AN ABSTRACT OF THE THESIS OF

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Title: Organic Soil Amendments: Impacts On Snap Bean Common Root Rot And Soil Quality

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Richard P. Dick

Common root rot is a major disease of commercially grown snap bean (*Phaseolus vulgaris* L.) on the irrigated sandy soils of central Wisconsin. The objective of this study was to determine the relationships between soil properties and suppressiveness to common root rot of snap bean (causal agent *Aphanomyces euteiches*) in soils. The soils had been annually amended for three years in a field trial on a Plainfield sandy loam in Hancock, WI. Soils were amended each year from 1998 to 2001 with three rates of fresh paper-mill residuals (0, 22 or 33 dry Mg ha^{-1}) or composted paper-mill residuals (0, 38 or 76 dry Mg ha^{-1}). Soil was removed from each treatment in April (one year after last amendment) and brought to the laboratory. This was repeated with a field soil sample taken in September, 2001. The soils from the two samplings were incubated at room temperature and periodically assayed (days 9, 44, 84, 106, 137, 225 and 270 for April sampling) (days 13, 88 and 174 for September sampling) for suppressiveness of snap bean root rot (0 to 4 where 0 = healthy and 4 = dead plant). The same days, incubated soils were characterized for β-glucosidase, arylsulfatase and fluorescein diacetate

activities; microbial biomass C (by chloroform fumigation); water stable aggregation (WSA) and total C. In the first incubation, there were large differences between field amendment treatments in terms of snap bean root rot incidence. The disease was suppressed by both fresh and composted amendments, but compost was most suppressive at high compost rates with disease incidence <40% which are considered healthy plants that can reach full yield potential. In the second incubation, disease severity difference among treatments were similar to the first incubation. This would indicate the suppression was induced prior to initiation of this experiment.

Disease severity of bean plants grown in unamended field soil was high but in amended soils tended to decrease in intensity over time. Root rot severity was negatively related to β-glucosidase, and microbial biomass at the beginning and the end of the first incubation period, respectively. FDA hydrolysis was not correlated with disease severity and WSA moderately correlated with disease. The best indicator of disease severity was arylsulfatase which was significantly and negatively correlated with disease severity in 4 of 5 sampling periods.

Organic Soil Amendements: Impacts on Snap Bean Common Root Rot and Soil Quality

by
Maria Cecilia Cespedes Leon

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Maria Cecilia Cespedes Leon, Author

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CONTRIBUTIONS OF AUTHORS

Dr. Alexandra Stone was instrumental in the design and implementation of this study, the interpretation of the data and editing of the final manuscript.

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For my sons, Felipe and Nicolas, because they are the future and deserve to live in a sustainable world. And for my husband, Agustin, for helping me see the importance of this work.

ORGANIC SOIL AMENDMENTS: IMPACT ON SNAP BEAN COMMON ROOT ROT AND SOIL QUALITY

CHAPTER 1

GENERAL INTRODUCTION

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Modern agriculture has resulted in intensive management of soils and significant use of pesticides. This has raised concerns about degradation of soil quality due to erosion and loss of organic matter. Furthermore, the public is requesting a reduction in pesticide use for environmental and food safety protection. There is interest in developing economical sustainable systems to meet these challenges.

Sustainable agriculture, which includes integrated and organic agricultural systems, aims to reduce the use of chemical fertilizers, pesticides, additives and growth regulators while improving soil quality. However, to be successful, these systems need to effectively control diseases, pests, and weeds and supply adequate nutrients.

One important principle of sustainable systems is to suppress or control diseases by natural processes. Some agronomic practices have been shown to enhance disease suppression in soils.

Root rot is a major disease of commercially grown snap bean on the irrigated sandy soils of central Wisconsin (Pfender and Hagedorn, 1982; Kobriger et al., 1998). Combined infections by *Aphanomyces euteiches* and *Pythium ultimum* are associated with the disease (O'Brien et al., 1991).

DISEASE SUPPRESSION

Disease suppression of soil-borne diseases has been explored intensively for decades (Davey and Papavizas, 1961; Baker, 1987; Campbell, 1989; Chen et al. 1988; Boehm and Hoitink, 1992). The ability of organic amendments to suppress diseases has been demonstrated, but relatively little is known about the utility of soil quality properties as indicators of the disease-suppressive potential of soils.

There is evidence that soil organic amendments can induce disease suppression by stimulating resident antagonists (Cook, 1990). The addition of readily available C to the soil, as a green manure, compost or natural litter, stimulates microbial activity and may cause intense competition. Kundu and Nandi (1985) showed that when the C:N ratio of soil increased, fungal population activity decreased, but actinomycetes and bacterial populations increased. This is closely related to the decomposition level of organic matter in amendments. Composted tree bark with high content of degradable C suppresses diseases if the soil is colonized by appropriate microflora (Hoitink et al., 1997).

Paper-mill residuals are industrial byproducts produced in large amounts which could be used as amendment. For example, Stora Enso Company in Wisconsin Rapids, produces approximately 46,000 wet tons annually. While a small percentage of the paper-mill residual produced is spread on cropland, most is added to landfills. Paper-mill residuals amendment has been shown to increase water and nutrient retention and improve the soil's ability to suppress crop diseases (Cooperband, 2001; Stone et al., 2001).

The mechanisms involved in disease suppression are complex and may vary depending upon the disease organism involved. Disease suppression can be viewed as a manifestation of ecosystem stability and health. Thus, indicators for soil health possibly could function as indicators for disease suppressiveness (van Bruggen and Semenov, 2000). Identifying soil conditions that generate disease suppression may provide guidance for developing disease-suppressive soils. Microbial activity level is an indicator of the potential of an organic material or amended soil to suppress soil-borne diseases (Lumsden et al., 1983; Boehm and Hoitink, 1992; Stone,1997; Dissanayake and Hoy, 1999).

SOIL QUALITY AND DISEASE SUPPRESSION INDICATORS

Microbial biomass-C and enzyme activities have been found to be sensitive in reflecting soil quality because they reflect long-term soil management with organic matter inputs (Bandick and Dick, 1999; Ndiaye et al., 2000), they are relatively simple to run and do not have temporal variability. Organic residue-decomposing organisms are probably the major contributors to soil enzyme activity; from a soil quality perspective, enzymes involved in the C cycle are probably good indicators of soil quality (Dick et al., 1996). From a disease suppression perspective, the choice of an indicator depends on the mechanisms specific for suppression of each disease. Disease depends on the characteristics of the amendment, for example its chemical composition (van Bruggen and Semenov, 2000). It may be that enzymes involved in the C cycle and microbial biomass-C are likely candidates as indicators of disease-suppressive soils. Carbon cycling enzymes may be related to parasitism because degradation of the host cell wall can be an important mechanism for suppressing a disease. Total C is an index of soil organic matter and is a general indicator of soil quality. It is related to microbial activity, aggregation and many other qualities of soil structure such as infiltration, water-holding capacity and bulk density. An integrative index for soil structure is water stable aggregation; this also measure has been considered as a possible indicator of soil suppressiveness.

The objective of this study was to determine the effect of paper-mill residuals as organic amendment on the suppression of common root rot of snap bean (causal agent *Aphanomyces euteiches*) and the relationships between soil properties and suppressiveness in an amended sandy soil over time.

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CHAPTER 2

LITERATURE REVIEW

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ROOT ROT OF BEAN

Root rot is a major disease of commercially grown snap bean (*Phaseolus vulgaris* L) on the irrigated sandy soils of central Wisconsin (Pfender and Hagedorn, 1982; Parke and Rand, 1989; Kobriger et al., 1998). A complex of fungal pathogens cause bean root rot, including A*phanomyces euteiches*, *Pythium* spp, and probably *Rhizoctonia solani* (Kobriger et al., 1998), *Fusarium solani* and *Thielaviopsis basicola* (O'Brien et al., 1991). The most important organisms in the complex depend largely on the climatic and soil conditions of a particular area (Pfender and Hagedorn, 1982). In green bean production areas which receive higher rainfall, *Aphanomyces euteiches* and *Pythium* spp are the most important (O'Brien et al., 1991). The major causal agent of this disease seems to be *Aphanomyces euteiches* (Pfender, 1991a).

Aphanomyces species cause root rot on several other crops: peas (*Pisum sativum*), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), yellow sweet clover (*Meliliotus officinalis*), common vetch (*Vicia sativa*), cowpeas (*Vigna sinensis*), black medic (*Medicago sativa*), faba bean (*Vicia faba*), spinach (*Spinacea oleracea*), flax (*Linum usitatissimum*), redroot pigweed (*Amarantus retroflexux*), and sugarbeet (*Beta vulgaris* L. *spp. vulgaris*) (Sherwood and Hagedorn, 1962; Ayers, 1974; Lamari and Brenier, 1985; and Parke and Grau, 1993).

The strain of *Aphanomyces euteiches* which causes severe root and hypocotyl rot in beans was designated *A. euteiches* f. sp. *phaseoli* (Pfender and Hagedorn, 1982) This strain is also pathogenic to alfalfa, but does not infect peas, soybeans, or common hosts of several other *Aphanomyces* spp. (Pfender, 1991a). A*phanomyces euteiches* f. sp. *pisi*, the pea pathogen, can be isolated from field-

grown beans but causes much less damage on this host than does *A. euteiches* f. sp. *phaseoli* (Pfender, 1991a).

Disease Description

Aphanomyces euteiches causes dark lesions on roots; it does not cause seed rot or pre-emergence damping-off. Lesions on root tissues are initially yellow-brown and fairly firm. They rapidly coalesce to involve most of the roots which become softer as the pathogen destroys the cortex. The infected roots soon darken from the activity of secondary invaders (Pfender, 1991). The malfunction of roots and lower stems caused by the pathogen results in stunted, chlorotic, and eventually necrotic stems and leaves, followed by brown discoloration and death (Parke and Grau, 1993).

Aphanomyces root rot is caused by the fungus *Aphanomyces euteiches* f. sp. *phaseoli*, which is a water mold of the family Saprolegniaceae, order Saprolegniales, and class Oomycetes (Parke and Grau, 1993). The aseptate mycelium of this fungus can produce two kinds of spore stages. The thick-walled oospores, formed by sexual fusion of the oogonia and antheridia, can persist in a dormant state in the soil for years and may be dispersed long distances in infested soil. When oospores germinate, they form hyphae or sporangia; sporangia generate the hyphae or produce asexual swimming spores or zoospores (Pfender, 1991a). *Aphanomyces euteiches* is considered a poor saprophyte. Oospores form in infected plant tissues and can survive in field soils for month or years after host residue decomposition. Many plants species are hosts to *A. euteiches*, so populations can be maintained even in the absence of an economic host crop by infection of alternate hosts (Mitchell et al., 1968).

Aphanomyces spp. is almost always associated with *Pythium ultimum* Trow in infected plants in Wisconsin. Combined infections by the two pathogens increase disease severity synergistically and cause increased mortality of infected plants, especially at higher temperatures (Pfender, 1991a).

Beans can be damaged by several Pythium species, which also are water molds of the family Pythiaceae, order Peronosporales, and class Oomycetes (Martin, 1993). *Pythium ultimum* survives in soils as thick-walled oospores, which begin to germinate when they are stimulated by an exogenous source of nutrients such as seed or root exudates. Secondary infections occur from zoospores released from sporangia (Pfender, 1991b). Because of their broad host ranges, Pythium species are almost omnipresent in soils. Not all the Pythium species are plant pathogens; there are some strict saprophytes, parasites and mammalian pathogens, but there are an important number of species which are facultative saprophytes as well as plant pathogens (Martin, 1993). Pythium diseases affect seeds, seedlings and roots of all plants, causing pre-emergence and postemergence damping-off, stem rot, root rot, blight, and stunting (Agrios, 1997; Pfender, 1991b).

Disease Factors

Soil temperature and moisture are typically the most important determinants of disease severity. Infection can occur at all temperatures favorable to bean growth, but disease is more severe at 20-28°C (Pfender, 1991a). In soils where other root rot pathogens exist, there is an optimum temperature for infection at about 16°C and an optimum temperature for symptoms development at 28°C. Therefore, crops planted early may escape Aphanomyces root rot to a greater extent

than those of later plantings because more plant growth occurs before the threshold temperature for symptom development is reached (Papavizas and Ayes, 1974).

Because *Aphanomyces* spp. are water molds, they cause most severe disease in wet seasons and in fields irrigated frequently (Pfender, 1991a). Flooding, which results in low oxygen diffusion rates in soil, is particularly conducive to disease development (O'Brien et al., 1991). There is practically no information on the exact moisture relationships of mycelial growth, oospore germination and zoospore production, and frequency of infection by *Aphanomyces euteiches*. However, root rot of peas is most severe in heavy, poorly drained, and compact soils or soils in which water may be held by impervious subsoil or by sub-irrigation (Papavizas and Ayes, 1974).

In fields with more than one soil type, disease usually appears first in soils with the greater water-holding capacity or that have poor drainage areas. Thus, soils that naturally retain water or have impervious subsoils provide the most favorable conditions for of the disease development (Papavizas and Ayes, 1974).

Disease Control

The most effective control of disease is avoidance. Regular rotation of crops may be helpful in delaying buildup of pathogen population (Pfender, 1991a). When beans are grown without rotation on infested soils, the population of *Aphanomyces* spp. can increase rapidly (Pfender, 1991); thus snap bean yield is significantly reduced in plots where snap bean was grown the year before (Parke and Rand, 1989).

Chemical control is not available at present because none of the available fungicides are effective for *Aphanomyces* control (Parke et al., 1991). Metalaxyl,

which controls Pythium root rot, is not effective against *Aphanomyces* spp (Pfender, 1991a). Moreover, there are no commercial cultivars with resistance to this pathogen (Parke et al., 1991).

Very little has been published concerning the epidemiology and cultural practices of Aphanomyces root rot of bean. However, *A. euteiches* f. sp. *phaseoli* is very similar to *A. euteiches* f. sp. *pisi* (the causal agent of pea root rot) in morphology and life cycle (Pfender, 1991a). For this reason information about this former species will be included.

A. euteiches sp. *pisi* can spread from an infected plant to another plant 18 cm away within a row or to plants in other rows during the croping cycle (Pfender and Hagedorn, 1983). In small field plots with soil infested at various inoculum levels, the disease incidence increases rapidly early in the season at high inoculum level. At lower inoculum levels, the increase in disease incidence was similarly rapid, but occurred later in the season (Pfender and Hagedorn, 1983). The inoculum level can decrease about 50% per year in absence of a pea crop (Pfender and Hagedorn, 1983).

Mineral deficiencies and toxicities can complicate diagnosis of disease symptoms. The type and abundance of nutrients in soil also can influence the severity of disease. Pea root rot may be reduced by applications of NO_3-N and increased by applications of NH_4-N due the influence of N on the composition of root exudates and microbial interactions (Campbell and Neher, 1994).

Because a complex of fungal pathogens causes bean root rot in the field, and control procedures have been difficult to develop, an integrated approach is needed (Kobriger et al., 1998). Cultural methods are required to reduce disease severity and allow greater development of the root system through improved conditions of drainage and aeration. Sowing in raised beds, shallow planting, or greater use of manures may help to achieve an improved soil environment

(O'Brien, 1991). Disease severity can be reduced by early planting and use of short season cultivars on soils with high fertility levels so that plants mature before environmental conditions become favorable for *A. euteiches* (Papavizas and Ayers, 1974).

DISEASE SUPPRESSION: DEFINITION AND MECHANISMS

Soil is a very complex environment that controls microorganisms, pathogens and plants to favor or limit disease development. The ability of a soil to allow the expression of pathogenicity by inoculum in a population of susceptible host plants is called soil receptivity. Soil receptivity can be conducive or suppressive, depending on the disease expression of a certain inoculum density (Rouxel, 1991).

"Conducive soils" are those in which a pathogen can develop and cause severe disease even with a low inoculum density. "Suppressive soils" are those that provide an environment in which disease development is reduced, even when the pathogen is introduced to a susceptible plant (Baker, 1987).

There are several processes that result in disease suppression. Suppresiveness is basically linked to specific physicochemical or biological properties. Soils in which disease cannot develop due to abiotic characteristics of the soil are known as physicochemical suppressive soils. However, the most numerous and typical suppressive soils can be classified as "microbial suppressive soils" (Rouxel, 1991).

Physicochemical Suppression

Physicochemical suppression is an intrinsic property related to the chemistry, mineralogy or physical condition of the soil. Thus, Fusarium wilt of cotton (*Gossypium* spp) is more severe in light sandy soils than in heavier clays which are suppressive (Campbell, 1989).

Combinations of physical factors make possible the diversity of microorganisms in the soil. Thus, soil water and soil aeration selectively affect the activity of microorganisms in at least four ways: a) gas exchange, b) diffusion of solutes, c) motility of organisms, and d) availability of water for growth and metabolism (Cook and Baker, 1983).

