/s Rye	<0.0001	<0.0001

NS: Not Significant at $P < 0.05$

Table 3.7 Jar bioassay. Analysis of variance for *Rhizoctonia solani*.

ANOVA Pr > F	
Cover crop	< 0.0001
Tissue	< 0.0001
Cover crop X Tissue	< 0.0001
Time	< 0.0001
Cover crop X Time	< 0.0001
Tissue X Time	< 0.0001
Cover crop X Tissue X Time	< 0.0001

NS: Not Significant at P < 0.05

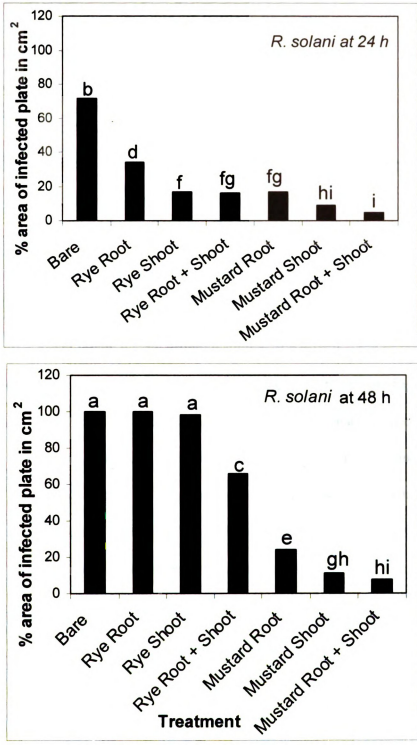


Figure 3.7. Jar bioassay. Growth of *Rhizoctonia solani* on petri plate observed at 24 h and 48 h. Fungal growth expressed as % area of growth on plate.

JAR BIOASSAY FOR DOSE RESPONSE

Pythium ultimum – The dose response of mustard treatments on *P. ultimum* showed a similar trend for all tissues and tissue combinations. Figure 3.8 shows that the infection at 48 h was greater in all treatments than at 24 h. The more the amount of tissue used, the lesser was the fungal growth. This was true for all treatments (Figure 3.8). The amount of glucosinolate present in shoots is significantly greater than the amount present in roots. This explains greater inhibition of fungi in treatments involving mustard shoot. The best treatment for controlling *P. ultimum* was 10 g of mustard shoot at 24 and 48 h. This could be related to this treatment having maximum concentration of glucosinolate.

Fusarium solani – Since this is a slow growing fungus, the effects were observed at 24, 48 and 120 h. At all times, 10 g of tissue inhibited fungal growth the most (Figure 3.9). This can be explained by the fact that more the amount of tissue, greater is the glucosinolate concentration. Comparing figure 3.9 and table 3.5, it is noticed that the mustard root and mustard shoot only treatments in this experiment did allow some growth of *F. solani*, though this was much lesser than growth in the bare treatment (Figure 3.9). Analysis of variance for *P. ultimum*, and *F. solani* are shown in table 3.8.

Table 3.8. Jar bioassay. Least square means and analysis of variance for *Pythium ultimum*, and *Fusarium solani* in dose response jar bioassay.

	% Area of Growth on petri dish														
	Pythium ultimum						*Fusarium solani*								
	24 h			48 h			24 h			48 h			120 h		
	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g
LS MEANS															
Mustard Root	95	52.8	48.3	100	93.4	91.9	0	0	0	1.7	0.95	0.38	2.2	1.5	0.96
Mustard Shoot	26.1	9.63	6.96	56.5	24	17.4	0	0	0	1.4	0.96	0.47	1.6	1.2	1.02
Mustard Root + Shoot	44.6	24.2	16.8	100	85.5	35.8	0	0	0	1	0.7	0.42	1.4	0.9	1.06
ANOVA Pr > F															
Tissue	< 0.0001						0.0164								
Amount	< 0.0001						<0.0001								
Tissue X Amount	0.0245						0.1052 NS								
Time	< 0.0001						<0.0001								
Tissue X Time	0.0022						0.041								
Amount X Time	0.1240 NS						<0.0001								
Tissue X Amount X Time	0.0006						0.2889 NS								

NS: Not Significant at P < 0.05

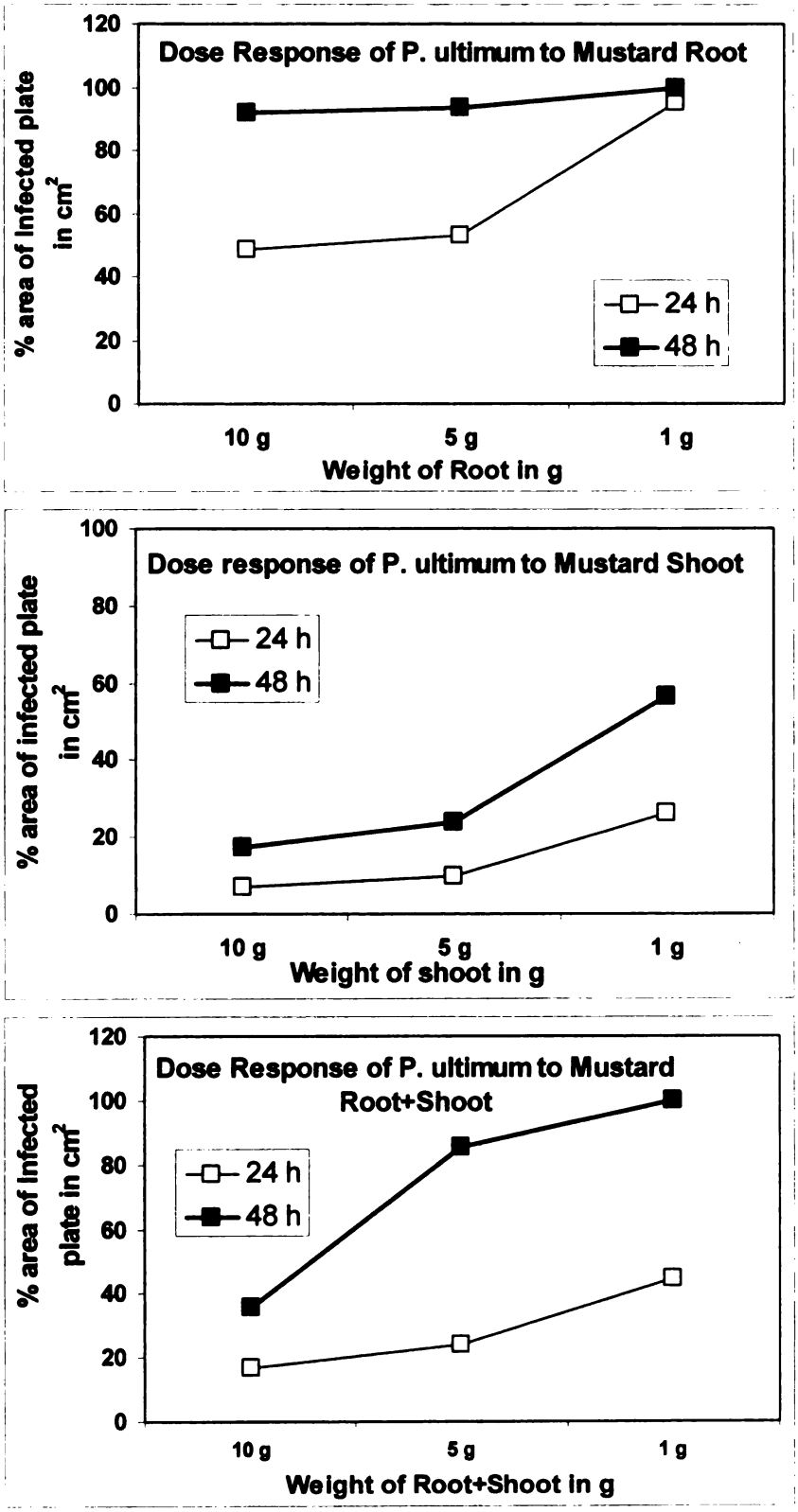


Figure 3.8. Dose response of *Pythium ultimum* to mustard tissue over time.