Chemical factors also influence the microbial activity in soils. The most classic example is alkaline soils which suppress clubroot of brassicaes caused by *Plasmodiophora brassicae*, where there exists a positive relationship between soils pH levels and disease resistance (Rouxel, 1991). Amending the soil with $CaCO_3$ provides significant control of this disease (Engelhard, 1989). Mineral nutrition also may accentuate disease symptoms. For example, the disease take-all on wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) caused by *Gaeumannomyces graminis* is known to be more serious in phosphorous-deficient plants, and the addition of phosphate fertilizer is effective in reducing the disease (Campbell, 1989). Also, root rot and cortical diseases caused by *Fusarium, Rhizoctonia, Aphanomyces, Cercosporella, Poria* and *Armillaria* species, may be increased by applications of NH_4-N and reduced by applications of NO_3-N. Conversely diseases caused by species of *Ophiobolus, Diplodia, Phythium* and *Streptomyces* respond in an opposite manner (Huber and Watson, 1974). In addition, N may influence microbial interactions. For example, an increase of bacteria and actinomycetes antagonistic to *Aphanomyces euteiches* has been correlated with suppression of pea

root rot in soil amended with NO_3-N but not with NH_4-N (Campbell and Neher, 1994).

Biological Suppression

Research in disease suppressiveness suggests that the principal soil component involved is biological, because steam sterilization or fumigation of the soil renders the soil conducive to disease if the pathogen is reintroduced (Malajczuk, 1983; Campbell, 1989). Biological soil suppression occurs in the presence of the pathogen and the susceptible host. The pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil (Cook and Baker, 1983).

Two types of microbial disease suppression, general and specific, have been described. General suppression is directly related to the total microbial biomass and activity at a time critical to the pathogen, during propagule germination and penetration and growth in the host rhizosphere, rather than to a single microorganism or specific group of microorganisms (Cook and Baker, 1983). In this mechanism, many diverse kinds of microorganisms function as biocontrol agents; the species of active soil microorganisms are less important than the total active microbial biomass which competes with the pathogen for C and energy in some cases and for N in other cases (Cook and Baker, 1983). This has been demostrated for diseases caused by *Pythium* spp. and *Phytophthora* spp. (Nelson, 1987; Hoitink et al., 1991).

Specific suppression usually involves a small number of microbial antagonists to the disease (Campbell, 1989) or an individual microorganism that

specifically and qualitatively affects a given pathogen during a stage in its life cycle (Cook and Baker, 1983; Chung and Hoitink, 1990). The specificity of antagonistic microbial groups or a single species are more closely related to physiological features of the pathogen than to their taxonomic position. For example, *Rizoctonia solani* produce large nutrient-independent propagules know as sclerotia and a narrow group of microorganisms is responsible for suppressing or eradicating the pathogen (Chung and Hoitink, 1990).

Suppression may vary according to the antagonists involved and there are many mechanisms in which an antagonistic organism can operate which include direct parasitism or predation, competition, antibiosis (Cohen el at., 1998; Chernin and Chet, 2002), and systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Zhang et al., 1996). In the case of organic matter-mediated suppression, those mechanisms are supposed within the context of microbial communities and their response to soil and plant-introduced energy reserves (Hoitink and Boehm, 1999).

Predation and Parasitism

Predation and parasitism occurs when the biological control agent feeds directly on or inside the pathogen. When one fungus feeds on another fungus, it is called mycoparasitism. This process results in the direct destruction of pathogen propagules or structures (Chernin and Chet, 2002). Organic amendments have contributed to increased hyphal lysis and formation of abortive sporangia of *Phytophthora* spp (Malajczuk, 1983). Pathogen propagules may be destroyed after incubation in suppressive organic substrates. Apparently mycolysis would seem to be most destructive to organisms that do not produce resistant structures

(Lockwood, 1960) vegetative hyphae of many fungal species have been found to be susceptible (Chernin and Chet, 2002). Mycelia of *Aphanomyces euteiches* have been observed to be rapidly lysed in field soils (Lockwood, 1960).

Mycoparasitism is based mainly on the activity of lytic exoenzymes responsible for partial degradation of the host cell wall. Briefly, this process involves "recognition" of the host, positive chemotropic growth, attachment, and synthesis of a cell-wall-degrading enzymes that allow the parasite to penetrate the host and complete its destruction (Chernin and Chet, 2002). Mycoparasitism has been divided into necrotrophic and biotrophic. Necrotrophs kill their host, sometimes without infecting it. They then utilize the nutrients released from the dead hyphae (Cook and Baker, 1983). Biotrophs are favored by a living host structure, they obtain nutrients while causing little harm to their fungal host, at least during the early stages of mycoparasitism (Cook and Baker, 1983; Chernin and Chet, 2002).

The most promising and studied fungal biocontrol agents are the fungus *Gliocladium virens* (=*Trichoderma virens*) and *Trichoderma* spp. Many *Gliocadium* and *Trichoderma* spp. isolated from natural habitats have been used in biocontrol against several soil-borne plant pathogenic fungi. They kill the host by direct hyphal contact, causing the affected cells to collapse or disintegrate (Chernin and Chet, 2002). The hyphae of *Trichoderma* spp may penetrate growing hyphae as well as resting structures such as sclerotia (Campbell, 1989). *Trichoderma* spp. have been involved in the stimulation of oospore formation, lysis of hyphae and chlamidospore production in *P. cinnamomi*.

Many different soil microorganisms, including bacteria, phycomycetes, chytridiomycetes, hyphomycetes and actinomycetes have been observed parasitizing oospores of *Phytophthora megasperma* var *sojae*, *P. cactorum*, *Pythium* sp, and *Aphanomyces euteiches*, with 60-80% parasitism not uncommon in

soils (Cook and Baker, 1983). Protozoa and fungal seeding mites have been observed ingesting and lysing zoospores (Malajczuc, 1983).

Competition

Competition is an interaction between organisms, both of which need a limited resource such as nutrient or space, so that the preferential use of it by one organism harms the other or reduces its growth rate (Campbell, 1989). Antagonists more efficiently able to use essential resources compete effectively with the pathogen for an ecological niche, leaving the pathogen less able to harm the plant. Many plant pathogens require exogenous nutrients to germinate and colonize the host. For example, oospores and sporangia require exogenous amino acids, carbohydrates to stimulate germination (Nelson, 1987). Therefore, competition for nutritional factors, mainly carbon, nitrogen and iron, may result in the biological control of plant pathogens (Chenin and Chet, 2002). Moreover, under certain conditions, soil nutrient limitation may cause fungistasis, which is the imposition of fungal spore dormancy by nutrient limitation (Campbell, 1989; Benson, 1994).

Competition for limiting nutrients in soil and rhizosphere is particularly important. Soils with very high levels of microbial biomass and activity are suppressive to Pythium diseases. In compost-amended soilless potting mixes, the active microbial community scavenges the root or seed exudates and eliminates the germination stimulant (Chen et al., 1988). Addition of sucrose, asparagines, or seed exudates to compost-amended suppressive potting mixes has been demonstrated to reduce the level of suppressiveness in a dose-dependent, linear relationship (Chen et al., 1988). In addition, compost harvested from the center, high temperature region of a hardwood bark compost pile was conducive and of

lower microbial activity and biomass and higher reducing sugars than the suppressive, lower temperature outer region of the same pile. However, within days, the conducive material (incubated at room temperature), becomes suppressive; during the same period, the microbial activity increases and the reducing sugar contents decline a levels comparable to those in the suppressive, outer region compost (Chen et al., 1988).

Biocontrol agents require sources of organic matter, and its quality and quantity are critical to efficacy and survival of those agents (Hoitink and Boehm, 1999).

Addition of organic amendments to soil may not immediately suppress diseases. Control does not occur in fresh, undecomposed organic matter (for example immature or inadequately stabilized composts). This type of organic substrate may enhance the growth of pathogens with highly competitive saprophytic ability. Fresh organic matter supports high microbial activity (serving as a food base for biological control agents) but also supports the growth and potential infection of saprophytic plant pathogenic fungi such as *Pythium* spp (Asirifi et al., 1994; Hoitink and Boehm, 1999). For example, *R. solani* causes disease in fresh compost, but, in mature compost (with low concentrations of free nutrients), sclerotia are killed by *Trichoderma* and biological control is generated (Chung and Hoitink, 1990; Hoitink et al., 1997). *Pythium* spp. are generally considered good colonizers of fresh organic residues, but they are not good competitors. Fresh plant residues incorporated into soil generally initially increase *Pythium* spp. populations and disease severity. However, suppression is typically generated after several weeks of decomposition (Grunwald et al., 2000). There are differences in the degree of saprophytism between *Pythium* species. *Pythium ultimum* propagules have been reported to germinate, grow saprophytically on organic matter, and produce new sporangia within 44 h of organic matter

incorporation, then populations decline slowly thereafter (Hancock, 1981). *Aphanomyces euteiches* is considered a poor saprophyte (Papavizas et al., 1974). Saprophytic hypal growth has been observed in sterilized soil columns, but not in natural field soils (Sherwoood and Hagedorn, 1962). Competitive saprophytic ability may not have an important role in the survival of *A. euteiches* in soil (Sherwoood and Hagedorn, 1962). For *Phytophthora* spp. there are differences in the saprophytic abilities. *P. cinamomi* and *P. cactorum* can survive as either parasites or saprophytes, depending on environmental conditions. *P. infestans* and *P. megasperma* are considered biotrophs with very little saprophytic potential (Weste,1983).

Adequately stabilized composts seem to have a major impact on disease suppression, generated when the organic matter is fully colonized by soil microorganisms and the pathogen can no longer effectively compete for resources (Hoitink and Boehm, 1999).

Excessively stabilized organic matter does not support adequate activity of biocontrol agents. When the degradable organic matter becomes limiting, biocontrol begins to fail and soil-borne diseases can be severe (Hoitink and Boehm, 1999). Decomposed Canadian sphagnum peats (dark) were consistently conducive to root rot of poinsettia (*Euphorbia pulcherrima*) caused by *Pythium ultimum* while light-colored Canadian sphagnum peats varied in suppressiveness (Boehm and Hoitink, 1992). Suppression of damping-off of cucumber (causal agent *Pythium ultimum*) in a compost-amended sand was sustained for more than one year by the degradation of the less decomposed coarse and mid-size particulate organic matter fractions of the compost (Stone et al., 2001). In this case, severe damping-off was observed in the peat mix at all times after potting, confirming the conducive nature of this particular peat. The disease severity in pure sand not amended with peat or compost also was very high.

A gradual increase in the incidence of some diseases may coincide with a gradual depletion of soil organic matter (Asirifi, 1994). Suppressiveness can be sustained for several months to years after organic amendment. Municipal solid waste compost supported suppression up to six month of Phytopthora root rot of citrus (causal agent *Phytopthora nicotiana*) in a sandy field soil (Widmer et al., 1998). Paper-mill residual amended for two years to a loamy sand one month before planting suppressed common root rot of snap bean (causal agent *Aphanomyces euteiches* and *Pythium* spp.) for up to fifteen months after amendment (Stone et al., submited).

Antibiosis

Antibiosis is the inhibition or destruction of one organism by a metabolic product of the antagonist (Cook and Baker, 1983; Chernin and Chet, 2002). These compounds are toxic or inhibitory to the pathogen, resulting in destruction of its propagules or suppression of its activity. Antibiosis plays a key role in biological control of crown gall, caused by *Agrobacterium radiobacter* var. *tumefasciens*. Biological control is generated by a nonpathogenic *A. radiobacter* strain 84, which has a plasmid that codes for production of a highly specific nucleotide bacteriocin called agrocin 84 that selectively inhibits most pathogenic agrobacteria (Fravel and Engelkes, 1994).

Aphanomyces species are very sensitive to some antibiotics (Sherwood and Hagedorn, 1962). When certain bacteria were applied to pea seeds, disease severity was reduced and plant emergence and pea yield were increased. Compared with the control (Captan) *Pseudomonas cepacia* was the most effective, *P. fluorescens*, the second and *Corynebacterium* sp the third (Parke et al., 1991).

Systemic Acquired Resistance

Plants possess various inducible defense mechanisms to protect themselves against pathogen attack. Classic examples are induced systemic resistance (ISR) or systemic acquired resistance (SAR), which is the ability of a plant to exclude or overcome, completely or to some degree, the effect of a pathogen or other damaging factor (Agrios, 1997). Induced systemic resistance involves the activation in the host plant of chemical and physical self defense responses against a pathogen, resulting in a partial or complete resistance to a variety of diseases (Chernin and Chet, 2002). Induced resistance to Pythium root rot of cucumber has been reported in compost-amended soilless potting mixes (Zhang et al., 1996).

Multiple Mechanisms

An antagonist may use more than one form of antagonism, and the action of some antagonists may fit under more than one mechanism. The most effective biological control agents use two or thee different mechanisms (Chernin and Chet, 2002). For example, some *Gliocladium spp.* cause death and dissolution of their host hyphae by secreting one or more antibiotics; these antagonists, coiled around the host hyphae, are then able to grow on the dead cell contents. The antagonism begins with a form of antibiosis but is usually classified as necrotrophic parasitism (Cook and Baker, 1983). *Pseudomonas* species produce a siderophore with a very high affinity for iron; the host plant and the pathogen also produce siderophores. There is, therefore, intense competition for iron in the rhizosphere and if the pathogen does not get enough, it neither germinates nor grows and the disease decreases. Thus, *Pseudomonas* can control take-all by both methods antibiotic production and by the use of siderophores (Campbell, 1989).

STRATEGIES TO INDUCE SUPPRESSION

To be successful in management of soil-borne diseases the plant, pathogen, biocontrol agent, environment conditions and interactions among them must be considered. Better knowledge will produce better results, maximum benefits to crop production, with and minimum expenditure of resources (Fravel and Engelkes, 1994).

Introduced Antagonists

Specific suppression is transferable to a conducive soil (Baker, 1987; Campbell, 1989) because the microorganisms are transferred as well. Adding suppressive soil to a conducive soil can reduce disease severity by introducing microorganisms antagonistic to the pathogen. A conducive Colorado soil inoculated with a soil from Colombia was made suppressive to *Rhizoctonia solani*. In this case, the suppressive Colombia soil contained high populations of *Trichoderma hamatum*, an antagonist to the pathogen (Cook and Baker, 1983; Baker, 1987). Soil transfers are more effective if the addition is made to sterile or steamed soils so that the microorganism are able to colonize rapidly without competing with a resident population (Campbell, 1989).

If the microorganism can be isolated from the suppressive soil, it can be equally effective and cheaper to transfer just the isolate or even, in some cases, their metabolic products like antibiotics (Campbell, 1989). At present, there are many biocontrol organisms commercially available, including Trichoderma 2000 Bio-Fungus, Binab T, RootShield, Supresivit (*Trichoderma harzianum*) and Galltrol-A, Nogall, Norbac 84c(*Agrobacterium radiobacter*). To be successful in

the use of those products, information is needed on environmental effects on biocontrol agents, interaction among microbes, microbes and plants, and how the environment modifies these interactions (Fravel and Engelkes, 1994).

Cropping Systems Management to Generate Suppressiveness

The more that is known about why some soils are suppressive, the more successfully farmers will be able to manage farming systems (Cook and Baker, 1983).

Four traditional cultural practices have been used to enhance biological control of soil-borne plant pathogens in the field: tillage, crop rotations, green manures, and the addition of organic amendments.

Tillage can be important in generating biological control. Tillage increases the population and activities of resident antagonists in crop residues through the exposure of new sites for colonization on the residue and through the intensification of microbial activity that accompanies disturbance (Cook, 1990). Reduced, minimum or even no tillage creates a surface build-up of crop residues that are not plowed into the soil. In dry conditions, the pathogens from the last crop may survive in the residue and increase disease, but in wetter conditions decomposition and pathogen control may take place on the surface of the soil (Campbell, 1989). Tillage associated with crop rotation further contributes to biological destruction of inoculum by fragmenting and accelerating the breakdown process of crop residues infected with pathogens (Agrios, 1997). There may, however, be confusion with the crop residue interpretation since that residue also acts as organic amendment. In general, crop residues have a negative effect on

disease suppression in the short term, but as the residue decomposes, the pathogen may be suppressed in the long term.

Crop rotation is an accepted method of disease suppression to prevent yield decline in many crops. Rotation allows time for resident antagonists to sanitize the soil or for propagules of specialized pathogens to die (Cook, 1990). If similar crops which may host the same pathogens do not follow one another, then there is a good chance that any inoculum left in the soil will die from starvation in the absence of its host or will be parasitized and lysed by other microorganisms (Campbell, 1989). Sugar beet (*Beta vulgaris* L. *spp vulgaris*), corn (*Zea maiz*), or spring wheat in rotation decreased basal rot of onion (*Allium cepa*) caused by *Fusarium oxysporium* f. sp. *cepae* in Japan, and isolates of the pathogen from onion in rotation were less virulent than the isolates from onion in continuous culture (Sumner, 1994). In eastern Washington, *Cephalosporium gramineum*, which causes stripe of winter wheat, can be controlled by a three-year rotation (potatoes, snap bean and cucumber). Because of the potentially greater economic return from winter wheat, growers repeatedly risk sowing it after a one year break; losses of 50-75% were common in many fields when winter condition favored the disease (Cook and Baker, 1983).