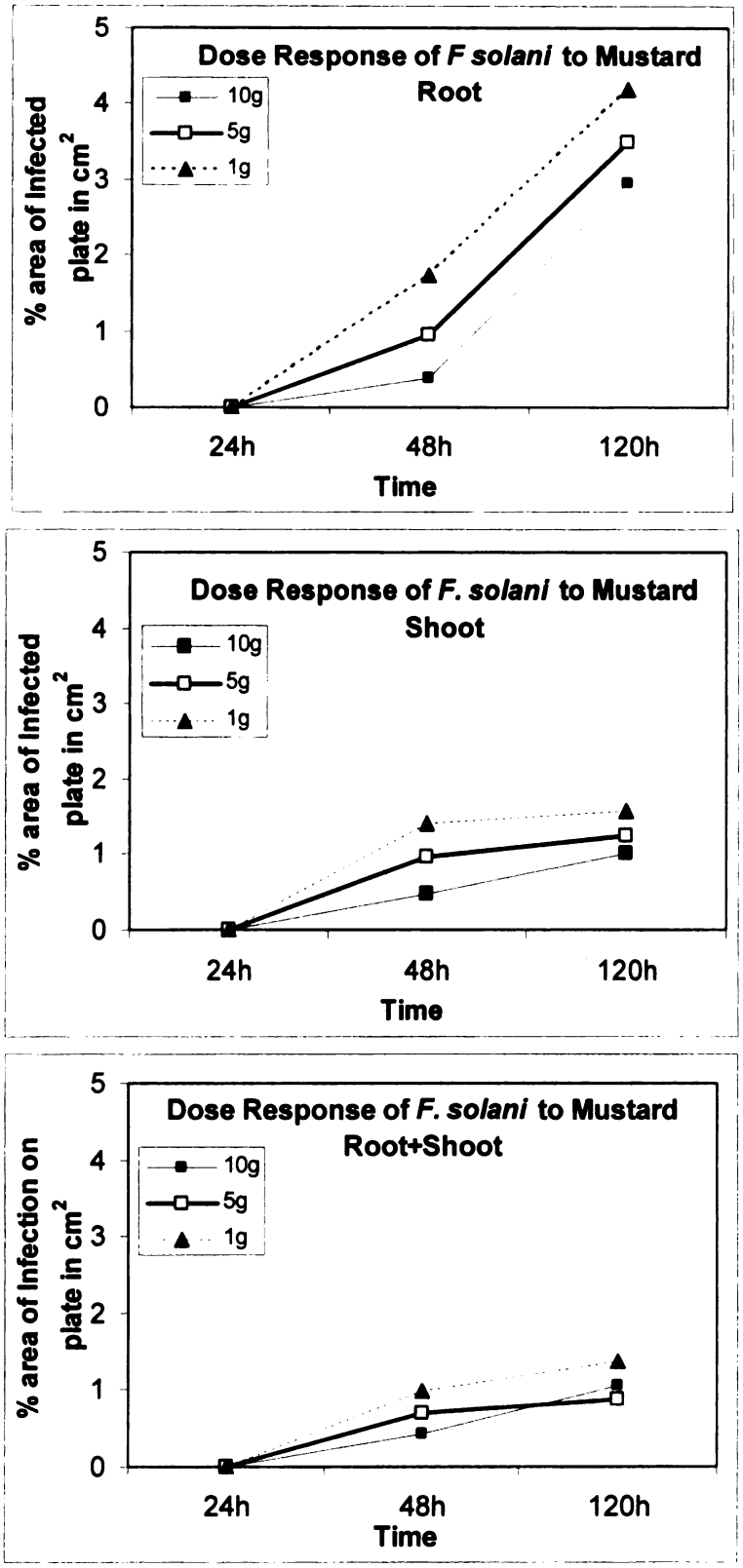


Figure 3.9. Dose response of *Fusarium solani* to Mustard tissue over time.

Rhizoctonia solani – In this experiment along with mustard, effect of rye on *R. solani* was also studied. In case of mustard tissues, fungal response to dose was most clearly seen with shoot tissue (Table 3.9). It was less obvious in mustard root + shoot. In mustard shoots, 10 g of tissue inhibited growth of *R. solani* best, while 1 g of tissue gave the poorest result. Except for shoots, in all the other cases by 48 h, *R. solani* had completely colonized the entire petri plate. With rye tissues, the trend was not as clear. At the highest dosage, which is with 10 g of tissue, rye root + shoot provided most growth suppression at 24 h (Table 3.9). Analysis of variance for *R. solani* are shown in table 3.9.

Table 3.9. Jar bioassay. Least square means and analysis of variance for *Rhizoctonia solani* in dose response jar bioassay

	% Area of Growth of *Rhizoctonia solani* on petri dish											
	Rye						Mustard					
	24 h			48 h			24 h			48 h		
	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g	1 g	5 g	10 g
LS MEANS												
Root	23.9	25	24.4	100	100	100	46.3	43	45.6	100	100	100
Shoot	21.6	18.7	15.5	100	100	31.3	17.2	4.3	1.4	100	35.4	18.1
Root + Shoot	16.3	15.5	12.3	100	100	25.1	39.3	35	31.5	100	100	100
ANOVA Pr > F												
Tissue			< 0.0001						< 0.0001			
Amount			< 0.0001						< 0.0001			
Tissue X Amount			< 0.0001						< 0.0001			
Time			< 0.0001						< 0.0001			
Tissue X Time			< 0.0001						< 0.0001			
Amount X Time			< 0.0001						< 0.0001			
Tissue X Amount X Time			< 0.0001						< 0.0001			

CONTAINER TUBER BIOASSAY

In this experiment, the bioassay provided information about soil-borne disease presence 3 times over the entire season. The first time, before the incorporation of cover crops, a baseline was established to see effect of existing soil borne disease presence on tubers. This showed that there was no difference by rep or by treatment in terms of preexisting soil borne disease. Second time, the bioassay was carried out 7 days and third time 40 days after incorporation of cover crop tissues. A similar trend was observed 2nd and 3rd time (Figure 3.13) with most of the treatments. The treatment with mustard root + shoot had healthiest tubers (table 3.10). These were comparable to the fumigated control (figure 3.10). In case of rye, the treatment with root + shoot had moderately healthy tubers (overall rating 1.25). Tubers from this had slightly more disease than tubers from fumigated control at 7 days incorporation (rating 1.12), but at 40 days (rating 1.37), the health of tubers deteriorated with this treatment (figure 3.12). Overall effect of the cover crop and tissue were significant, at 7 days incorporation but at 40 days, the interaction was not significant (Table 3.10). When estimates between different treatments were drawn, it was observed that there was no significant difference between mustard and fumigated. Fumigated control being better than the rye treatment, significant difference was observed between them (Table 3.11).