In some diseases, monoculture in a conducive soil, after some years of severe disease, eventually leads to reduction in disease through increased populations of microorganisms antagonistic to the pathogen. This monoculture provides the time required for biological destruction of pathogen inoculum by resident antagonists in the soil. Take-all disease of wheat caused by the soil-borne fungus *Gaeumannomyces graminis* var. *tritici* becomes less severe and yields usually increase with the fifth or sixth successive wheat crop (Christensen and Hart, 1993). This sort of decline is known for several diseases, e.g. potato (*Solanum tuberosum*) scab (causal agent *Streptomyces scabies*), Rhizoctonia root rot on

radish (*Raphanus sativus*), damping off and Fusarium wilt on watermelon (*Cituillus lanatus*). Suppressiveness in monoculture is thought to be due to an increase in antagonistic soil flora in the continued presence of the pathogen (Campbell, 1989).

There are many reports on the favorable effects of organic amendments and green manures on disease suppression; for this reason, this topic will be discussed in a separate section.

ORGANIC SOIL AMENDMENTS

A variety of organic amendments may be applied to soils to improve soil quality. Crops can be grown with the explicit purpose to incorporate green biomass to soils or this may be done to recycle materials such as animal and human manures and industrial, or yard wastes.

Addition of organic matter to the soil has several beneficial effects: a) improvements in plant nutrient status, b) improvements in soil structure, c) stimulation of antagonists (Campbell, 1989), and in some cases, d) suppression of pathogen life cycles (Papavizas and Lewis, 1971).

Amendments have been widely used in agriculture because they are known to improve plant growth by providing nutrients and improving soil physical characteristics. Prior to incorporation, organic materials may be composted. This is done to stabilize the organic matter into more humic type fractions, reduce microbial activity, reduce the C to N ratio, reduce the phytotoxicity, and reduce the biomass of the organic waste (to lower transportation costs of recycling these materials in agriculture and forest ecosystems) (Zibilske, 1999).

More recently, there is growing evidence that organic amendments added to the soil can induce disease suppression by stimulating resident antagonists (Cook,

1990). The addition of readily available C to the soil (as a green manure, compost or natural litter) stimulates microbial activity and may cause intense competition to develop, leading to C limitation and fungistasis (the imposition of dormancy, especially for fungal spores, by nutrient limitation). When available C is added above the needs of the saprophitic competitors, germination of certain pathogens as *Pythium* spp may be stimulated as fungistasis is broken (Campbell, 1989).

The evidence for these effects has been noticed and researched in several conditions and crops. Nursery container media with peat as the sole source of organic matter was conducive to damping-off; however, media amended with various types of manure compost suppressed the disease (Inbar et al., 1991). Mixes containing more than 20% (v/v) composted pine bark supported a significant level of suppression to Pythium damping-off (Hoitink et al., 1991). However, for nursery crops, compost must be of consistent quality and maturity to be successfully used as a disease suppression agent (Hoitink et al., 1991). Composts applied as mulches to the soil surface readily control Pythium and Phytophthora root rots (Hoitink and Boehm, 1999).

Green organic matter incorporated in the planting trench of potato increases general microbial activity, which antagonizes *Streptomyces scabies*; and suppresses potato scab (Campbell, 1989). Several cruciferous plant amendments as well as rhubarb (*Rheum rhabarbarum*), swiss chard (*Beta vulgaris* var. *cicla*) and soybean (*Glycine max*) tissue reduced damping-off of sugarbeets (*Beta vulgaris* L. *spp. vulgaris*) caused by a fungus complex (*Aphanomyces cochlioides* Drench, *Rhizoctonia solani* Kuehn, *Phoma betae* Frank, and *Pythium* spp) (Lewis and Papavizas, 1971).

Cruciferous amendments also reduced root rot in peas (Papavizas, 1966; Papavizas and Lewis, 1971; Chan and Close, 1987; Parke and Rand, 1989; Muehlchen et al., 1990; Smolinska et al., 1997). Disease suppression with

cruciferous amendments is believed to result from the effects of volatile toxic substances produced as a result of decomposition of cruciferous amendments. Those substances may accumulate in soil to such an extent that one or several vital phases in the cycle of life of the pathogen may be suppressed or arrested before penetration of the host (Papavizas, 1966). Glucosinolates are found in Brassicaceae with higher concentrations in the seeds. Glucosinolates are degraded enzymatically to yield a variety of important compounds including isothiocyanates, nitriles, thiocyanates, oxazolidinethione and epithionithiles (Larsen, 1981). Although it has not been specifically demonstrated that glucosinolate degradation products are responsible for toxic effects toward the pathogen, the evidence suggests a relationship between volatile glucosinolates hydrolysis products and suppression of *Aphanomyces euteiches* (Smolinska et al., 1997). Papavizas and Ayers (1974) reported that a rye (*Secale cereale*) green manure reduced pea root rot (causal agent *Aphanomyces euteiches* Drenchs) whereas barnyard manure was ineffective. Oats (*Avena sativa*), corn, soybean and rye plant materials added to soils reduced *Aphanomyces* root rot of peas; the oat amendment was consistently the most effective (Davey and Papavizas, 1961).

Amendment Types

Generally, a wide range of materials can suppress diseases. The incidence of lettuce (*Lactuca sativa*) soft rot caused by *Sclerotinia sclerotiorum* was best reduced by most organic amendments. Stable manure, fowl manure, lucerne hay, cow manure, and mushroom compost and significantly reduced this disease compared to the control (Asirifi et al., 1994). Compost potting mixes have been shown to effectively suppress some soil-borne pathogens (*Phytophthora spp.*,

Pythium spp., Rhizoctonia solani, and *Fusarium* spp.) (Hoitink et al., 1991). Composted pine bark suppressed *Phytophthora spp.* and *Pythium* spp., but *Rhizoctonia spp.* was not consistently suppressed (Kwok et al., 1987; Hoitink et al., 1991). Dissanayake and Hoy (1999) evaluated the effects on sugarcane root rot of compost prepared from cotton gin trash, cotton wood bark, mixed hardwood bark, municipal solid waste, municipal yard waste, municipal biosolids, a sugar mill by-product and filterpress cake, incorporated into natural soil and steam-treated field soil in greenhouse experiments. Non-sterile cotton gin trash compost, filterpress cake, and biosolids suppressed disease and increased plant growth in field soils, but this ability was reduced after steam treatment. Bark composts suppressed root rot and increasing plant growth in either non-sterile or steamed field soils. Lumsdem et al. (1983) showed that addition of 10% of composted swage sludge to the soil suppressed Aphanomyces root rot of peas; Rhizoctonia root rot of bean, cotton, and radish; Sclerotinia drop of lettuce; Fusarium wilt of cucumber; and Phytophthora crown rot of pepper. Pythium damping-off of pea and bean, Fusarium root rot of pea and Thielaviopsis root rot of bean and cotton were not affected or were increased in severity. Nevertheless, all compost increased in effectiveness with increased time after incorporation into soils, suggesting that time allows the development of antagonists and removal of material from amended soil, reducing pathogen aggressiveness

Paper-mill residuals are industrial byproduct produced in large amounts. Spectroscopic studies showed that this material consisted of cellulose type I, which is present in ordinary pulp, with a minor uronic acid and lignin contribution (Jackson and Line, 1997). Long-term paper-mill residuals applications can be managed to effect positive changes in soil physical properties such as aggregation and moisture- holding properties that are correlated with soil quality (Zibilske et al., 2000).

Paper-mill residual has been used on sandy, vegetable producing soils in Wisconsin. A six-year study was initiated in 1998 to investigate the impact of organic amendment quality and quantity on disease incidence (Stone et al., 2001). Fresh and composted paper-mill residuals were applied at two rates each year. The crops were potatoes, snap beans and cucumbers (*Cucumis sativus*) in 1998, 1999, and 2000, respectively. Both the fresh and the composted amendments strongly suppressed common root rot of snap beans (causal agents *Aphanomyces euticheS* and *Pythium ultimun*). In contrast, only the composted paper-mill residual suppressed foliar bacterial diseases, brown spots of snap beans(causal agent *Pseudomonas syringae* pv *syringae*) and angular leaf spots of cucumbers (causal agent *Pseudomonas syringae* pv *lachrymans*). The same trends were observed on these soils in short-term root rot and foliar disease plant bioassays conducted in growth chambers (Stone et al., 2001).

Chemical Properties of Amendments

There is some evidence that chemical properties of amendments are important in conferring disease suppression. Levels of inorganic N associated with organic amendment may affect disease suppression potential. High NH_4-N and low NO_3-N levels in composted municipal sewage sludge have been shown to increase *Fusarium* wilt (Hoitink et al., 1997). Total N in tomato tissue and NO_3-N concentrations in soil were positively correlated with corky root severity (Workneh et al., 1993).

Compost salinity enhances diseases caused by *Pythium* and *Phytophthora* species. High salinity can be ameliorated by applying compost a month ahead of planting to allow leaching to remove salts from soils (Hoitink et al., 1997).

Accumulation of sodium in soils produces dispersion and disintegration of soil structure (Cook and Baker, 1983) reducing the habitats for microbial antagonists.

SOIL QUALITY AND DISEASE SUPPRESSION INDICATORS

Disease suppression is a complex phenomenon. It can be viewed as one manifestation of ecosystem stability and health. However, suppression may vary depending upon the disease organism involved, the antagonists, and the environmental conditions. Thus, indicators for soil health could function as indicators for disease suppressiveness (van Bruggen and Semenov, 2000). Identifying soil conditions related to disease suppression may help to develop soil management practices that induce and maintain disease suppression.

Soil organic amendments have a variety of effects on soils, all of which could influence disease suppression. There is considerable experimental evidence that microbial activity level is an indicator of the potential of an organic material or amended soil to suppress soil-borne diseases (Lumsden et al., 1983; Nelson et al, 1983; Boehm and Hoitink, 1992; Stone,1997; Dissanayake and Hoy, 1999). There is little information on the temporal dynamics of microbial activity and biomass during decomposition of organic amendments relative to disease suppression (Hoitink et al., 1991). A first step would be to characterize disease suppression in terms of soil structure, biological activity and microbial biomass.

Biological soil properties have been found to be superior to some chemical properties in detecting effects on soil management. In particular, microbial biomass-C and enzyme activities can be sensitive soil quality indicators because they reflect long-term soil management with organic matter inputs and cover cropping (Bandick and Dick, 1999). Enzyme activities can reflect recent changes

(2 years) in management, such as cover cropping, and have less seasonal variability than mineralizable N and CO_2 respiration (Ndiaye et al., 2000).

Enzyme assays and microbial biomass-C are attractive as biological indicators because they are relatively simple to run. Furthermore, enzyme assays can be conducted on air-dried soil samples by providing the same ranking of treatments (Bandick and Dick, 1999). This could facilitate the adoption of these assays for practical or commercial applications as soil test assessments of soil quality and for disease suppression.

Soil enzyme activities could be useful as soil quality indicators because they can be sensitive to organic C inputs into soil (Bandick and Dick, 1999). Enzyme reactions are involved in a wide range of soil functions. Consequently, soil enzyme activities in soil may be used to assess microbial functional diversity, biochemical processes, microbial ecology and to provide indicators of soil quality (Nannipieri et al., 2002). Enzymes exist in soil in the biotic form associated with viable microorganisms or soil fauna and in forms not associated with living cells (abiontic). Abiontic enzymes can remain catalytic as excreted enzymes, within dead cells, or complexed with organic and mineral colloids. (Dick, 1994; Dick et al., 1996).

β-glucosidase Activity

β-glucosidase has been suggested as an assay that reflects soil management effects (Bandick and Dick, 1999) and has microbial ecological significance because of its role catalyzing the releases of low-molecular-weight sugars which provide an important energy source for microorganisms in soil (Tabatabai, 1994). β-glucosidase activity had the least variability within treatment and across

experimental sites in studies comparing winter fallow and soil amendment winter cover crop over three years (Ndiaye, et al., 2000).

Arylsulfatase Activity

Arylsulfatase is important in nutrient cycling because it hydrolyzes ester sulfates and releases plant-available sulfate. Since microbial ester sulfates are found mostly in fungi (Saggar et al., 1981) they may be indirect indicators of fungal biomass. Evidence for this is that arylsulfatase has been shown to be highly correlated with ergosterol (biochemical compound of fungal biomass) and direct counts (microscopy) of fungi (R. P. Dick[1], *personal communication*). Arylsulfatase has been sensitive to disturbance by reflecting conservation practices (0-8 cm) in the no-till fields where as total C was unaffected by tillage (Bergstrom et al., 1998).

Fluorescein Diacetate Hydrolysis

Fluorescein diacetate hydrolysis (FDA) has been widely accepted as an accurate and simple method for measuring total microbial activity (Adam, G. and Duncan, H. 2001). FDA provides a broad-spectrum indicator of the biological status of soils. It can be hydrolyzed by many enzymes such as proteases, lipases and esterases and its hydrolysis has been found among a wide array of the primary decomposers, bacteria and fungi. (Dick et al., 1996). Some studies demostrated FDA activity to be related to suppressiveness of root pathogens including *Pythium ultimum* (Inbar et al., 1991; Boehm and Hoitink, 1992; Boehm et al., 1997),

Pythium arrhenomanes (Dissanayake and Hoy, 1999) and *Pyrenochaeta lycopersici* (Workneh et al., 1993). Nevertheless, FDA is not always closely correlated with disease suppression, because it may not account for the quality and stage of organic matter decomposition which are important factors in disease suppression (van Bruggen and Grunwald, 1996; van Bruggen and Semenov, 2000). The product of FDA hydrolysis, fluorescein may be absorbed by soil organic matter (Inbar et al., 1991) and its low activity in sandy and clayey soils (Adam and Duncan, 2001) has limited its potential for practical applications as an indicator of disease-suppressive soils.

Microbial Biomass-C and Microbial Respiration

Microbial biomass-C is an indicator of the amount of C in the viable population of soil. It reflects the mass of the microbial pool but indicates nothing about the community composition. After addition of organic amendments, changes in total soil microbial biomass often occur quickly (Goyal et al., 1993). There are a diverse number of methods available for its determination. A frequently used method is the soil fumigation-incubation method which involves fumigating the soil with chloroform and measuring the amount of C released upon subsequent incubation (Jenkinson and Powlson, 1976). Microbial respiration is obtained from measurement of CO_2 released during the incubation period from non-fumigated samples.

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Chen et al. (1988) have shown that disease severity caused by *Pythium ultimum* was negatively correlated with microbial biomass-C for container media suppressive to cucumber damping-off.

Total Carbon

Direct measurement of total C in soils quantifies inorganic and organic forms of C. In humid regions, nearly all C is in organic form (Nelson and Sommers, 1996). Organic C makes up approximately 58% of soil organic matter by weight, so total C provides an indicator of organic matter in soils (Sikora and Stott, 1996). Soil organic matter is related to soil quality because it is a source of plant nutrients, increases soil aggregate stability, reduces erosion, increases gas exchange and water infiltration, promotes water-holding capacity and decreases potential for soils to become saturated (Sikora and Stott, 1996). Furthermore, highly significant correlations between soil organic C and microbial biomass-C have been found in an experiment with different levels or farmyard manure, suggesting that soil organic C was an important factor in the development of soil microbial biomass (Goyal et al., 1993).

Water Stable Aggregation

Aggregate stability is the resistance of soil aggregates to break down by water and mechanical manipulation. Aggregation has been suggested as a central and integrative characteristic of soil quality (Taylor and Dick, unpublished). This is because it affects many other soil properties such as aeration, water-holding

capacity, infiltration, drainage, bulk density and resistance to erosion (Gale et al., 2000), influencing the soil ecosystem function. Increased aggregation increases soil porosity and improves the movement of water and gases through soils; this is beneficial for resident microbial populations, plant root penetration, and nutrient uptake (Taylor and Dick, unpublished) and it may affect the growth, health and degree of susceptibility of roots to plant diseases.

Soil organic amendments improve biological growth and activity, which cause formation of aggregates. Soil with improved structure can provide a better habitat for a larger and more diverse microbial population directly related to general disease suppression mechanisms and many important direct and indirect effects on root disease epidemiology (MacDonald, 1994).

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CHAPTER 3

ORGANIC SOIL AMENDMENTS: IMPACTS ON SNAP BEAN COMMON ROOT ROT AND SOIL QUALITY

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ABSTRACT

Common root rot (causal agent *Aphanomyes euteiches*) is a major disease of commercially grown snap bean (*Phaseolus vulgaris* L.) on the irrigated sandy soils of central Wisconsin. The primary objective of this study was to determine the effect of amendment with paper-mill residuals on common root rot of snap bean. The secondary objective was to determine relationships between soil properties and root rot severity. This study was conducted in a field trial on a Plainfield sandy loam in Hancock, WI that had annual soil amendments from 1998 to 2001 with three rates of fresh paper-mill residuals (0, 22 or 33 dry Mg ha^{-1}) or composted paper-mill residuals (0, 38 or 78 dry Mg ha^{-1}). Soil was removed from each treatment in April 2001 (one year after last amendment) and on September 2001 (four months after amendment) and brought to laboratory. Soils were incubated moist at room temperature and periodically bioassayed in a bean seedling test (9, 44, 84, 106, 137, 225 or 270 days after removal from the field) for snap bean root rot. Soils were sampled on the same day as the root rot bioassay and assayed for β-glucosidase, arylsulfatase and fluorescein diacetate activities (FDA); microbial biomass-C (MBC) (by chloroform fumigation); water stable aggregation, and total C. There were large differences in snap bean root rot incidence between field amendment treatments. The disease was suppressed by both fresh and composted paper-mill residuals, but compost was the most suppressive with high rates supporting healthy plants with the lowest incidence (<40%) of disease. The unamended field soil maintained high levels of disease incidence throughout of the experiment while disease tended to decrease over time in amended soils. Root rot severity was strongly negatively related with total C and arylsulfatase. β-glucosidase activity was negatively correlated with disease severity while soil

microbial activity was high early in the incubation; but this high correlation was lost after 106 days incubation.

INTRODUCTION

Common root rot is a major disease of commercially grown snap bean on the irrigated sandy soils of central Wisconsin (Pfender and Hagedorn, 1982; 1989; Kobriger et al., 1998). Combined infections by *Aphanomyces euteiches* and *Pythium ultimum* are associated with the disease.

Disease suppression of soil-borne diseases has been explored for decades (Davey and Papavizas, 1961; Baker, 1987; Chen et al., 1988; Campbell, 1989; Boehm and Hoitink, 1992). The ability of amendments to suppress diseases has been demonstrated, but little is known about how soil properties may related to disease suppression. This is important in developing practical management systems and indicators of quick inducement of disease suppression.

The mechanisms involved in disease suppression are varied and complex and may differ depending upon the disease organism involved. There is evidence that organic amendments added to the soil can induce disease suppression by stimulating resident antagonists (Cook, 1990). The addition of readily available C to the soil, as a green manure, compost, or natural litter, stimulates microbial activity, causes intense competition, and may produce C limitation and fungistasis. Conversely, when C is added above the needs of the saprotrophic competitors, germination of pathogens may be stimulated and fungistasis broken (Campbell, 1989). Kundu and Nandi (1985) found that when the C:N ratio of soil increased, fungal populations decreased, but populations of bacteria increased. This is closely related to the decomposition level of organic matter in amendments. Compost from

tree bark, will suppress diseases if it is colonized by an appropriate microflora (Hoitink et al., 1997).

Pathogen propagules may be destroyed after incubation in suppressive organic substrates. Predation and parasitism occur when the biological control agent feeds directly on or inside the pathogen resulting in a direct destruction of pathogen propagules or structures (Chernin and Chet, 2000).

Disease suppression mechanisms can be viewed as a manifestation of ecosystem stability and health. Thus, indicators of soil health may be useful as indicators for disease suppression (van Bruggen and Semenov, 2000). Identifying soil conditions that are conducive for disease suppression may provide soil indicators for identifying disease-suppressive soils and guiding soil management to induce and maintain disease suppression. Microbial activity can be an indicator of the potential of an organic material or amended soil to suppress soil-borne diseases (Lumsden et al., 1983; Boehm and Hoitink, 1992; Stone, 1997; Dissanayake and Hoy, 1999).

Microbial biomass-C and certain enzyme activities have been found to be sensitive in reflecting soil quality because they reflect long-term soil management with organic matter inputs (Bandick and Dick, 1999), they are relatively simple to run, and can have less seasonal variability than other soil properties. Because organic residue-decomposing organisms are a major portion of the soil community and many of these have been shown to involve in disease suppression, hydrolytic enzymes hold potential as indicators of soil quality (Dick et al., 1996). A disease suppression depends on the specific material used as amendment and its chemical composition (van Bruggen and Semenov, 2000). Amendments can increase microbial activity and microbial competition by providing C compounds for energy. Consequently, enzymes involved in the C cycle and microbial biomass-C may be useful as indicators of disease-suppressive soils. For example, parasitism

might be expected to be related to enzymes involved in degradation of the host cell wall. Total C is related to total soil organic matter. Soil organic matter content is considered central to soil quality, because it affects microbial activity, aggregation, infiltration, water-holding capacity and bulk density. Likewise, water stable aggregation has been considered as another possible indicator of soil suppressiveness.

Paper-mill residuals are industrial byproducts produced in large amounts, which could be used as amendments. For example, Stora Enso Co. (Wisconsin Rapids, WI.) produces approximately 46,000 wet tons annually. While a small percentage of the paper-mill residual produced is spread on cropland, most is buried in landfills. Paper-mill residuals amendment can increase water and nutrient retention and improve the soil's ability to suppress crop diseases (Cooperband L., 2001; Stone et al., 2001).

The objective of this study was to determine the effect of paper-mill residuals as an organic amendment on the suppression of common root rot of snap bean (causal agent *Aphanomyces euteiches*) and the relationships between soil properties and suppressiveness in amended sandy soil decomposing over time.

MATERIALS AND METHODS

Field Trial and Soil Sampling

The field trial was initiated in 1998 at the University of Wisconsin Agricultural Experiment Station in Hancock, Wisconsin. The soil type is a Plainfield loamy sand (sandy, mixed, mesic, Typic Udipsamment; U.S. Soil

Taxonomy) with 87%, 5% and 8% of sand, silt and clay respectively (general soil characteristics are shown in Table 3.1). This field was naturally infested with *Aphanomyces euteiches* when the study began. The disease severity index was below 40% (Alexandra Stone, *personal communication*[2]). The experiment was randomized in complete block design consisting of three replications of plots 4.6 x 7.6 m. This was a 2 factor experiment with 2 amendments (composted or fresh paper-mill residuals) and 3 rates (none, low and high rates). The amendments each year in April were applied on a dry weight basis, spread manually, and rotovated to a 15 cm depth.

Paper mill residual amendments were applied two weeks prior to planting in 1998 and four weeks prior to planting in 1999, 2000 and 2001 to allow sufficient time for residue decomposition. Fresh paper-mill residuals treatment were applied at two rates that approximated 50 and 100% of crop N requirement, with the assumption that 25% of the total N content would become available to the crop over the growing season. To meet this requirement in 1998, fresh paper-mill residual was applied at 22.4 or 44.8 dry Mg ha^{-1} for potato (*Solanum tuberosum*, N requirement of 224 kg N ha^{-1}). In 1999 and 2000, fresh paper-mill residuals were applied at slightly lower rates (22.4 and 33.6 dry Mg ha^{-1}) to supply the lower N requirements of snap bean and cucumber (*Cucumis sativus*) (Table 3.2.).
Fresh paper-mill residuals, marketed to growers as ConsoGro™, was obtained from Stora Enso North America (formerly Consolidated Papers Inc.), in Wisconsin Rapids. ConsoGro™ is a combination of the wood fiber fines clay, calcium carbonate, and other mineral fillers collected from the primary wastewater settling process, as well as microbial biomass generated during the secondary aeration process.

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Table 3.1. General soil characteristics sample in April, 2001.

Treatment	pH	PO_4-P	NH_4-N	NO_3-N	Bulk density	Total C	Total N	C:N
		----------	$\mu g\ g^{-1}$	----------	$g\ cm^3$	--------- %	--------	
C	6.67	65.77	3.24	7.49	1.58	0.47	0.05	10.1
CH	7.07	64.23	2.87	11.24	1.43	1.32	0.11	12.0
FH	6.80	54.53	2.77	16.18	1.49	0.86	0.07	11.1
CL	6.60	55.50	2.73	10.23	1.48	0.92	0.08	12.2
FL	6.53	51.07	1.85	9.43	1.50	0.72	0.07	10.9

The composted paper-mill residuals treatment had no bulking agent, it was obtained from the Oneida County landfill in Wisconsin (Rhinelander, WI). The paper-mill residuals used to make the compost were produced by the Rhinelander Paper Company (Rhinelander, WI). These residuals were "composted" (managed in outdoor windrows) for approximately 5 months before land application.

The composted residuals were not applied to meet N crop requirements but rather a very high rate (78.4 Mg ha^{-1}) was applied to increase the potential to disease suppression. The low rate was then applied at one half (38.1 Mg ha^{-1}). The high rate was approximate the high rate of fresh residuals.

All plots were fertilized based on soil test recommendations in mid June 2000 prior to cucumber planting at 135 and 90 kg ha^{-1} of N and K, respectively. After cucumber harvest during the last week of August 2000, the remaining fruits were removed and all plots were sprayed with Gramoxone extra ®(a.i.Paraquat dichoride) at rate of 1.8 L ha^{-1}. During the first week of September 2000, cereal rye (*Secale cereale*) was seeded at rate of 56 kg ha^{-1} and the entire field was rototilled to incorporate the seed to a depth of 5 cm. Rye biomass was harvested and analysed on October 2000, and dry weight, total C and total N are shown in Table 3.3.

From each field plot, soil was collected to a 20 cm depth in April and September 2001 by taking 28 kg soil randomly across each plot with a shovel. The soil was sampled before cover crop incorporation in April and when potatoes were growing on September. Each treatment was sampled by replication and this replication was maintained for subsequent incubations, bioassays, soil analysis and statistical analysis. The soil was passed through 2 cm sieve to remove large pieces of plant material. The soils sampled collected from the field study were placed in

plastic bags at 23°C temperature for the duration of the experiment. Moisture was maintained at approximately 50% field capacity.

Root Rot Bioassay Experiment

A subsample of soil from each bag was periodically removed for the root rot bioassay. This was conducted in the greenhouse by placing 80 cm^3 of vermiculite at the bottom and 150 cm^3 of the test soil in 250 cm^3 plastic cones (Stuewe & Sons, Inc., Corvallis, OR). Each cone contained 5 synthetic cosmetic puffs in the bottom to prevent vermiculite leakage. Four seeds of snap bean (*Phaseolus vulgaris* "Oregon 91G" (Rogers Seeds) previously treated with Allegiance® dry (a.i. metalaxyl) were sown into each cone tube done with three replication for each incubated soil.

Snap beans were grown at 21°C (day) and 10°C (night) in a greenhouse with a 14-hr photoperiod. A month after planting, the plants were lifted carefully without damaging the roots, washed and individually rated on an disease severity scale with the following disease class: 0 = healthy; 1=slightly discolored roots, hypocotyl firm; 2 = moderately discolored rots, hypocotyl collapses under pressure; 3 = darkly discolored roots, hypocotyl collapses easily under pressure; 4 = dead or dying plant. Dry plant biomass was measured and disease severity index (DSI) was calculated for each plant according to the method of Kobriger et al. (1998) as follows:

DSI= [Σ(disease class*number of plants in class)*100]/[total plants)*4]

The bioassay was conducted six times with soils collected in April: May 9, June 13, July 23, September 14, and December 11of 2001 and January 25, 2002.

Table 3.2. Field organic treatments applied in late spring each year at Hancock research station, University of Wisconsin on the long-term disease suppression study site.

Treatment	Amendment	Amendment rate			
		1998 (potato)	1999 (snap bean)	2000 (cucumber)	2001† (potato)
		Mg ha^{-1}			
Control	Non-amended soil	-	-	-	-
CH	Composted paper-mill residuals at high rate	78.4	78.4	78.4	78.4
FH	Fresh paper-mill residuals at high rate	44.8	33.6	33.6	44.8
CL	Composted paper-mill residuals at low rate	38.1	38.1	38.1	38.1
FL	Fresh paper-mill residuals at low rate	22.4	22.4	22.4	22.4

†Soils were sampled just before application of amendments in April 2001 and four month later in September 2001.

These dates represent 9, 44, 84, 137, 225 and 270 days of incubation after soil was removed from the field, respectively.

The bioassay was conducted three times with soils collected in September: September 27, and December 11of 2001 and March 6, 2002. These dates represent 13, 88 and 174 days of incubation.

Soil Analyses

Soil from each bag was collected on the same day as the root rot bioassay and passed through a 2-mm sieve. Enzyme assays and microbial biomass measurements were conducted 48 h after sampling of moist soil, which was stored at 4°C. The water stable aggregates assay was conducted within 2 weeks after sampling on soils that were immediately air-dried after sampling.

Table 3.3. Biomass C and N analysis of cereal rye cover crop in October, 2000.

Treatment	Dry weight	Total C	Total N	C/N
	$g\ m^2$	----------%----------		
Control	74	42.6	4.1	10.3
CH	108	41.6	4.9	8.4
FH	93	42.6	3.6	11.9
CL	95	43.1	4.1	10.4
FL	108	41.1	4.2	9.9

β-glucosidase and arylsulfatase activities were determined on field-moist soil in duplicate as described by Tabatabai (1994). For β-glucosidase, in brief, the substrate was p-nitrophenyl-β-D-glucoside solution, which was incubated (buffer pH 6.0) for one h at 37°C. After incubation and filtration, the product ρ-nitrophenol (PNP) was determined by measuring absorbance at 420 nm. Controls were run without the substrate, and the absorbance was subtracted from the sample with substrate. For arylsulfatase, the same procedure as for β-glucosidase was used, but instead the substrate was p-nitrophenyl sulfate solution (PNS) and the buffer pH was 5.8. Activity was calculated as μmol PNP g soil^{-1}.

Fluorescein diacetate hydrolysis was determined on field-moist soil as described by Zelles et al. (1991). Soil (3 g) was weighed into each of four 125 mL Erlenmeyer flasks; 50 mL of sodium phosphate buffer (pH 7.8) with fluorescein diacetate (3',6'diacetyl fluorescein) substrate was added to three flasks, and 50 mL sodium phosphate buffer with no substrate to the fourth flask (control). Flasks were stoppered and placed on a platform shaker (160 strokes/min) for 3 h at room temperature (25° C). The flasks were removed from the shaker, 2 mL acetone was added, the flask was swirled, and about 30 mL were transferred to a centrifuge tube. Tubes were centrifuged for 5 minutes at 31,000 *g* (15,750 rpm). The suspension was passed through Whatman #42 filter paper and absorbance was measured with a visible light spectrophotometer at 490 nm wavelength. Activity was calculated as μmol fluorescein hydrolized g^{-1} soil h^{-1} with a standard curve, and the control value was subtracted.

Microbial biomass-C was measured on field-moist soil by the chloroform-fumigation incubation method (Jenkinson and Powlson, 1976). Soil (10 g) was weighed into glass scintillation vials. Soil was exposed for 24 h to ethanol-free chloroform under vacuum. Soils were incubated in the dark at 25°C for ten days.

The same procedure was done on a non-fumigated soil to determine respiration rates. For both fumigated and unfumigated procedures the CO_2 produced was measured by thermal conductivity gas chromatography . A k_c of 0.41 (Voronry and Paul, 1984) was used to calculate MBC without subtraction of the control.

Soils collected on the first bioassay day were used to quantify culturable microbial populations by a plating technique. Briefly, 10 grams of soil (dry weight) was added to a flask containing 95 ml of saline solution (0.85% NaCl) and shaken on an orbital shaker for 20 min at 180 rpm. About 20 glass beads (2 mm diameter) were added to each flask before shaking to help soil dispersion. Serial dilutions (10^3 to 10^8) from these soil suspensions were carried out under aseptic conditions using sterile saline solution (0.85% NaCl) as a diluent. Aliquots of 0.1 mL from these dilutions were inoculated in Petri dishes containing about 25 mL of each of the following solid media: rose bengal-streptomycin agar (Martin, 1950); starch-casein agar (Kluster, 1966) and nutrient agar diluted thousand times (Alef, 1995). These media were used as selective media for fungi, actinomycetes and oligotrophic bacteria, respectively. The aliquots were spread over the media surface using a glass rod loop until no excess of free liquid was noted and the plates incubated upside-down at 25°C in the dark. Colonies were counted under magnification after 4 days for fungi and after 10 days for actinomycetes and bacteria. Petri dishes with fewer than 30, or greater than 300, were not considered for enumeration of actinomycetes and bacteria, and those with fewer than 10 colonies were not considered for fungi enumeration. Microbial counts were determined in triplicate and results expressed as colony forming units (CFU) per gram of dry soil.

Total C was determined by dry combustion as described by Nelson and Sommers (1996) with a C 144 Leco Carbon Determinator. In brief, a sample of approximately 0.35 g was placed in a ceramic boat in especially designed

horizontal resistance furnace maintained at constant temperature at 1358°C under O_2 flow. Oxygen is directed onto the sample and carries the CO_2 released through dust and water vapor traps and into an infrared detection system.

Water stable aggregation was determined on air-dry soil as described by Kemper and Rosenau (1986), modified as follows. Soils were passed through a 2-mm sieve and then allowed to air dry for 48 hours. A subsample was placed in a 1 mm sieve to eliminate aggregates smaller than 1 mm. Four g of the retained soil sample was placed in a screen cup (3.6 cm diameter with 0.250 mm stainless steel screen). Containers with 100 mL de-ionized water were placed on the stationary platform under the 0.25 mm sieves and the screen cup was cycled into the water for 3 min at 35 cycles min^{-1}. After that, containers with water and dissolved soil aggregates were removed and containers with 80 mL sodium polyphosphate 2 g L^{-1} (dispersing solution) were placed on the stationary platform. Screen cups were cycled through this solution until only sand particles remained on the screen. Both water and dispersing solution containers were placed in a 110°C oven overnight and weighed. Percent of water stable aggregates was calculated as follow:

WSA (%) = (g soil in dispersing container-0.16g)*100/(g soil in both containers-0.16g)

Subtraction of 0.16g was to compensate for the mass of the dispersing solution.