Overall from this experiment, it was observed that there was unknown interaction between the roots and shoots of the 2 cover crops that enhanced suppression of the fungi. It is possible that the mixed quality of residues from roots+ shoots with a range of available carbon and nitrogen increased the soil microbial community which proved to be

a greater competition for the fungi. In case of mustard, the glucosinolate released by the shoots proved to be anti-fungal and inhibited growth and infection of fungi.

Figure 3.13 shows a comparison between the different treatments used in this bioassay. The sterile soil treatment (Figure 3.11e) provided the healthiest tubers with no infection. Mustard root + shoot (Figure 3.11a) amended soil had comparable results to the fumigated control (Figure 3.11d) and sometimes was better than the fumigated control. When soil was inoculated with different fungi, tuber infection by *Pythium ultimum* consistently had high levels of rotten tubers (Figure 3.14).

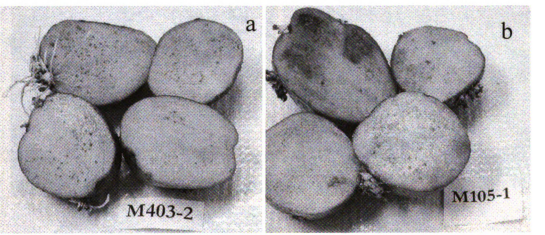


Figure 3.10. Container tuber bioassay. Comparison between tubers from 2 treatments. a) tubers in soil with mustard root+shoot as treatment. b) tubers in soil which was fumigated.

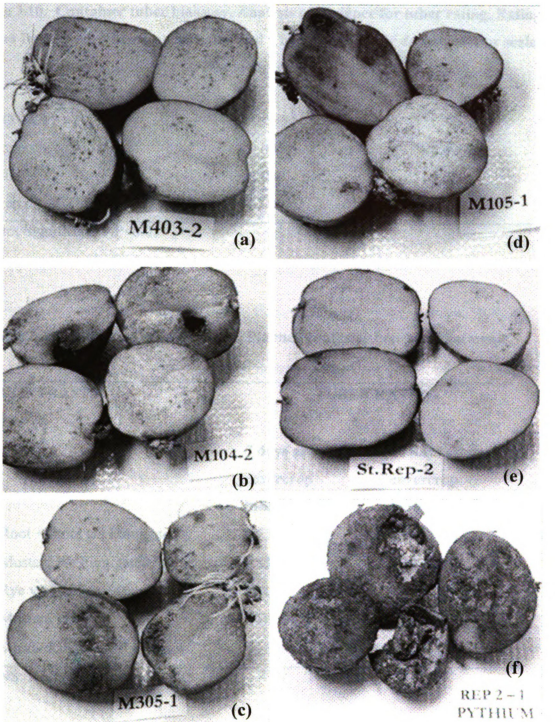


Figure 3.11. Container tuber bioassay. Tubers showing effect of different treatments. (a) Mustard root + shoot, (b) Rye Root + Shoot, (c) Mustard Shoot, (d) Fumigated, (e) Sterile soil, (f) *P. ultimum* inoculated soil.

Table 3.10. Container tuber bioassay. Analysis of variance for tuber rating. Rating tubers from 0 – 5, 0 being no infection and 5 being completely destroyed. For scale, refer to figure 3.8.

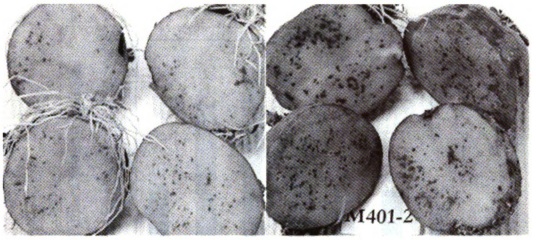
ANOVA Pr >F		
Covercrop	<0.0001	<0.0001
Tissue	<0.0001	<0.0001
Covercrop X Tissue	<0.0001	0.4377 NS

NS: Not Significant at P<0.05

Table 3.11. Container tuber bioassay. Planned contrasts between treatments.

CONTRASTS	**TUBER RATING**	
Pr > |t|		
	7 days after covercrop incorporated	**40 days after covercrop incorporated**
Root +Shoot v/s Fumigated	0.5536 NS	0.7692 NS
Mustard v/s Fumigated	0.8339 NS	0.1490 NS
Rye v/s Fumigated	<0.0001	<0.0001
Mustard v/s Rye	<0.0001	<0.0001
Mustard shoot v/s Rye shoot	<0.0001	<0.0001

NS: Not Significant at P<0.05



a b

Figure 3.12. Container Tuber Bioassay. Comparison between treatment with rye root + shoot at, a: 7 days after incorporation of cover crop and b: 40 days after incorporation of cover crop.

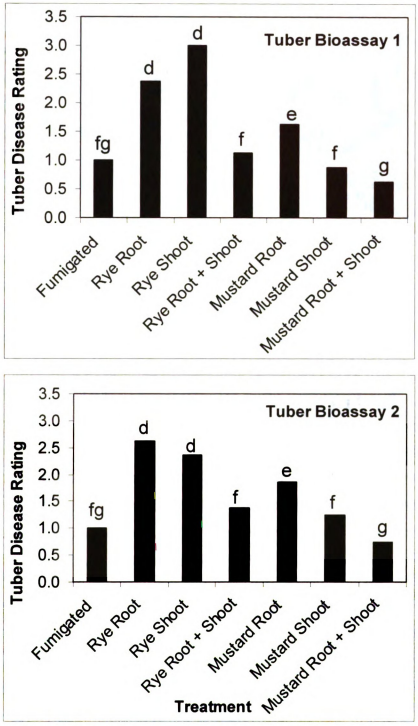


Figure 3.13. Container tuber bioassay. Tuber infection for different treatments. Tuber Bioassay 1 = 7 days after incorporation of tissue. Tuber Bioassay 2 = 40 days after incorporation of tissue. For scale refer to figure 3.8.

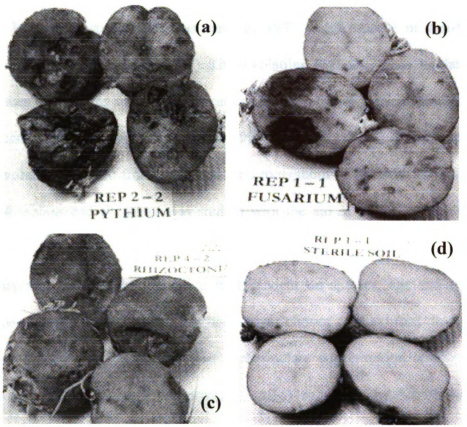


Figure 3.14. Container tuber bioassay. Comparison between tubers placed in inoculated soil and tubers placed in sterile soil. a – soil inoculated with *Pythium ultimum*, b – soil inoculated with *Fusarium solani*, c – soil inoculated with *Rhizoctonia solani*, d – sterile autoclaved soil.

DISCUSSION

There have been studies since as early as 1937 which release of anti-fungal volatiles from mustard (Walter et al., 1937). These volatiles, termed as glucosinolates are present in tissues of *Brassica* spp. at concentrations high enough to prevent development or spread of certain soil pathogens (Kirkegaard 1998 and Sarwar 1998). The suppressive effect of the volatiles released from root, shoot and root+shoot of *Brassica juncea* L. on *P. ultimum*, *R. solani* and *F. solani* was studied with the aid of the above mentioned bioassays.