Statistical Analysis

For all experiments, the design was a randomized complete block. The results were analyzed as split plot with amendments as main plot and time as subplot. Treatment effects were analyzed for each month with analysis of variance.

Main effects of means were separated with the Fisher LSD method ($P<0.05$). Simple regression was performed with DSI data for each treatment and replication. The slopes were then subjected to ANOVA and a means separations test of the slopes were done with the Fisher LSD method ($P<0.05$). Data was analyzed using S-PLUS 2000 statistical software package (MathSoft, Data Analysis Products Division). Pearson correlations were performed to determine which soil properties were best related to disease severity using SAS (SAS Institute, 1996).

RESULTS

Root Rot and Dry Plant Biomass

Analysis of variance showed there was a significant overall treatment effect on both disease severity index (DSI) and plant biomass (Table 3.4). The DSI for Aphanomyces root rot of snap bean is shown in Fig. 3.1. The control, which received no organic amendment, had high disease severity, ranging from 59 to 72%. There was a significant effect of time on DSI and plant biomass ($p<0.05$). However, there was no significant interaction between treatment and time ($p<0.05$). The significant effect of time was due to a significant change in treatments CL, LF, and FH between days 44 and 225. The disease severity index increased again between day 225 and 270 in those three soil amendments (Fig. 3.1a) but, there was no overall significant difference between the first and last sampling day. The soils were sampled from three field replications (blocks) and these same field replications were maintained in the incubation study. Therefore, the results of Table 3.4 provide some information about spatial variability within the field experiment where there was no significant block effect for DSI or plant biomass.

This would indicate that the spatial variability at the Wisconsin site these factors were not significant across the experimental site.

Compost applied at the highest rate generated the strongest suppression of root rot, ranging from 42% DSI at the beginning of the experiment to 20% after 225 days incubation. This treatment was always significantly ($p<0.05$) lower than the control for all measurement periods. Comparing composted with fresh material suggests that compost at the high rate was always more suppressive than the fresh paper residuals as either high or low rates. For the first 44 days, the FL treatment was the least effective treatment with a DSI of 58 to 69%. However, from days 84 to 225 this treatment had a consistent decrease in DSI or an increase in disease suppression. The other treatments tended to be intermediate in disease severity and DSI was variable, with no trend over time. Treatments CL and FH were statistically different ($p<0.05$) from the control for every sampling period with the exception of day 137, and days 44 and 137, respectively. There was never a statistical difference between CL and FH. There was a decrease in DSI for all treatments. Linear regression showed that there were not statistical differences between slopes of any treatment. The estimate of the slope was 0.117 and the intercept varied from 41.02 on CH to 82.58 on the control (Fig 3.2.).

For the soils collected in September, treatment effects follow a similar trend and had the same ranking as the first incubation. In the incubation at the second sampling at September 14, all treatments decreased but again increased some on the third sampling date (Fig.3.1). Compost applied at the highest rate generated the strongest suppression of root rot, ranging from 25% DSI at the beginning of the experiment to 23% after 323 days after amendment (Fig. 3.1b.). This treatment was always significantly ($p<0.05$) lower than the control for all measurement periods.

Table 3.4. Analysis of variance for DSI, plant biomass and soil properties.

Source of Variation	DSI	Plant Biomass	β-glucosidase	Aryl sulfatase	FDA	MBC	WSA	Total C
Treatment	***	***	***	***	***	***	***	***
Time	***	**	***	***	***	***	**	ns
Block	ns	ns	ns	ns	***	ns	ns	**
Treatment*Time	ns	ns	ns	ns	ns	ns	ns	ns
Treatment*Block	ns	ns	ns	*	ns	ns	*	ns
Time*Block	ns	ns	ns	**	***	ns	ns	*

ns, no significant differences at $p<0.05$,

*, **, *** Indicate statistical differences at $p< 0.05$, $p< 0.01$ and $p<0.001$ respectively.

Plant biomass is shown in Fig. 3.3. The largest difference between the control and amended soils was at day 84. At this time, the control plant biomass ranged from 0.3 to 0.5 g plant^{-1}, while the CH treatment had the most plant growth of 0.7 to 0.8 g plant^{-1}. The CH treatment always had the highest biomass, and was significantly different ($p<0.05$) than the control for every sampling period except day 137. The CH was not significantly different ($p<0.05$) than the other amendments until day 270, when it was statistically lower than the other treatment ($p<0.05$).

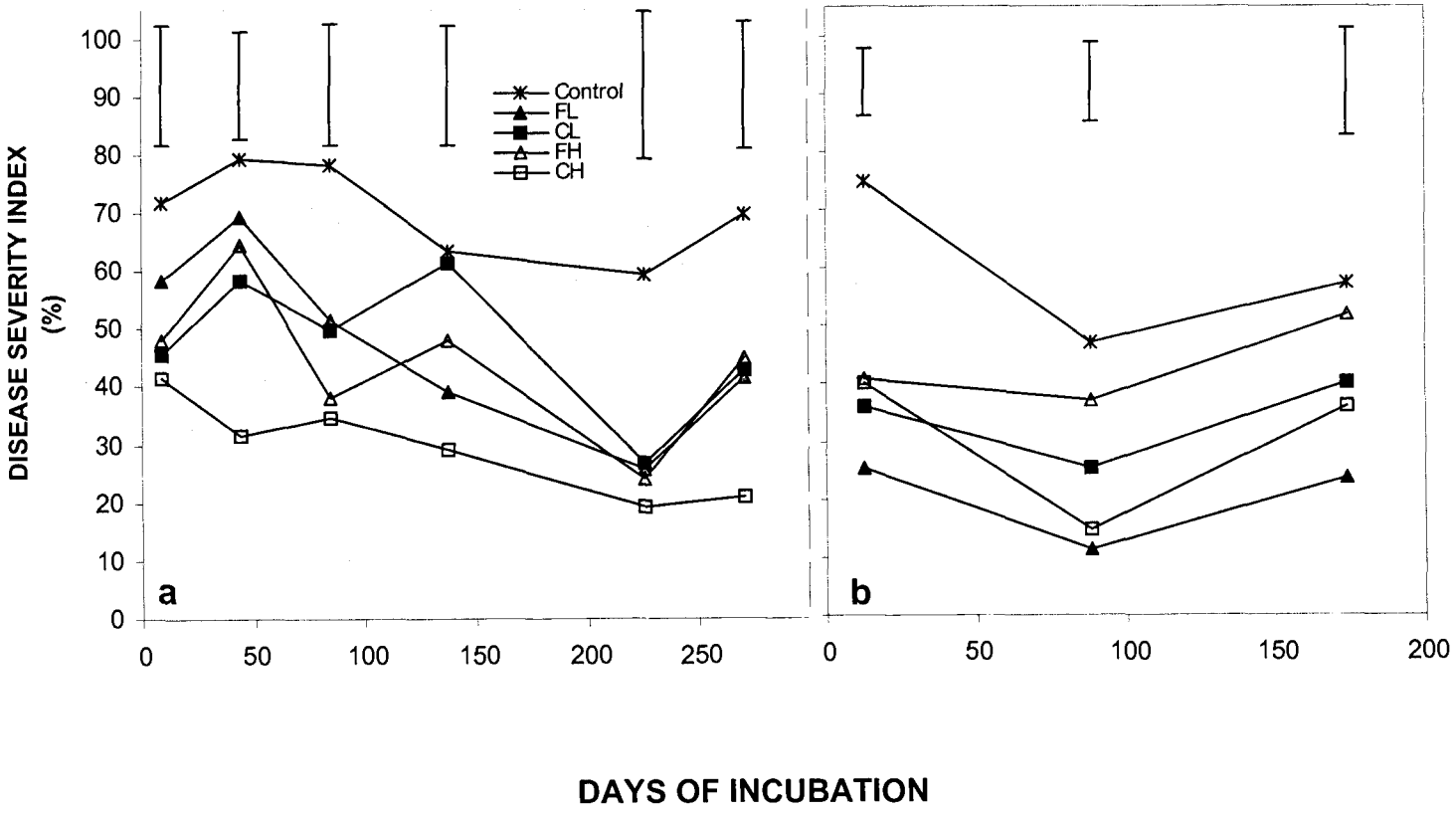

Figure 3.1. Soil amendment effects on DSI on soils collected in April (a) and on soils collected in September (b) over time (LSD bars $p<0.05$).

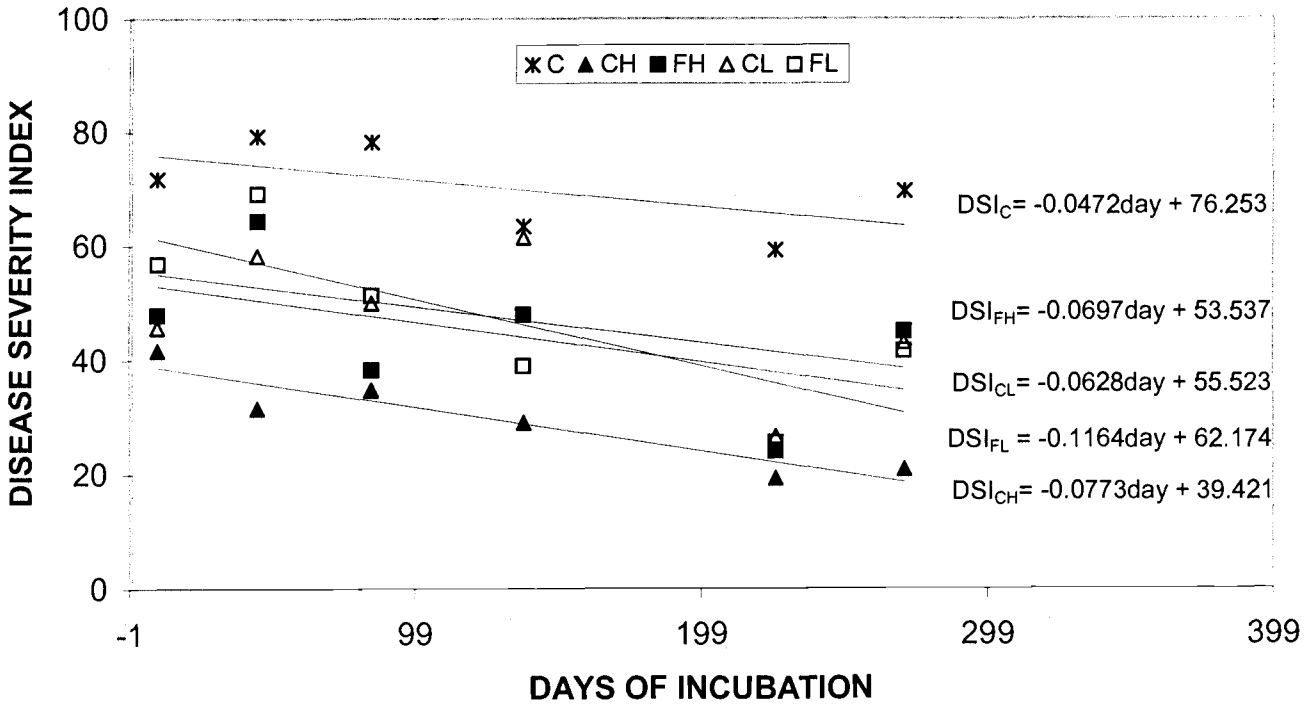

Figure 3.2. Linear regression of organic amendments for the relationship of DSI and time.

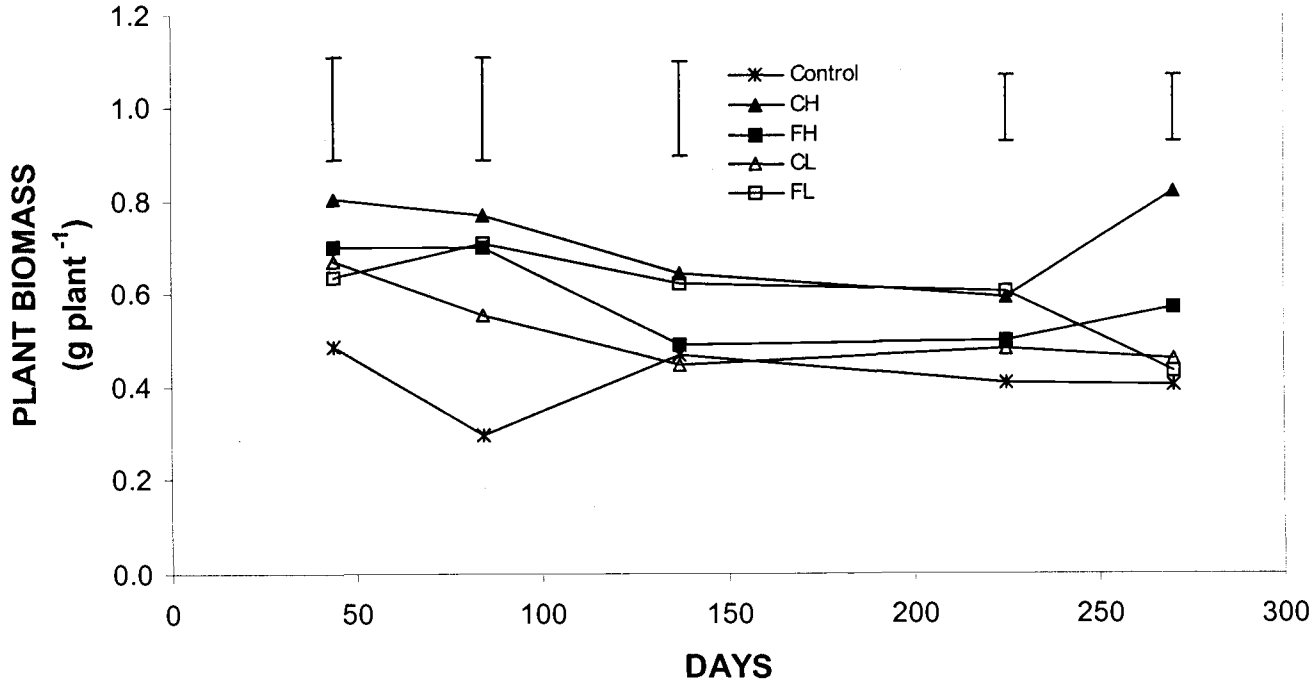

Figure 3.3. Soil amendment effect on dry biomass of plant tissue (LSD bars $p<0.05$).

Soil Indicators

β-glucosidase

Analysis of variance had a significant overall treatment effect for β-glucosidase activity as well as significant time effect (Table 3.4). Nevertheless, there was no significant interaction of treatment and time ($p<0.05$), suggesting that the change in treatment effect over time was linear and independent of when the soil was sampled (Table 3.4). The lack of a field block effects indicates that spatial variability is low across the experiment.

β-glucosidase activity was highest (up to 1 μmol PNP g^{-1} h^{-1}) and the treatment differences were stronger on the first sampling date (Fig 3.4). For all treatments, β-glucosidase activity steadily decreased over time up to day 106, after which activity remained stable at about 0.1 μmol PNP g^{-1} h^{-1}. The one exception to this was CH, which had a slight increase from day 137 to day 225.

For the first 44 days, the β-glucosidase activity for CH was significantly ($p<0.05$) higher than the other treatments. On day 106 it was significantly ($p<0.05$) higher than control, FL, and FH, and from day 137 to 225 it was significantly ($p<0.05$) higher than the control and FL.

The control always had the lowest levels of β-glucosidase activity relative to the other treatments, ranging from 0.52 to 0.05 μmol PNP g^{-1} h^{-1}. In the first 106 days, it was statistically lower ($p<0.05$) than all the other treatments. At day 137 the control was statistically lower for CL, FH or CH. On day 225, the control was statistically lower than for only CH.

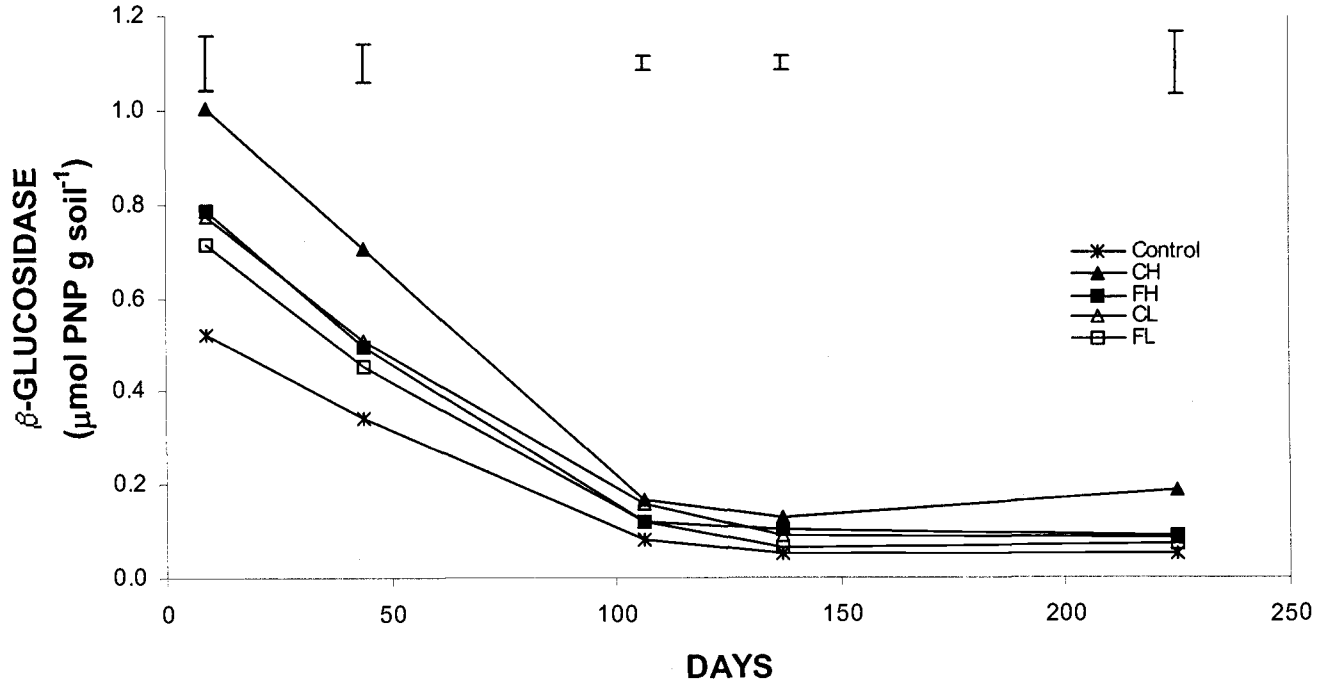

Figure 3.4. β-glucosidase activity over time (LSD bars $p<0.05$).