Effectiveness of fungal suppression is dependent on species, age and type of Brassica tissue (Kirkgaard et al., 1996). *B. juncea* was chosen as it exhibits the highest fungicidal activity and also has the highest glucosinolate concentration among other Brassica spp (*B. napus, B. rapa, R. sativus, S. alba*) as shown by Smolinska and Horbowicz, 1999.

In the jar bioassay, the treatment with mustard shoots only was most effective in suppressing growth of the fungi. Similar result was also observed in the dose response bioassay. This may be due to the fact that the concentration of glucosinolate differs in different part of the plant and has been shown to be the highest in leaves (Sang et al., 1984). Mustard root only at 48 h limited growth of *P. ultimum* (7.56%) and *R. solani* (24.32%) but was not as effective as mustard shoot only. This result can be correlated to concentration of glucosinolate being higher in mustard shoots than in roots.

Growth of *P. ultimum* was significantly controlled by treatment with mustard root+shoot. In this treatment, the growth was 0 % at 24 h and was restricted to 5.54 % by 48 h. A similar trend was observed with *R. solani* and *F. solani* also where the growth

had slightly increased by 48 h. It is possible that the amount of isothiocyanate being released by the tissues had reduced as time passed. This is consistent with the findings of Lewis and Papavizas, 1970 who in a container study determined that volatiles released from leaf and stem of crucifers were maximum at the 1st week and had completely stopped by week 4. This is also corroborated by Doughty et al., 1996 who found that most isothiocyanates were released from *Brassica* spp for the first 9 days after incorporation of residue. This same effect was observed in the container tuber bioassay. Soil collected 7 days after incorporation of mustard residues was better at suppressing tuber infection than the soil collected at 40 days after incorporation. This may explain maximum reduction in disease intensity soon after incorporation of residue. Although, this contradicts findings by Lazzeri et al., 2001, which show that fungal growth is not just limited to glucosinolate and their hydrolysis derived products and addition of fresh organic material increased fungal population in their study.

Reduction of fungal population immediately after incorporation of residue has a significant effect on reducing the overall fungal infection later in the season. As reported by Papavizas, 1962, sensitivity of a fungus (*R. solani*) to *Brassica* is greater during the early stage of saprophytic colonization of substrate than after it has already established itself. Initial reduction in population can considerably lower population later in the season and can be an important factor of disease suppression in the field.

Rye root had mixed effects on the 3 fungi although it was significantly better than control with no residue at suppressing fungal growth. It is not determined but this may be due to some allelopathic effect of the root. Overall for all times monitored, control with no residue gave the least suppression of disease.

In the dose response bioassay, a general trend was observed where more the residue biomass greater was the disease suppression. This is consistent with the findings of Charron et al., 1999 who determined that higher the isothiocyanate concentration, greater was the *P. ultimum* and *R. solani* concentration. In the container tuber bioassay, overall a similar trend was seen at 7 days and 40 days after cover crop was incorporated with the difference being that the tuber infection had on the whole increased at 40 days after incorporation. Soil amended with mustard shoot (rating 1.25) was associated with the healthiest tubers. This treatment was comparable to the fumigated soil (rating 1.00).

CONCLUSION

We derived the following conclusions from the results of the jar bioassay and container bioassay:

1) Mustard shoots in the bioassay were a very effective tool in suppressing fungal infection even more than mustard root + shoot. In field study however, mustard root+shoot proved to be equally effective against *P. ultimum*, *F. solani* and *R. solani.*
2) Rye roots and shoots together can minimize intensity of fungal colonization. This can be attributed to certain allelochemical properties of rye.
3) Interaction between root and shoot of either mustard or rye may have increased beneficial microbial population which competed with the fungus and can decreased infection.
4) Higher the amount of mustard tissues incorporated, greater will be the benefits of disease suppression.
5) Rye should continue to be used as a winter cover crop and growers should consider trying mustard if soil borne disease is a major problem in potato production.

FUTURE WORK

It would be useful to derive at a seeding rate for mustard which will be most beneficial for decreasing incidence of soil fungal pathogens. Effect of the different tissues on soil microbial communities can be studied. This can be correlated to disease infection.

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Chapter 4: Effect of organic fertility amendments on soil fungi.

ABSTRACT

This experiment investigated various soil fertility amendments and their effect on fungal infection of potato (*Solanum tuberosum*) roots. The experiment was balanced by amount of carbon inputs applies to the soil with different organic amendments. The field experiment was repeated in 2003 and 2004. In 2003, at the 1st harvest, the plot without any soil amendment (bare) had the highest % of diseased root – 54.85%. The lowest root infection of 26.64 % was for potatoes grown in soil amended with rye + 5000 lb/a compost. This soil amendment had the highest amount of carbon input to the soil (2390 lb/a). In 2004, the same pattern of root rot suppression was observed. However in 2004, treatment effects to root rot response were much more evident. At both monitoring time periods, rye + 5000 lb/a compost was associated with the lowest fungal infection which was significantly different than bare. A comparison of treatments was conducted on the basis of Carbon added. The significance of 0 lb/a Carbon, 1200 lb/a Carbon and 2300 lb/a Carbon were looked at cumulatively at both years. In 2003, there was no significant treatment effect for any of the contrasts. But in 2004, root rot was significantly higher 0 lb/a Carbon versus 2390 lb/a Carbon. Overall a consistent pattern was noted. The amount of Carbon input and the quality of residue affected the % of disease infection. The specific gravity of tubers and the dry weight of potato roots did not exhibit any particular changes with the soil amendments. A significant difference in yield was observed between 0 lb/a Carbon and 1200 lb/a Carbon in 2003 while in 2004 this effect was not apparent. In 2004, 0 lb/a Carbon versus 2390 lb/a Carbon exhibited significant difference in the yield.

INTRODUCTION

Organic amendments in general are thought to suppress soil pathogens, and have been long used as method of biocontrol (Cook and Baker, 1983). The surface layer (about 0-15 cm in depth) in agricultural soil is the zone of highest organic C and N pools, soil microbial biomass and microbial activity (Doran, 1987; Janzen et al., 1992; Van Gestel et al.,1991), and is consequently important for providing nutrients to plants (Campbell, 1978; Paul, 1984; Woods, 1989). Application of cover crops residues or manure are possible means of improving nutrient dynamics in the surface layer of intensively managed crop systems.

Cover cropping may influence other aspects of the agroecosystem, either positively or negatively for agricultural purposes. The mechanism may be biotic (e.g. microbial interactions, plant pest interactions) or abiotic (e.g. soil physical factors; Lockwood, 1988). Cover crops and crop rotations can have various effects on soilborne diseases. Results of greenhouse tests have shown that green manures of cover crops differed significantly in their suppression of root rot severity and damage to plant growth (Abawi et al., 2000). In addition, differential effects of green manures of various cover crops on the severity of root rot and yield have also been observed under field conditions (Abawi et al., 2000). Incorporated cover crop residues may provide either a food base which encourages pathogen growth (Phillips et al., 1971) or conversely, some such as crucifers may actually decrease soil pathogen populations (Lewis and Papavizas, 1971; Subbarao et al., 1994b). More complex interactions also occur in which cover crops may cause one soilborne pathogen to increase while suppressing another (Gardner and Caswell-Chen, 1994). It has been demonstrated that increasing N inputs, either in organic

or inorganic form may increase the severity of root rot, probably due to higher evaporation and plant water stress (Papendick and Cook, 1974; Cook, 1980)

Animal manures have been used as soil amendments in plant production for centuries. With an increase in the human consumption of poultry meat and eggs in the USA, more poultry manure and litter is available for disposal on agricultural fields. Poultry manures and litters are known to have the potential to provide biological control of nematodes (Gonzalez and Canto-Saenz, 1993, Kaplan et al., 1992; Kaplan and Noe, 1993; Riegel et al., 1996) and soilborne pathogenic fungi (Homma et al., 1979; Osunlaja, 1990). In some research on control of nematodes and soilborne pathogenic fungi, rates used in greenhouse and laboratory experiments exceeded 20 MT/ha (Conn and Lazarovits, 1999; Kaplan and Noe, 1993). There has been limited research on the influence of environmentally acceptable rates of poultry litters on root diseases and nematodes.

Frequently litter is removed from poultry houses and spread immediately on fields. Very little composting is done before spreading. The litter may be high in cellulose and lignin (Fauci and Dick, 1994). The different litters may have an effect on population densities of *Rhizoctonia solani* Kuhn, *Pythium* spp., and other fungi in soil. Poultry litters may influence population densities of fungi and bacteria that may be saprophytic competitors or antagonists of soilborne pathogenic fungi (Riegel and Noe, 2000).

Baker (1965) discusses the different stages in the life cycle of a pathogen where suppression may take place. At spore germination the soil may have fungistasis activity, that is, the exogenous energy source may be deficit due to competition from other microorganisms or suppression of germination can be caused by toxic compounds

excreted by the soil microflora. However, after germination the growth of the pathogen and the process of host infection may also be inhibited by competition, antibiosis or exploitation (Baker, 1965; Schneider, 1982). Suppression of soil-borne plant pathogens may be enhanced by the incorporation of organic amendments into soil. Sivapalan et al., 1993 in a field study showed that high compost rates (120 tons per hectare) increased the total microbial populations and the number of species of fungi compared to the lower compost rate (80 tons per hectare). Lumsden et al., 1987 studied soils from the Chinampa region in Mexico and found that their agroecosystem involves management of high quantities of organic matter thus maintaining a high organic level in the soil which stimulated soil microbial activity. This indirectly resulted in higher suppression of *Pythium* sps in their soils. Similar results were found by Nitta, 1991 where increased microbial diversity caused greater disease suppression in soil.

Farming practices such as crop rotation, stubble retention and fertilizer use, all affect ecological niches available for microorganisms. Disease suppression may be tied to communities of microorganisms associated with a specific substrate, under certain environmental and management conditions (Hoitink et al., 1991).

A study by Sumner et al., 2002 investigated the effect of continuous applications of poultry litter on root diseases, nematodes, and weeds with different tillage practices in vegetable production in Georgia where they did not detect permanent changes in crop yield or population densities of soilborne pathogenic or saprophytic fungi and root-knot nematodes with the long-term use of poultry litters. *Rhizoctonia solani* is a common saprophyte in soil and many isolates will grow on cellulose (Papavizas, 1970), and some may grow on lignin (Sherwood, 1970). But in the same study by Sumner et al., 2002,

diseases induced by *R. solani* were not increased by any of the litter amendments in either tillage system. In contrast to this, in Nigeria air-dried poultry manure reduced Fusarium stalk rot but increased charcoal rot in corn (Osunlaja, 1990). Though the two diseases here are different, these investigations show the opposite effect that an organic amendment treatment can have on different soil fungal pathogens.

Rye has been effectively used as a winter cover crop as it provides plenty of ground cover and holds the soil in place. Besides being one of the easiest crops to grow, rye has a profusely branched root system which prevents soil compaction in soils that are annually tilled. Not only that, the extensive root system also enables it to scavenge nutrients efficiently from the soil profile. Rye, by nature is very competitive. In a study by Creamer et al., 1997, rye comprised of at least 80% of the above ground biomass just before kill.

The overall objective of this study was to improve quality and health of potato roots and tubers and reduce disease incidence, comparing the effect of rye in combination with other soil amendments.

MATERIAL AND METHODS

Plot information

The field experiment was conducted at Sandhill research plot near Michigan State University in East Lansing, Michigan in 2004. The plot was 54 x 106 m in area. The soil was very well drained and sandy type. The top soil had been removed 15 years ago and was then fallow until continuous potatoes 2 years ago. This made the field more susceptible to potato pathogens. The cover crop grown was winter rye (*Secale cereale*). The seeding rate of rye was 34-40 kg/ha. The amount of biomass incorporated into the soil was calculated by using a standard 0.5 x 0.5 m quadrat. The biomass by dry weight was determined to be 2820 kg/ha for rye shoot, and 28 kg/ha for rye root.

Treatments

The experiment was monitored in the year 2003 and 2004. The 2 basic treatments were 1) rye grown as a cover crop and 2) bare soil. In addition to this, the plots were amended with different amounts of poultry compost and sugar beet pulp. The treatments were balanced by the amount of Carbon input. The plots were split plots with or without each amendment. The treatments are listed in table 4.1.

Table 4.1. Rye with amendments field experiment. Treatments used in the study

No.	Treatment
1	Bare (+/- 10,000 lb/a compost)
2	Bare (+/- 5,000 lb/a beet pulp)
3	Bare (+/- 5,000 lb/a compost)
4	Fumigated
5	Rye (+/- 5,000 lb/a compost)
6	Rye (+/- 2,500 lb/a beet pulp)
7	Bare (+/- 2,500 lb/a beet pulp)

Inoculation of the plots

The first year (2003), the plots were inoculated with *Pythium ultimum*, *Fusarium solani* and *Sclerotinia sclerotiorum*. All three were obtained from the Department of Plant Pathology at Michigan State University. The spore counts were; 4.5 X 10^5 spores/ml for *P. ultimum*, 16 X 10^5 spores/ml for *F. solani* and 10 sclerotia/g of soil for *S. sclerotiorum*. Even though the field was not inoculated with *Rhizoctonia solani*, during analysis, it was seen that the potato roots were infected with *R. solani*. No infection by S. sclerotiorum was observed. Hence, the next year (2004), the soil was inoculated with *P. ultimum*, *F. solani* and *R. solani*, the inoculum levels being 20 X 10^7 spores/ml suspension 16 X 10^7 spores/ml suspension and 490 g dry inoculum/plot (875 sq ft) respectively. The fungi were cultured on Potato Dextrose Media and then inoculated on Millet seed. The amount of inoculum needed for the field was determined on a weight basis. The millet seed was spread evenly on the entire plot.

Potato variety and fertilization

The variety of potato used was "Onaway". Seed pieces (52 g) were planted on 30th June 2004 at 34 in distance within rows and 12 in between rows. Nitrogen fertilizer was applied in 3 splits at 200 N kg/ha as recommended. 100 N kg/ha was applied at planting, 50 N kg/ha at hilling and 50 N kg/ha at tuberization. Potassium (0-0-60) and phosphorous (19-17-0) were applied at 180 K_2O and 40 P_2O_5 kg/ha respectively before planting. The plot was irrigated using a traveler irrigation system.