The other treatments tended to be intermediate in β-glucosidase activity. There were significant differences between CL and FH on day 106 and between FL and FH on day 137.

Arylsulfatase

A significant overall treatment and time effect ($p<0.001$) on arylsulfatase activity is shown in Table 3.4. However, there was no significant interaction of treatment and time ($p<0.05$). This would suggest the treatment effect change over time was linear and independent of when the soil was sampled for arylsulfatase. There were interactions between block and treatment ($p<0.05$), and block and time ($p<0.01$), suggesting that blocks generate a different effect over time. The lack of a block effect (Table 3.4) indicates there is low spatial variability for arylsulfatase is low across the experimental study.

Arylsulfatase activity had a trend similar to β-glucosidase, with high activity at the beginning of the experiment and a steady decrease of activity over time (Fig. 3.5.). The high level of arylsulfatase activity did not decrease as rapidly as β-glucosidase activity; the decrease was more gradual. There was a dip in activity between day 106 and 137, especially for the CH treatment.

There were no differences in arylsulfatase in the control over time, although values fluctuated from 0.1 to 0.2 μmol PNP g^{-1} h^{-1}. Treatments were significantly different at every sampling period except on day 44, when only the control was statistically lower than FH, CL and CH. Similarly on day 137 only CH was significantly different ($p<0.05$) than the control.

Composted paper residuals at the highest rate (CH) consistently had the highest arylsulfatase activity, from 0.7 μmol PNP g^{-1} h^{-1} at day 9 to 0.3 μmol PNP

g^{-1} soil h^{-1} on day 137. This treatment was always significantly ($p<0.05$) higher than in the control.

The other treatments tended to be intermediate in arylsulfatase activity. For the first 44 days, FH was significantly higher ($p<0.05$) than FL. There were statistical differences ($p<0.05$) between CL and FL on day 44, but CL and FH were never significantly different ($p<0.05$).

Fluorescein Diacetate Hydrolysis

There was a significant overall treatment effect for FDA activity ($p<0.001$) (Table 3.4). In addition, there was a significant effect of time and block ($p<0.001$). The change in treatment effects over time was linear and independent for FDA because there was no significant interaction of treatment. Therefore, FDA did have a significant block effect (Table 3.4.) indicating low spatial variability at the Wisconsin site. Moreover, there was a significant interaction of block and time ($p<0.001$), suggesting that block effect changed over time.

Fluorescein diacetate hydrolysis (FDA) activity over time is shown in Fig 3.6. Data were obtained on only days 44, 106 and 137 because of technical problems on the other days. The level of FDA activity did not vary much over time. However, FDA followed a similar trend with other enzymes with values from $49x10^{-3}$ μmol FDA $g^{-1}h^{-1}$ to $37x10^{-3}$ on day 44 and from $21x10^{-3}$ to $22x10^{-3}$ μmol FDA $g^{-1}h^{-1}$ on day 137.

Treatment effects were found on days 44 and 137. At day 44, CH was significant higher ($p<0.05$) than the control and FH, and on day 137 the control was significant lower than CL. Significant differences ($p<0.05$) were found between blocks for every sampling day.

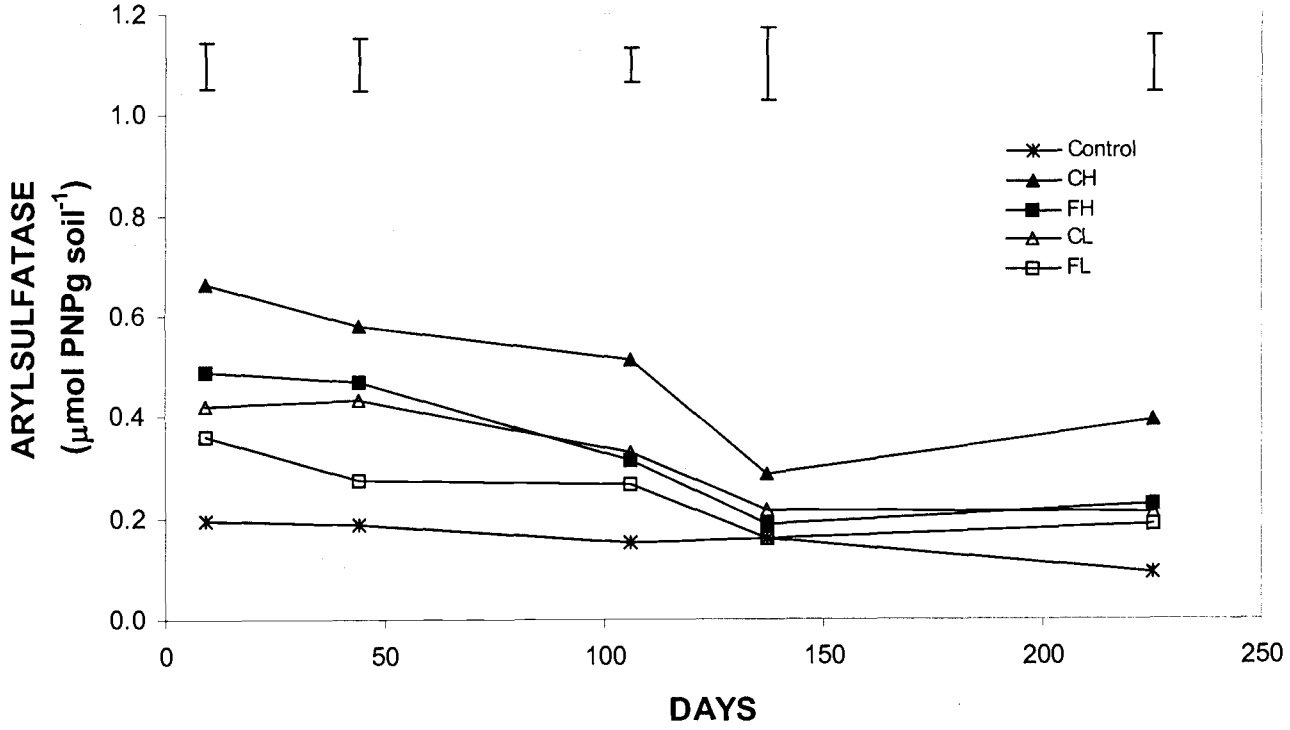

Figure 3.5. Arylsulfatase activity over time (LSD bars $p<0.05$).

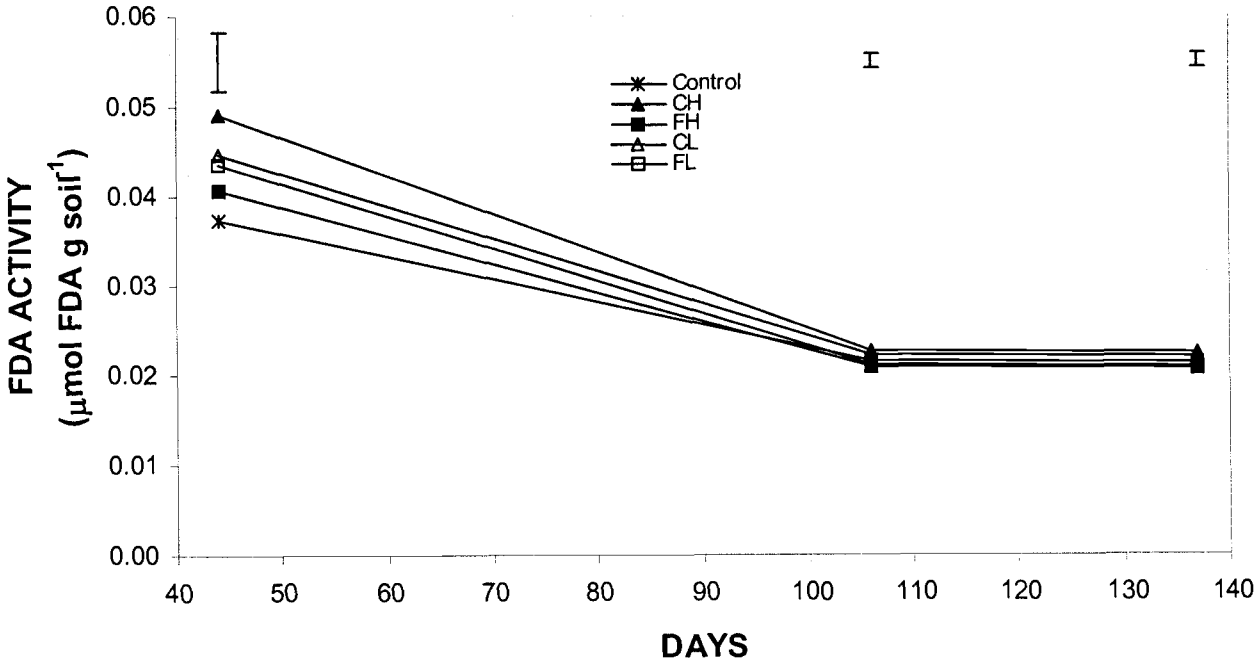

Figure 3.6. Fluorescein diacetate hydrolisis (LSD bars $p<0.05$).

Microbial Biomass and Respiration

There were significant effects of treatment and time for MBC but no interactions. Apparently spatial variability for MBC was at the field site because there was no significant block effect (Table 3.4).

Microbial biomass-C had higher values at the beginning of the study (Fig. 3.7.) with the lowest values at day 106 for all treatments.

Microbial biomass-C in the composted amendment at the highest rate started at 147 μg CO_2–C g^{-1} decreased to 55 μg CO_2–C g^{-1} by days137, then increasing to 118 μg CO_2–C g^{-1} at 225 days incubation. This treatment was significantly ($p<0.05$) higher than the control for all measurement periods except for day 137. Compared to the other amendments, the control always had the lowest microbial biomass (from 67 to 32 μg CO_2–C g $soil^{-1}$). These differences were statistically different with CL, FH and CH for the day 106 with FL at day 137 and with FH and CH at day 225. Treatments FL, FH and CL were intermediate in microbial biomass and were not significantly different ($p<0.05$) between them for any measurement period.

Microbial respiration (Fig.3.8.) had higher levels in the first day of the experiment and soils amended with compost had higher levels than soils with fresh amendments or the control. Compost treatment had values of about 50 and fresh material of about 20 μg C g $soil^{-1}$.

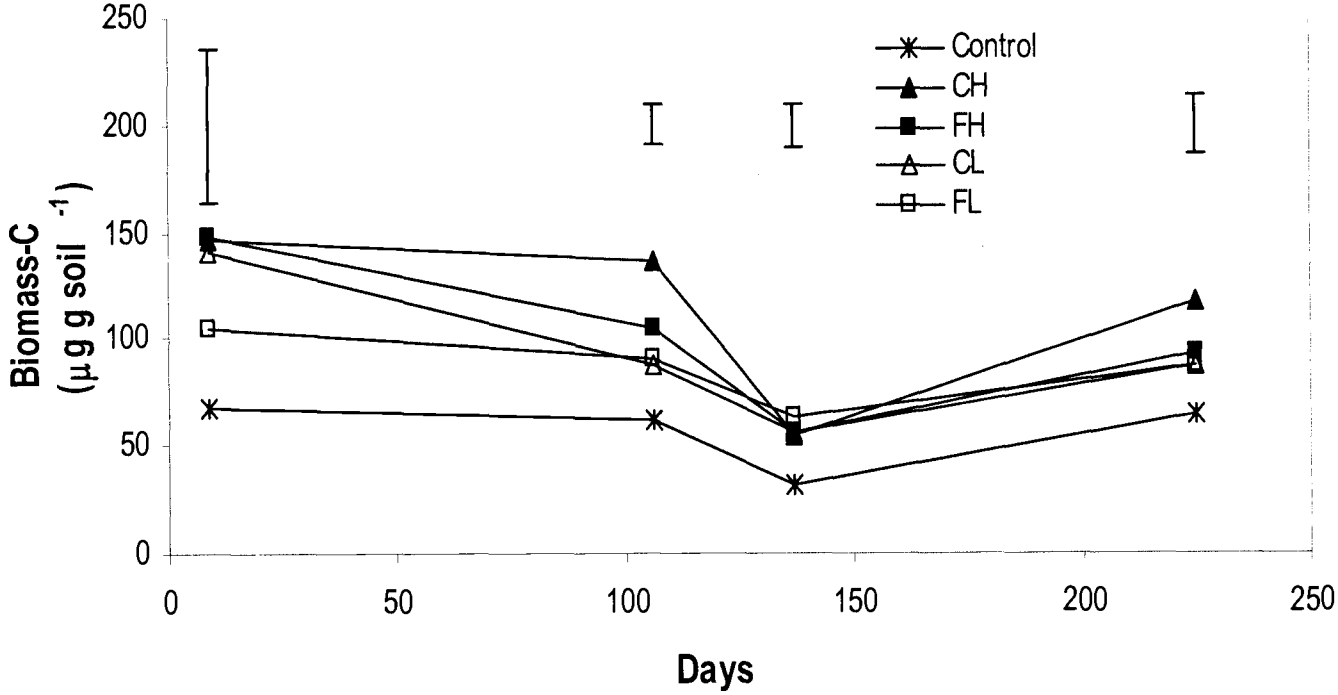

Figure 3.7. Effect of time and treatment on soil microbial biomass (LSD bars $p<0.05$)

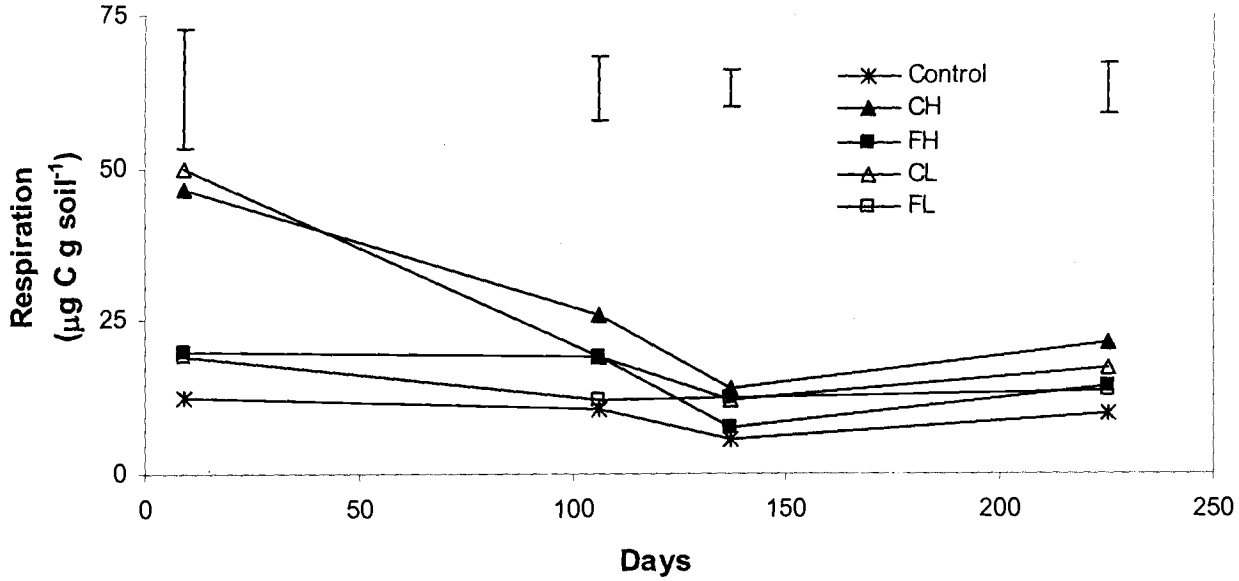

Figure 3.8. Respiration rate (µg C g soil^{-1})(LSD bars $p<0.05$).

Culturable Bacteria, Actinomycete and Fungi

In order to have a general knowledge of the community distribution, culturable bacteria, fungi and actinomycete were isolated, incubated and their colonies counted for soils collected at the beginning of the experiment (day 9). Actinomycete populations were higher ($p<0.05$) in CH than control, CL and FL. Fungal populations were significantly lower ($p<0.05$) in the control, (Fig. 3.9) than CH, CL and FL. There were no significant differences in bacterial counts among treatments ($p<0.05$).

Water Stable Aggregation

A significant overall treatment and time effect was found for WSA (Table 3.4). There was no significant interaction of treatment and time ($p<0.05$). The lack of block effect suggests that WSA was uniform across the Wisconsin experimental site. Nevertheless, there was significant interaction of time and block ($p<0.05$) indicating that treatment effect change across blocks.

Water stable aggregation was quite stable over time (Fig. 3.10). The CH had the highest WSA, starting at 71%, increasing to 89% by day 106, and then decreasing to 67%. For the first 137 days, CH was numerically higher than the other treatments but it was not always significantly different ($p<0.05$) than the other treatments. There was no significant difference ($p<0.05$) between the low and high rates of composted amendments suggesting that the lowest rate of amendment was sufficient to maximize water stable aggregation.

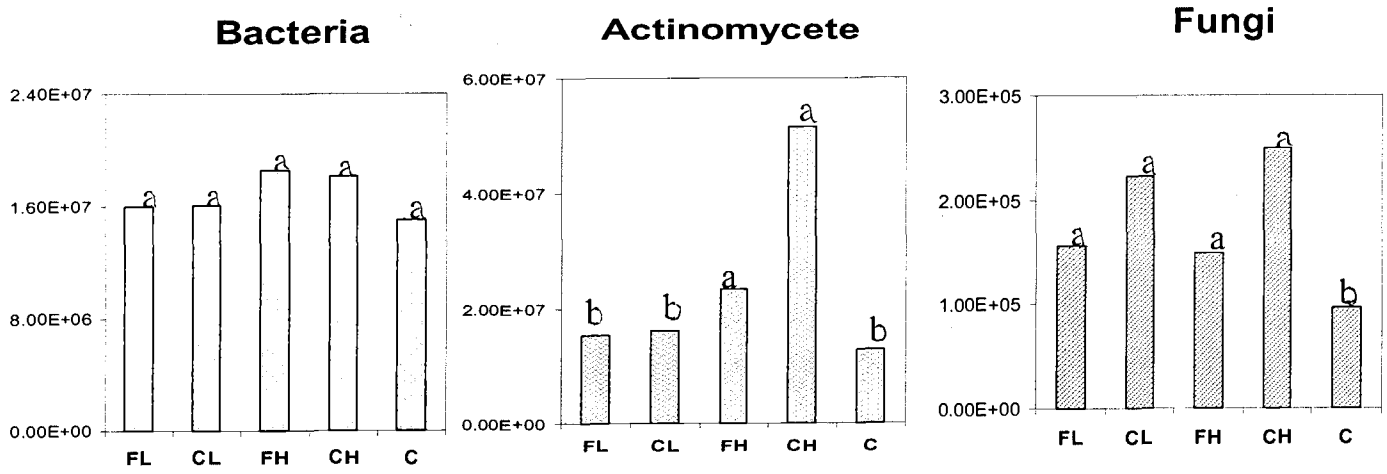

Figure 3.9. Culturable microbial population (different letters indicate significant differences between treatments $p<0.05$)

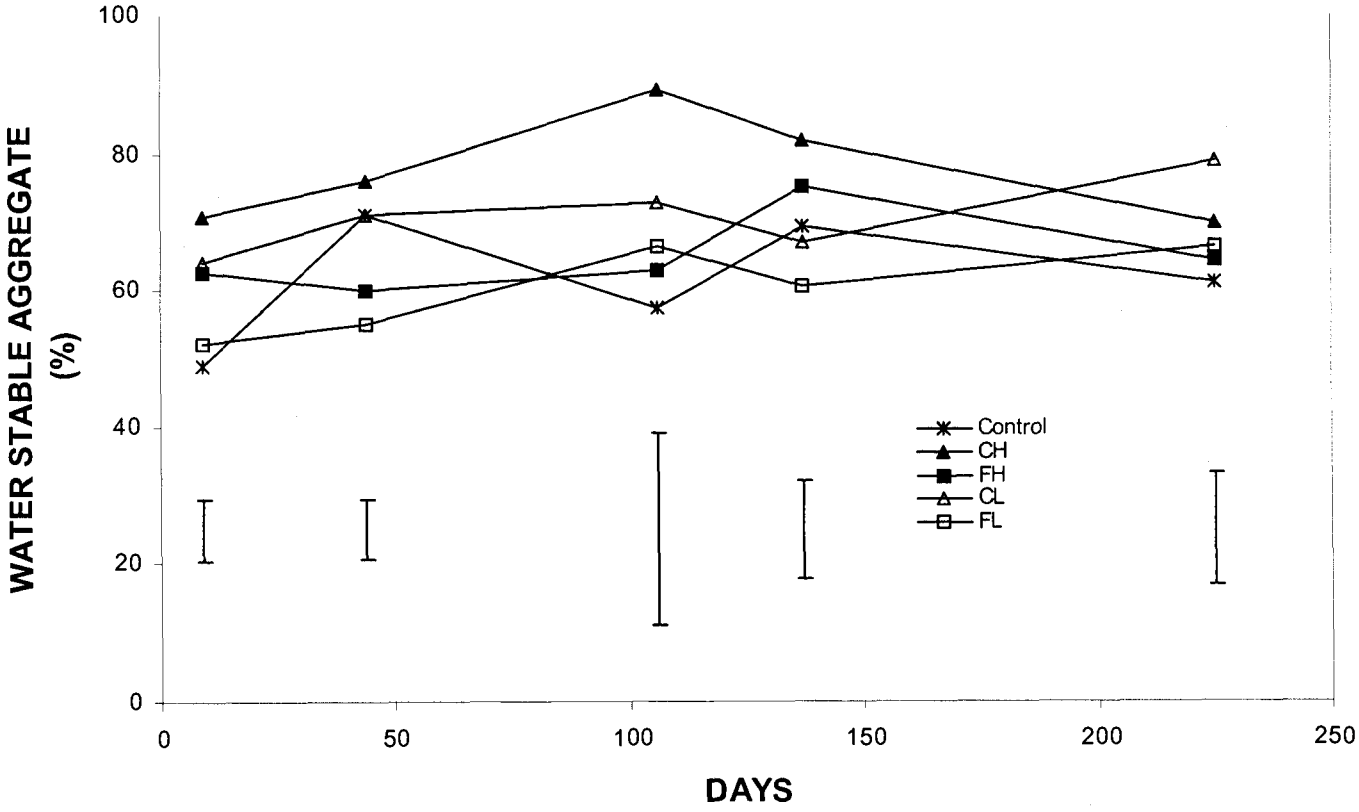

Figure 3.10. Effect of time and treatment on water stable aggregate (LSD bars $p<0.05$).

The control and treatments FL, FH and CL tended to be slightly lower in WSA than CH, and they varied slightly over time. For example, values ranged from 49% to 69%, from 52% to 67%, from 60% to 75% and from 64% to 79% for C, FL FH and CL treatments respectively.

Total Carbon

Total C was significantly affected by the amendments (Table 3.4). This would suggest the treatment effect change over time was linear and independent of when the soil was sampled. There was a significant block effect ($p<0.05$) and an interaction of time and block. The block effect suggests that total C was not uniform across the experimental Wisconsin site.

Total C was stable over time within each treatment (Fig. 3.11). Composting at the highest rate (CH) had the largest C total with levels around 1.3%. Treatment CH was always higher than the other treatments ($p<0.05$). Control had values of total C from 0.38 to 0.58%, it was always lower than the other treatments. Control values were statistically lower than the amended treatments every time with only one exception on day 106 when control was not statistically different than FL ($p<0.05$).

Treatments FL, FH and CL tended to be intermediate in total C. Values ranged from 0. 64% to 0.81%, from 0.82% to 0.89% and from 0.84% to 1% for FL FH and CL treatments, respectively.

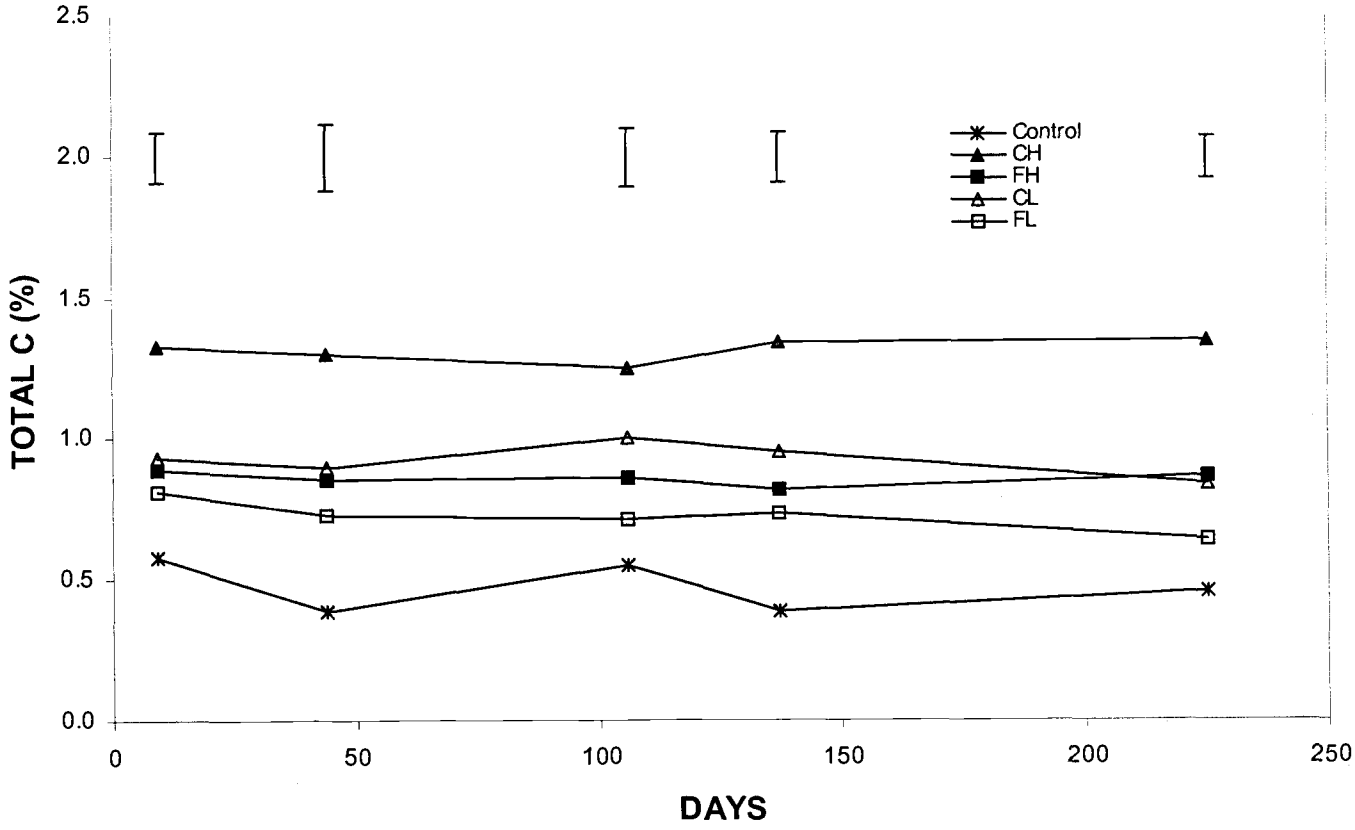

Figure 3.11. Carbon Total (%) (LSD bars $p<0.05$).

Correlations

Pearson correlation coefficients (r) between DSI and soil parameters were performed each month (Table 3.5.) and all dates combined (Table 3.6.).

Strong negative correlations were found between DSI and total C, with values from -0.41 to -0.81. When correlations were performed on all data (Table 3.6.), the correlation was -0.51 ($p<0.001$)

Among biological soil parameters tested in this study, arylsulfatase was the most negatively correlated with DSI, with values from -0.68 to -0.81 except for day 137 (-0.19) when most of the correlations were quite low. When correlations were performed on all data (Table 3.6.), the correlation for arylsulfatase was -0.26 ($p<0.001$)

β-glucosidase also was strongly negatively correlated with DSI (higher than 0.68) for the first 106 days, but a significant correlation was lost when β-glucosidase activity declined after 137 days (Table 3.5.). When correlations were performed on all data (Table 3.6.), the correlation for β-glucosidase was 0.24 ($p<0.05$)

No strong correlations were found for FDA activity at any time. Correlation values varied from -0.26 to 0.15, when correlations were performed on all data, it was 0.25

Between microbial biomass-C and DSI, correlations were found on days 106 (-0.62, $p<0.05$) and 225 (-0.65, $p<0.01$). When correlations were performed on all data (Table 3.6.), FDA the correlation was -0.28.

Table 3.5. Pearson correlation coefficients (r) between soil parameters and Disease Severity Index (DSI).

Day	Log β-glucosidase	Log Arylsulfatase	Log FDA	MBC	Respiration	Water Stable Aggregates	Total C
9	-0.68 **	-0.68 **	-0.26	-0.28	-0.51	-0.73 **	-0.69 **
44	-0.71 **	-0.71 **	-0.37	-0.51	-0.63	-0.38	-0.81 ***
106	-0.77 ***	-0.81 ***	-0.08	-0.62 *	-0.47	-0.34	-0.65 **
137	-0.22	-0.19	0.15	0.42	-0.58 *	-0.47	-0.41
225	-0.17	-0.70 **	- -	-0.65 **	-0.55 *	-0.35	-0.53 *

*, **, ***, **** Indicate $p< 0.05$, $p< 0.01$ and $p<0.001$ respectively.

Table 3.6. Matrix of Pearson correlation coefficients (r) for DSI and soil parameters (all dates together).

	β-g	Ar	FDA	MBC	R	WSA	Total C
Disease Index (DI)	0.24 *	-0.26 ***	0.25	-0.28 *	-0.22 **	-0.40 ***	-.051 ***
Log(β-glucosidase)(β-g)	- -	0.66 ***	0.47 ***	0.52 ***	0.54 ***	-0.15	0.31 **
Log (Arylsulfatase) (Ar)		- -	0.31 *	0.69 ***	0.60 ***	0.15	0.66 ***
Log(Fluorescein diacetate Hydrolisis) (FDA)			- -	0.10	0.04	0.05	0.09
Biomass-C (MBC)				- -	0.66 ***	0.05	0.42 ***
Respiration (R)					- -	0.17	0.47 ***
Water Stable Aggregation (WSA)						- -	0.32 **
Soil moisture (SM)							0.65 ***

*, **, *** Indicate $p< 0.05$, $p< 0.01$ and $p<0.001$ respectively.

Strong negative correlations were found on every date for DSI and soil moisture, with values ranging from -0.50 to -0.81. When correlations were performed on all data the correlation was -0.55 ($p<0.001$) (Table 3.6.).

When correlations were performed on all the data, β-glucosidase was strongly correlated with arylsulfatase (0.63, $p<0.001$) and microbial biomass was strongly correlated with arylsulfatase (0.69, $p<0.001$) and β-glucosidase (0.52, $p<0.001$). Total C was strongly correlated with soil moisture (0.65, $p<0.001$), corroborating that high C level in soils promote water-holding capacity because of the C effect on soil aggregation. In fact, there was strong correlation of 0.52 ($p<0.001$) between water stable aggregation and soil moisture.

DISCUSSION

The present study supports the theory that disease suppression may be generated through soil organic amendments and that it may have a long-term residual effect. Severity of snap bean root rot in all amended treatments was consistently lower than in the control over the incubation period, although treatment means separation were not always statistically significant.

Disease severity declined over the course of this experiment but this decline was similar between the control and the other treatments suggesting that there is a natural reduction of propagules over time and that suppression was induced at some point before this experiment began.

Mechanisms of suppression

There are many possible roles of the soil biological responses to organic matter that may be operating for induction of disease suppression. These are competition, pathogen destruction and systemic acquired resistance.

Competition

The stimulation of microbiota that causes competition with pathogens and therefore suppression of disease has been found for a variety of diseases (Lumsden et al., 1983; Boehm et al., 1992; Hoitink et al., 1997; Stone, 1997; Cohen et al., 1998; Hoitink and Boehm, 1999).

Competition can be an important mechanism for the suppression of Pythium and Phytophthora root rots in field and container systems. However, suppression through this mechanism is typically associated with rapidly decomposing organic substrates and microbial activity, and suppressiveness declines with time as the most labile constituents of the organic amendment are depleted (Stone et al., 2002; Boehm et al., 1997). The duration of suppression generated through this mechanism varies by the composition of the organic substrate; hardwood bark composts suppress for up to two years, pine bark composts for up to 9 months (Hoitink et al., 1997), and municipal solid waste composts (composed primarily of paper), for approximately 6 months (Widmer et al., 1998). It is not likely that fresh or composted paper-mill residuals would sustain competition and its associated suppressiveness for more than approximately 6 to 9 months; this experimental period began 12 months after the last amendment.