Potato harvest and disease measurements

Destructive harvest measurements were taken twice each season. The first measurement was taken a month after planting and the second 2 months after planting. At harvest, the potato vines were killed using two herbicides Matrix™ at 0.98 L/ha and Poast™ at 1.23 L/ha + 2.2 L crop oil concentrate. The last measurement was done at the final harvest. Twice over the season, a series of soil dilution bioassays were done to study effect of the treatments on soil fungi. The fungi obtained from these tests were isolated and cultured on selective media to ascertain the kind of fungal infection. For determining % of root infection, the potato roots were washed with distilled water and analyzed using the WhinRHIZO™ program (figure 4.1). Fungi were reisolated from the infected roots using selective media. The tubers were scored visually for infection on a scale of 0-3. The scale was based on % of infection. 0 was 0-25% infection, 1 was 25-50% infection, 2 was 50-75% infection and 3 was 75-100% infection (figure 4.2). The dry weights of the roots and tubers were obtained. Yield measurements were taken at final harvest along with tuber infection and size classification.

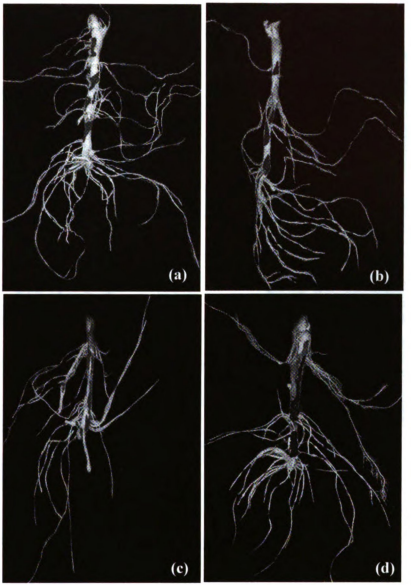

Figure 4.1. Roots scanned by WinRhizo showing different levels of infection. a) 0-25% infected b) 25-50% infected c) 50-75% infected d) 75-100% infected.

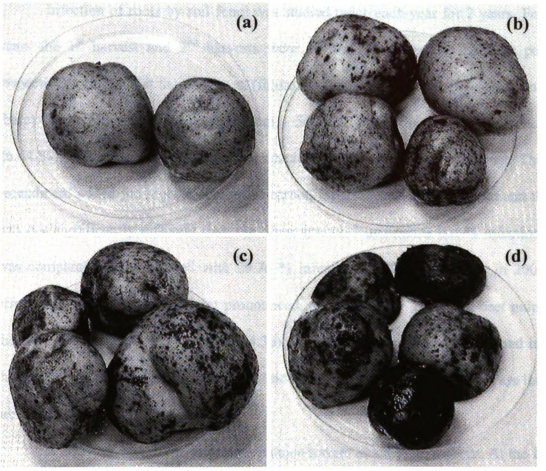

Figure 4.2. Rye with amendments field experiment. Scale used for rating tubers on basis of % infection. a) 0 (0-25%), b) 1 (25-50%), c) 2 (50-75%), d) 3 (75-100%).

RESULTS

Infection of Potato roots by soil fungi

Infection of roots by soil fungi was studied twice each year for 2 years. For both years the 1st harvest and 2nd harvests were 30 and 75 days after planting potatoes respectively. In 2003, at the 1st harvest (figure 4.3a), the plot without any soil amendment (bare) showed highest % of diseased root – 54.85%. While the lowest root infection – 26.64 %, for this harvest was for amendment with rye + 5000 lb/a compost. This soil amendment added the highest amount of carbon input to the soil. This treatment though was not significantly different than the other amended treatments but % infection wise was comparable to fumigated with 29.76 % infection. At the 2nd harvest in 2003, the effects of the treatments were not pronounced, although rye + 2500 lb/a beet pulp gave the lowest infection – 57.94% (figure 4.3b). In all the treatments, by the second harvest the amount of infection had increased considerably. Results for the ANOVA are listed in table 4.2.

In 2004, effects of the different treatments were much more evident. At the 1st and the 2nd harvest, rye + 5000 lb/a compost consistently was associated with the lowest fungal infection (figure 4.4a and 4.4b); this was significantly different than bare. At the 1st harvest, bare had the highest % of infection – 46.14 % while rye + 5000 lb/a compost was 12.64 %. By the 2nd harvest % of infection for rye + 5000 lb/a compost was contained at 16.02 %. Interestingly this treatment had the highest tuber yield. The fumigated plot in both years was effective at suppressing infection at the 1st harvest but by the second harvest, the effect of fumigation had waned.

The significance of 0 lb/a Carbon, 1200 lb/a Carbon and 2300 lb/a Carbon were looked at cumulatively at both years at both harvests. The results are given in table 4.3. In 2003, there was no significance for any of the contrasts. But in 2004, 0 lb/a Carbon versus 2390 lb/a Carbon was significant at both the harvests (table 4.3). % of fungal infection on roots at 0 lb/a Carbon versus 1200 lb/a Carbon was significant at the 1st harvest but the effect reduced considerably by the 2nd harvest.

From the results in 2003 and 2004, it was noticed that the amounts of Carbon input into the soil made a difference on the % of disease infection. It was also noted that along with the quantity of Carbon, the quality of carbon and the effect it has on soil microbes can also influence disease incidence.

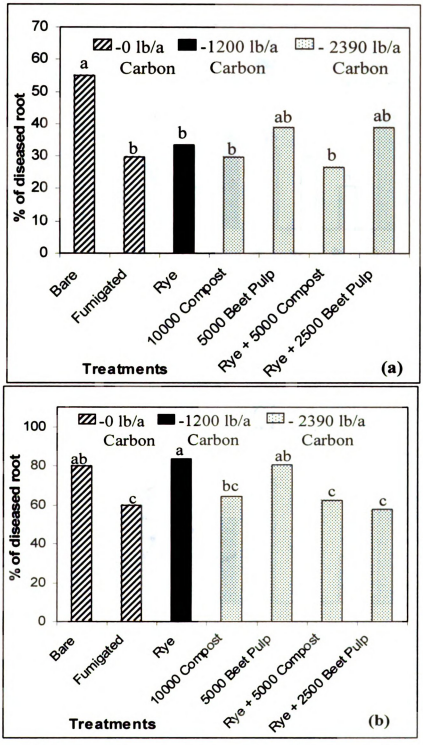

Figure 4.3. Rye with amendments field experiment. Graphs show % of diseased root for each treatment in 2003 at 2 harvests. a) Harvest 1 b) Harvest 2. Treatments followed by the same letter are not significant at $P < 0.05$.

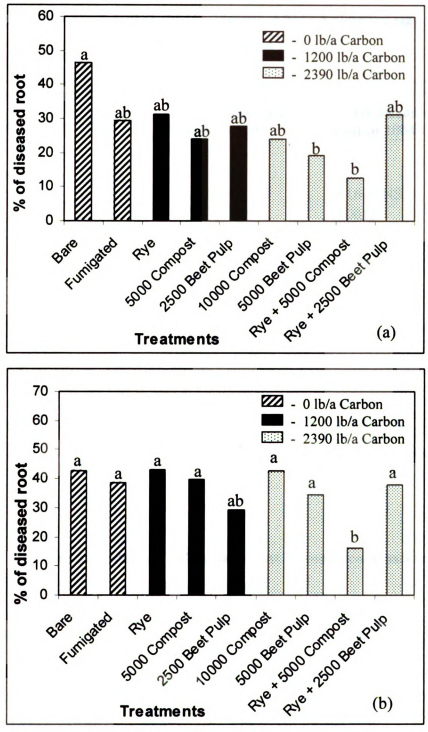

Figure 4.4. Rye with amendments field experiment. Graphs show % of diseased root for each treatment in 2004 at 2 harvests. a) Harvest 1 b) Harvest 2. Treatments followed by the same letter are not significant at $P < 0.05$

Table 4.2. Rye with amendments field experiment. ANOVA showing significance of factors for % of diseased root.

Analysis of Variance Pr > F	% Of diseased root in 2003	% Of diseased root in 2004
Harvest 1		
Factor	0.0449	0.1600 NS
Split	0.0947 NS	0.0013
Factor X Split	0.1174 NS	0.1598 NS
Block	0.0912 NS	0.2736 NS
Block X Factor	0.7533 NS	0.4333 NS
Block X Factor X Split	0.4057 NS	0.0040
Harvest 2		
Factor	0.4627 NS	0.4116 NS
Split	0.0135	0.0066
Factor X Split	0.1702	0.2012 NS
Block	0.3739 NS	0.9166 NS
Block X Factor	0.3261 NS	0.2094 NS
Block X Factor X Split	0.1236 NS	0.0117

NS: Not Significant at $P < 0.05$

Table 4.3. Rye with amendments field experiment. Planned contrasts for % of diseased root.

CONTRASTS	% OF DISEASED ROOT			
Pr > |t|	**2003**		**2004**	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
0 lb/a C v/s 1200 lb/a C	0.2708 NS	0.3714 NS	0.026	0.3539 NS
0 lb/a C v/s 2390 lb/a C	0.1223 NS	0.2895 NS	0.0004	0.0304
1200 lb/a C v/s 2390 lb/a C	0.9985 NS	0.0752 NS	0.2219 NS	0.2634 NS

NS: Not Significant at $P < 0.05$

Specific Gravity

The specific gravities of tubers were measured at the time of final harvest. The specific gravities in both 2003 and 2004 were not significantly different for any of the treatments. In 2003, the specific gravity was highest for treatment with rye + 5000 lb/a compost (figure 4.6) while in 2004, it was highest for the fumigated treatment (figure 4.5).

The ANOVA for the different factors involved in analyzing the data is presented in table 4.4.

Contrasts between the amounts of carbon deposited in the soil and its effect on the specific gravity is shown in table 4.5. None of the treatments were significantly different.

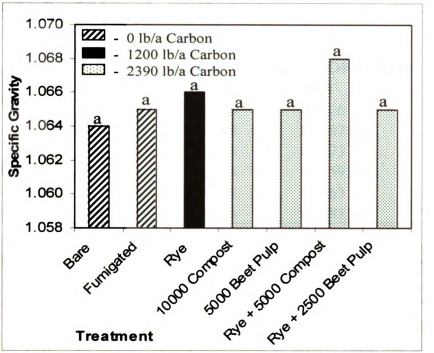

Figure 4.5. Rye with amendments field experiment. Specific Gravity for 2003. Treatments followed by the same letter are not significant at P < 0.05

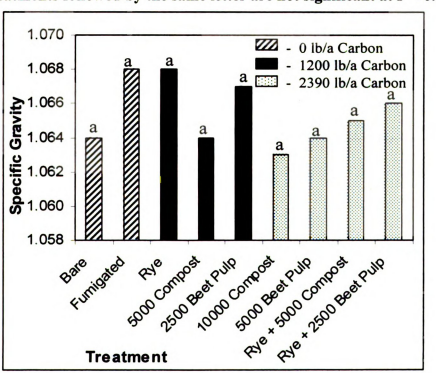

Figure 4.6. Rye with amendments field experiment. Specific Gravity for 2004. Treatments followed by the same letter are not significant at P < 0.05

Table 4.4. Rye field experiment. ANOVA for specific gravity of tubers.

Analysis of Variance Pr > F	SPECIFIC GRAVITY OF TUBERS	
	2003	**2004**
Factor	0.1566 NS	0.0426
Split	0.7353 NS	0.1487 NS
Factor X Split	0.2752 NS	0.0173
Block	1.0363 NS	0.3459 NS
Block X Factor	1.0000 NS	0.0363
Block X Factor X Split	0.3059 NS	0.2383 NS

NS: Not Significant at $P < 0.05$

Table 4.5. Rye with amendments field experiment. Planned contrasts for specific gravity of tubers.

CONTRASTS Pr > |t|	SPECIFIC GRAVITY OF TUBERS	
	2003	**2004**
0 lb/a C v/s 1200 lb/a C	0.3316 NS	0.4794 NS
0 lb/a C v/s 2390 lb/a C	0.1480 NS	0.1245 NS
1200 lb/a C v/s 2390 lb/a C	0.9489 NS	0.0517 NS

NS: Not Significant at $P < 0.05$

Dry weight of roots

The treatments at both harvests in 2003 and 2004 had no significant effect on the dry weights of root. Effect of treatments in 2003 and 2004 on dry weights of root is presented in figure 4.7 and 4.8 respectively.

The ANOVA for the different factors involved in analyzing the data is presented in table 4.6.

Contrasts between the amounts of carbon deposited in the soil and its effect on the dry weight of root is shown in table 4.7. Amounts of Carbon input had any no significant difference between the dry wt of roots.

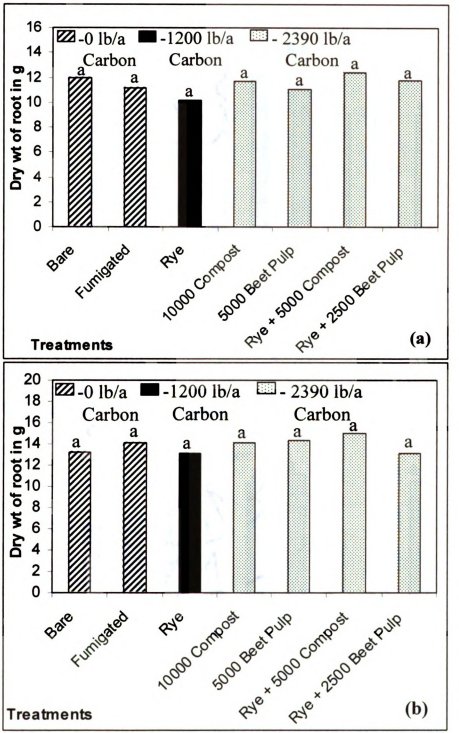

Figure 4.7. Rye with amendments field experiment. Dry weight of roots in 2003 at 2 harvests. a) Harvest 1 b) Harvest 2. Treatments followed by the same letter are not significant at $P < 0.05$

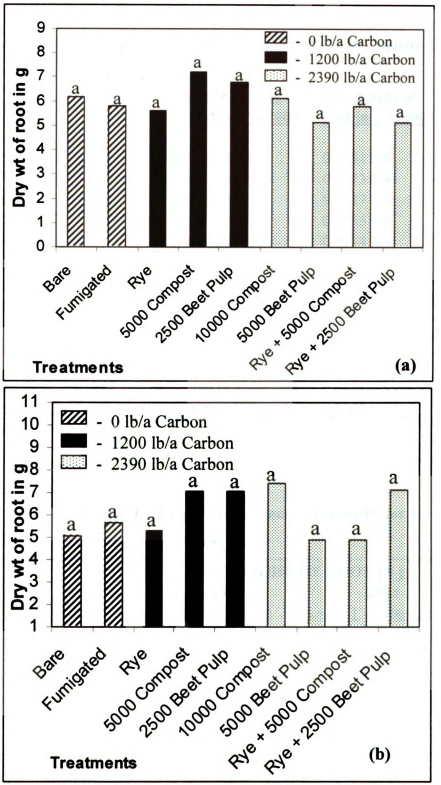

Figure 4.8. Rye with amendments field experiment. Dry weight of roots in 2004 at 2 harvests. a) Harvest 1 b) Harvest 2. Treatments followed by the same letter are not significant at P < 0.05

Table 4.6. Rye with amendments field experiment. ANOVA for dry weight of roots.