There is indirect evidence from β-glucosidase activity and FDA hydrolysis that competition may be low during our experiment because these assays declined

across all treatments the first 106 days whereas DSI did not show that decline. We hypothesize that these two assays reflect microbial activity because their decline in activity was likely due to the cover crop root residues that were in the soil when it was sampled. Thus, if competition was occurring we would have expected a concurrent decrease in DSI the first 106 days.

While it is likely that C competition generates strong levels of suppression during the growing season immediately after amendment (Stone et al., 2002) (Fig. 3.12), this mechanism does not appear to have generated the suppression observed during our experiment.

Pathogen Destruction or Loss of Viability

Organic amendments have caused an increase in the incidence of pathogen propagule lysis and loss of viability and thereby suppressing Phytophthora root rot of avocado (Malajcuk, 1983). Common root rot (causal agent *Aphanomyces euteiches* Drenchs) of peas has been reduced by incorporation of cereal rye (*Secale cereale*) (Papavizas and Ayers, 1974). Cruciferous amendments have also reduced common root rot in peas (Papavizas, 1966; Papavizas and Lewis, 1971; Chan and Close, 1987; Parke and Rand, 1989; Muehlchen et al., 1990; Smolinska et al., 1997), but it is believed to result from the effects of volatile toxic substances produced during decomposition of cruciferous amendments.

It is commonly understood that the viability of fungal propagules naturally declines over time (Malajczuk, 1983). Inoculum of *A. euteiches* f. sp. *pisi* was under field conditions reduced by 50% over a single year in a loamy soil in the absence of the host (Pfender and Hagedorn, 1983).

This loss of inoculum viability typically translates into reduced disease severity and is the basis for the adoption of crop rotation for the control of soil-borne diseases.

We hypothesized that amendments would increase the level of microbial antagonism and increase the loss of pathogen viability, reducing disease severity over time. Interestingly, DSI had a statistically significant decline over time but the slope of this decline was not significantly different between the control and amended treatments. Moreover, we hypothesized that a new amendment application would cause a reduction in DSI, but as is shown in Fig. 3.1., the incidence of common root rot is quite similar after amendement in soils sampled in September 2001. Therefore, it does not appear that pathogen destruction is a strong mechanism for disease suppression in these amended soils during the first 275 day incubation period, or after the new amendment in the second period of incubation. It is also plausible that Aphanomyces propagules were destroyed or rendered non-viable at some point prior to this experiment.

Systemic Acquired Resistance

Common root rot of snap bean was expected to have been relatively low at the beginning of field experiment in 1998 as there was no recent history of snap bean production in this field (>8 years). Systemic acquired resistance was demonstrated in 1999 by a very strong suppression of foliar brown spot on snap bean (Stone et al., 2002) (Table 3.7). However, only the composted paper-mill residuals generated reduced incidence of pod brown spot (Table 3.7). Foliar anthracnose of snap bean (causal agent *Colletotrichum lindemuthianum*) was suppressed only in the CH treated soils in the fall of 1999 (Table 3.7). Arabidopsis

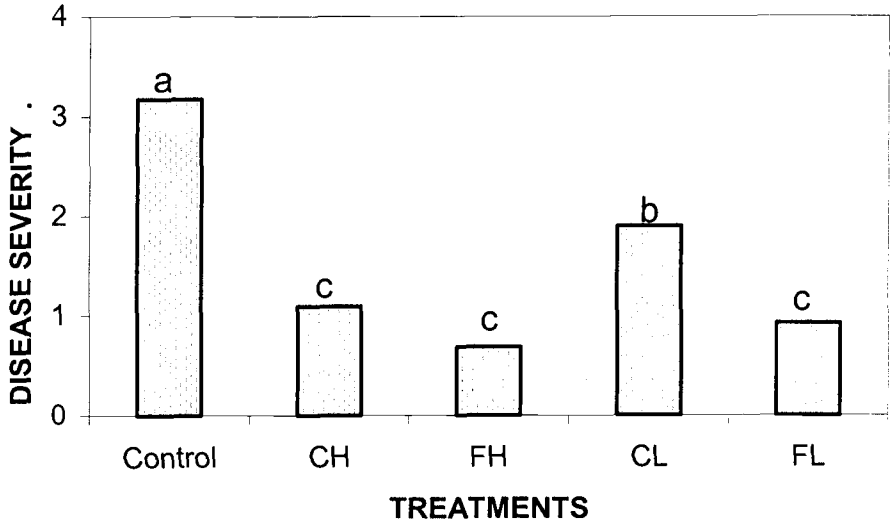

Fig. 3.12. Common root rot severity (based on Horsfall-Barratt scale) in field-grown snap bean of soil amended with fresh and composted paper-mill residual at Hancock experimental site. (Adapted from Stone et al., 2002)

Table 3.7. Foliar diseases of soil amended with fresh and composted paper-mill residual at Hancock experimental site. (Adapted from Stone et al., 2002)

Treatment	Field-grown snap bean 1999		Snap bean bioassay 1999
	Foliage brown spot[†] *(Pseudomonas syringae* pv *syringae)*	Pod brown spot[§] *(Pseudomonas syringae* pv *syringae)*	Anthracnose *(Colletotrichum lindemuthianum)*
FL	2.3 c[‡]	22.9 a[‡]	2.43 a[‡]
FH	3.4 b	34.3 a	2.53 a
C L	1.5 d	12.1 b	2.46 a
C H	1.1 d	7.6 b	0.75 b
Control	3.9 a	24.7 a	2.55 a

[†] Disease severity index based on Horsfall-Barratt scale
[‡] Values represented by different letters are significantly different at p=0.05
[§] Proportion of diseased pods

and tomato bioassays demonstrated that CH strongly induced plant defense genes involved in classic SAR (Gary Vallad[3], *personal communication*). In summary, CH was the strongest inducer of all the amendments (Table 3.7). Organic matter-mediated systemic acquired resistance has the potential to suppress root rots as well as foliar diseases (Zhang et al., 1996). An increase in plant resistance to infection in the 1999 field grown snap bean could have resulted in no build up of soil Aphanomyces inoculum at the end of the 1999 growing season. Aphanomyces is not a good saprophyte; and requires infection of host tissue to increase inoculum. Therefore, the other treatments which showed disease levels to varying degrees may likely have increased inoculum levels according to the degree of infection of the host. But because CH had little infection of host tissue there was minimal stimulation of inoculum.

Plants with enhanced resistance due to SAR (e.g. CH) would reduce infection and subsequent oospore formation. This could have occurred if there were not environmental or biological agents that reduce variability of these propagules. Therefore, organic matter-mediated SAR of snap bean in 1999 could have generated the differences in DSI that were observed in our study.

Soil Quality Indicators of Disease Suppression

To develop effective and consistent disease suppressive soils with organic amendments, it is important to understand the underlying soil ecological mechanisms and processes for eliminating or reducing disease severity. Furthermore, indicators are needed for assessing disease suppressiveness of soils to guide organic amendment management. Such indicators may have a direct

[3] Gary Vallad, University of Wisconsin. Department of Plant Pathology

mechanistic relationship with disease suppression or be a coincidentally correlated to disease suppression. Microbial biomass and activity measurements may be related to microbial competition with pathogens. Soil physical properties may relate to the microbial habitat status of a soil and rooting environment which could affect disease severity.

β-glucosidase catalyzes the release of low molecular weight sugars which are an energy source for microorganisms in soil (Tabatabai, 1994), playing an important role in the C cycle. Consequently it does reflect organic inputs in to soil which has been shown in studies where manures and cover crops have been incorporated into soils (Bandick and Dick, 1999; Ndiaye et al., 2000).

Our experiment was consistent with these early studies where β-glucosidase activity reflected treatment effects over the entire incubation period. However, it decreased steadily until day 106 and then remained relatively low. Higher values at the beginning of the experiment for each treatment, including the control, would suggest there was a residual effect from the cover crop because soils were taken for the study when the cover crop was in the field. Although the soil was sieved, many fine roots were likely retained in the soil and would be a substrate that stimulates production of hydrolytic enzymes involved in decomposition of compounds such as β-glucosidase.

β-glucosidase activity was significantly higher ($p<0.05$) with CH than the control suggesting that energy substrates for β-glucosidase were more available in CH than in other treatments. This could indicate that there was greater potential to release glucose and that CH provided a higher amount of readily available C sources to support microbial communities than the other treatments.

Although β-glucosidase consistently had treatment effects of the amendment, it would not be desirable as an indicator of disease suppression because it varied over time. This would make calibration and interpretation of this

assay difficult for practical applications. However, β-glucosidase activity should be tested under field conditions because it is common for enzyme activities to decrease under the artificial conditions of laboratory or greenhouse incubation in the absence of plants (Richard P. Dick, *personal communication*).

Fluorescein diacetate hydrolysis has potential to broadly represent soil enzyme activity and accumulated biological effects because it can be hydrolyzed by many enzymes such as proteases, lipases and esterases and has been found among a wide array of the primary decomposers, bacteria and fungi. (Dick et al., 1996). In this experiment, FDA had few significant differences between treatments. Differences between blocks were stronger than between treatments, suggesting that this assay was sensitive to spatial variation.

Fluorescein diacetate hydrolysis had no strong correlations with DSI at any time period. Correlation varied from –0.26 to 0.15; moreover, when correlations were performed on all data, it was 0.25. A positive value would not be expected because it is generally accepted that soils with higher microbial activity should have higher disease suppression. This coincides with van Bruggen and Grunwald (1996) who indicated that FDA hydrolysis was not always closely correlated with disease suppression. Nevertheless, FDA hydrolysis has been widely used in the literature as an indicator of disease suppression (van Bruggen and Semenov, 2000). It has been shown to be an indicator for suppressiveness of Pythium root rot (Inbar et al., 1991; Boehm et al., 1997), sugarcane root rot (causal agent *Phytium arrhenomanes*) (Dissanayake and Hoy, 1999) and *Phytohptora parasitica* and corky root of tomato (causal agent *Pyrenochaeta lycopersici*) (Workneh et al., 1993).

FDA activity has been correlated with disease suppression generated when the mechanism appears to be C competition between the microbial population and the pathogen (Stone, 1997; Boehm et al., 1992). As described above, it is unlikely

that C competition played a strong role in the suppression observed during this experimental period. Therefore, it may not be surprising that FDA activity was not shown to be closely related to disease suppression.

The lack of treatment effects may also be an inherent problem of what FDA hydrolysis measures. It may be too broad of an assay and for our study could not reflect the differential effect of different paper-mill residuals. Similar to β-glucosidase, it seemed to be affected by the residual effects of the cover crop (as show by its decline in the first 106 days on the first incubation), which was not related with disease suppression.

High MBC may be an indicator of general disease suppression because it may reflect a larger and more diverse community. In the present experiment, CH had significantly higher ($p<0.05$) MBC and significantly lower disease severity than the control, suggesting there was a larger microbial biomass involved in disease suppression. This coincides with results found for damping-off caused by *Pythium ultimum*, which was negatively correlated with microbial biomass (Chen et al., 1988).

Compost has less readily available C which is unfavorable for fast growing bacteria. At the same time, compost has most of its C as recalcitrant organic compounds that favors slow growing organisms such as actinomyces. *Trichoderma* spp. have been shown to stimulate oospore and clamydospore formation and hyphal lysis in *Phytophthora* species (Malajczuk, 1983). The mechanism proposed for *Gliocladium* spp. and *Trichoderma* spp. is antagonistisc mycoparasitism by lytic exoenzymes that partially degradate the host cell wall of these pathogens (Chernin and Chet, 2002). Results of culturable microbial population (Fig 3.9) suggest that composting amendments may have changed the microbial community composition in favor of certain actinomycetes and fungi. Elevation of these functional groups in the amended soil may have played a role during or prior to our study in causing

disease supression. Differences in populations and activities of antagonists supported by the different amendments treatments could explain the differences in DSI observed in our experiment.

The high activity of arylsulfatase in treatments with low DSI could be related to a larger fungal biomass. Arylsulfatase hydrolyzes ester sulfates, which are mainly in the fungal portion of soil microbial biomass. Fungi have up to 42% of their S as ester sulfate-S (Saggar et al., 1981). Furthermore, arylsulfatase has been strongly correlated with ergosterol (R. P. Dick, *personal communication*), a compound almost exclusively found in fungi (Newell et al., 1987). Therefore, it is possible that arylsulfatase activity could be an indicator of fungal biomass and may be related to an antagonistic fungal mechanism for disease suppression. Arylsulfatase may represent a subgroup of the microbial population associated with antagonists of the pathogen since it had the highest correlation of any of the microbial properties measured in our experiment.

All amendments caused an increase in water stable aggregation. Treatment CH was higher than the control and FL until day 137, but those differences were not always significantly different ($p<0.05$). Organic amendments not only are contributing disease suppression by providing an energy source for microbial activity but they can also improve soil structure, bulk density, water-holding capacity and gas exchange. Soil organic amendments improve aggregation by encouraging formation of micro-aggregates, which are mechanically bound by root and fungal hyphae to form larger aggregates (Puget et al., 2000). Aggregation influences soil ecosystem functions by providing a better habitat for a larger and more diverse microbial population, and improving root growth and health (Sikora and Stott, 1996). Therefore, aggregation may be related to soil disease suppressiveness from a soil ecology perspective.

When correlations were performed on all data (Table 3.6.), DSI was best correlated with total C. However, this may be a coincidence due to the fact that the soil was high in sand and low organic matter to start with and that extremely high rates of amendments were applied to the soil. It is unlikely that there could be measurable changes in total C in such a short time on other heavier textured and/or high organic matter soils and the lower amendments rates. Therefore, total C would not likely be a universal or temporally sensitive indicator of disease suppression for practical soil management decision making.

Disease severity index was correlated with arylsulfatase on nearly all sampling dates. β-glucosidase had a high correlation with DSI up to day 106 and MBC became correlated with DSI as the incubation proceeded, being highly correlated at day 225. Arylsulfatase was strongly correlated with total C, and microbial biomass-C. This is consistent with previous reports that arylsulfatase is a good soil quality indicator that reflects soil management (Bergstrom et al., 1998; Ndiaye el al., 2000).

There were stronger correlations between suppression and some soil properties of our study that reflect long-term effects of organic amendments (total C, WSA, and arylsulfatase), and weaker correlations between suppression and indicators that reflect short-term effects of organic amendments (FDA and β-glucosidase activities). Soil that had composted material appeared to have greater potential to sustain disease suppression will be expected because it likely contains more stable C. There was also an amendment rate effect where decreasing rates of either composted or fresh paper-mill residuals had significant or strong trends of decreasing disease. Total C in the amended soils also followed these same trends (Fig. 3.9).

CONCLUSION

The present study provides evidence that paper-mill residuals amended to Wisconsin Plainfield loamy sand soils generated suppression of snap bean root rot. Enzyme activity and microbial biomass analyses indicated that microbial properties were stimulated more by high rates than low rates and more by compost than by fresh paper-mill material. However, some of the microbial properties were not always closely correlated with DSI. Soil quality indicators of the short-term effect of organic amendments were not always closely correlated with disease suppression compared to indicators of the long-term effect which had more consistent correlations with DSI. The soil quality indicators that reflected the microbial response to decomposition of the more labile constituents of the organic substrate were not highly related to DSI and are consistent with the fact that amendments had been decomposing for of one year before this experiment began.

There are three possible mechanisms involved in the suppression of oomycete fungal pathogens such as *Pythium* and *Aphanomyces* spp. Carbon competition generating fungistasis, lysis and loss of pathogen propagule viability, and SAR. Carbon competition can cause strong suppression immediately after amendments. This may have been important earlier on specific period during of the field study. However, this mechanism would be unlikely in the current study because labile C would have been lost during the year since the last amendment.

Pathogen destruction also did not appear to play a major role in our experiment, as regression analysis of DSI showed that none of slopes were significantly different among the treatments; meaning the natural decline of DSI was the same with or without amendments.

Our results showed that compost particularly at the high rate caused a shift in microbial populations as evidenced by arylsulfatase (a possible biomarker for

fungal biomass) and by culturing. This could be evidence that the soil microbial community could be capable of competition and pathogen destruction. However, these types of suppression mechanisms were likely occurring earlier in the field experiment or just after organic amendments.

Systemic acquired resistance could have occured in 1999 for field-grown snap beans to generate differences in pathogen inoculum levels that may have been sustained over three years prior to our study. At the same time, soil amendments, especially CH, may have supported a microbial community that induced SAR during the bioassays of our experimental period.

Correlation coefficients suggest that total C and arylsulfatase activity were the best indicators of disease suppression of snap bean root rot for the soil used in this study. But because total C would not likely show easily measurable differences in other heavier-textured soils and it is not an indicator of the quality of soil organic matter; it is unlikely that it would be a practical indicator of disease suppressive soils. Arylsulfatase was well correlated with disease suppression in this experiment and was the best indicator of disease suppression under our experimental conditions. DSI was also strongly correlated with β-glucosidase at the beginning of this experiment, but there were residual effects that seemed to be due to cover crop residue and was independent of disease suppression. This suggests that this parameter may better reflect a soil microbial response to recent label C inputs that may be associated with competition as disease suppression mechanism.

Further investigation should be directed towards disease suppression under field conditions where it would be possible to evaluate not only enzyme activity under natural conditions, but also more realistic conditions for viability of pathogen propagules.

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