Analysis of Variance Pr > F	Dry weight of root in g in 2003	Dry weight of root in g in 2004
Harvest 1		
Factor	0.5338 NS	0.3631 NS
Split	0.2494 NS	0.8913 NS
Factor X Split	0.0434	0.7317 NS
Block	0.5364 NS	0.1735 NS
Block X Factor	0.2820 NS	0.1914 NS
Block X Factor X Split	0.7683	0.2500 NS
Harvest 2		
Factor	0.6212 NS	0.7098 NS
Split	0.1028 NS	0.1093 NS
Factor X Split	0.9590 NS	0.5606 NS
Block	0.0571 NS	0.2804 NS
Block X Factor	0.8972 NS	0.6891 NS
Block X Factor X Split	0.0925 NS	0.0060

NS: Not Significant at $P < 0.05$

Table 4.7. Rye with amendments field experiment. Planned contrasts for dry weight of roots.

CONTRASTS	DRY WEIGHT OF ROOT IN g			
Pr > |t|	2003		2004	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
0 lb/a C v/s 1200 lb/a C	0.2440 NS	0.3714 NS	0.3819 NS	0.0747 NS
0 lb/a C v/s 2390 lb/a C	0.8192 NS	0.2895 NS	0.5023 NS	0.2295 NS
1200 lb/a C v/s 2390 lb/a C	0.1480 NS	0.0752 NS	0.1536 NS	0.5565 NS

NS: Not Significant at $P < 0.05$

Yield

The yields were measured at the final harvest and were calculated on basis of fresh weight of tubers in g/plant. In 2003 and 2004, treatment with rye + 5000 lb/a compost gave the highest yield at 483.9 g/plant (figure 4.9) and 586.4 g/plant (figure 4.10) respectively. Both in 2003 and 2004, the yield for rye + 5000 lb/a compost was significantly different than the lowest yield for those years which was fumigated treatment (yield 341.4 g/plant) for 2003 and 5000 lb/a beet pulp (yield 367.8 g/plant) for 2004.

The ANOVA for the different factors involved in the experiment is presented in table 4.8.

When contrasts for the effect of amount of carbon inputs in the soil on yield were calculated, a significant difference was seen between 0 lb/a Carbon and 1200 lb/a Carbon in 2003 while in 2004 this effect was not observed (table 4.10). In 2004, 0 lb/a Carbon versus 2390 lb/a Carbon showed significant difference in the yield (table 4.10).

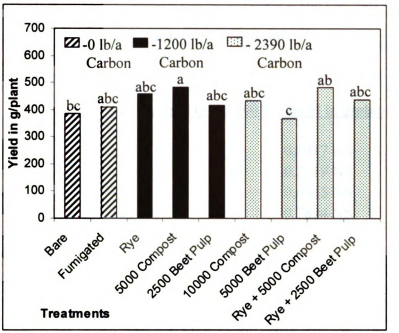

Figure 4.9. Rye with amendments field experiment. Graph shows yield of fresh tubers in g/plant in 2003. Treatments followed by the same letter are not significant at $P < 0.05$

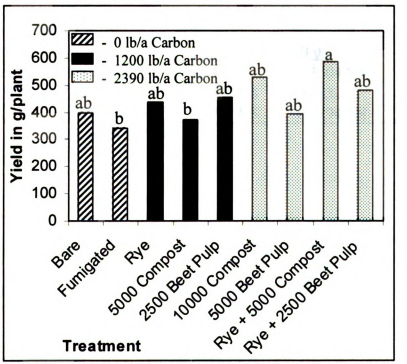

Figure 4.10. Rye with amendments field experiment. Graph shows yield of fresh tubers in g/plant in 2004. Treatments followed by the same letter are not significant at $P < 0.05$

Table 4.8. Rye with amendments field experiment. ANOVA for Yield as fresh weight of tubers in g/plant

Analysis of Variance Pr > F	Yield in g/plant in 2003	Yield in g/plant in 2004
Factor	0.1088 NS	0.5417 NS
Split	0.4686 NS	0.2919 NS
Factor X Split	0.4358 NS	0.6555 NS
Block	0.2446 NS	0.7824 NS
Block X Factor	0.5719 NS	0.0711 NS
Block X Factor X Split	0.0504	0.0029

NS: Not Significant at P < 0.05

Table 4.9. Rye with amendments field experiement. Planned contrasts for tuber yield.

CONTRASTS Pr > |t|	YIELD OF TUBERS	
	2003	2004
0 lb/a C v/s 1200 lb/a C	0.0401	0.3604 NS
0 lb/a C v/s 2390 lb/a C	0.2431 NS	0.0232
1200 lb/a C v/s 2390 lb/a C	0.3789 NS	0.2194 NS

NS: Not Significant at P < 0.05

DISCUSSION

The disease incidence in both years at both harvests was highest for treatment without any amendment (bare). In 2003 at harvest 1, the disease suppression was maximum for treatment with rye+5000 lb/a compost. Similar pattern was observed in 2004 at both harvest periods. This effect might be due to both the quantity and quality of organic matter added. Rye on its own may have controlled the soil fungal infection to a certain degree as reported in a field study by Sumner et al., 1995, where populations of *Pythium irregulare* and *Rhizoctonia solani* decreased after amending soil with rye (*Secale cereale*) residues. Addition of compost increases the soil microbial population which creates competition for the fungi. This is consistent with the findings of Pera et al., 1983 and Perucci , 1990) who reported that in addition to increasing organic matter of soil, amending with composts also increases the overall microbial population in soil. Interaction between the rye and compost might have proved beneficial for the overall root health of potatoes.

It is interesting to note that overall, treatments with highest carbon input (2390 lb/a Carbon) showed the maximum disease suppression. It is possible that the additional carbon acts as a food source to the microbial community and also plays a role in improving the overall health of the plant which indirectly causes greater disease suppression.

Although the specific gravities were not significantly different, in 2003, the specific gravity was highest for treatment with rye+5000 lb/a compost while in 2004, it was highest for fumigated treatment.

CONCLUSIONS

The soil amendments in this experiment were balanced by Carbon. Effect of the different amounts of Carbon on existing and introduced soil fungi was investigated in this study. Along with the disease incidence, effect on yield, specific gravity was also studied. In both the years, it was noticed that the amount of Carbon input into the soil made a difference on the % of disease infection. The quality of residue is also an important factor to consider when disease incidence is to be reduced. In this experiment, overall, treatment with rye + 5000 lb/a Carbon gave the best results. This treatment on the whole was successful in limiting disease infection on potato roots. This effect could be due to many reasons one of them being increase in beneficial soil microbes which indirectly competes with the antagonistic soil fungi. Greater amount of soil organic carbon (SOC) and carbon inputs to soil result in greater land productivity and contributes to favorable soil properties such as moisture and nutrient retention, which in turn buffer ecosystems from abiotic stresses (Woomer et al., 1994; Murage et al., 2000). This may result in greater resistance of plants to soil fungal diseases. Rye may also participate in its ability to produce certain allelochemicals which could reduce growth of fungi.

FUTURE WORK

It would be useful to study what effect soil organic carbon has on soil fungal populations. Data on allelopathic cover crops and their effect on fungal infections might be informative for farmers who use these as winter covers.

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