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Sonia G. Schloemann
University of Massachusetts Amherst

EVALUATION OF ANTI-FUNGAL ORGANISMS, SOIL SOLARIZATION, COVER CROP ROTATION, AND COMPOST AMENDMENTS AS ALTERNATIVES TO SOIL FUMIGATION IN COMMERCIAL STRAWBERRY PRODUCTION

A Thesis Presented

by

SONIA G. SCHLOEMANN

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Approved as to style and content by:

Wesley Autio, Chair

Prasanta Bhowmik, Member

Daniel Cooley, Member

Marvin Pritts, Member

Lyle E. Craker, Department Head
Department of Plant and Soil Sciences

To Mom

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ABSTRACT

EVALUATION OF ANTI-FUNGAL ORGANISMS, SOIL SOLARIZATION, COVER CROP ROTATION AND COMPOST AMENDMENTS AS ALTERNATIVES TO SOIL FUMIGATION IN COMMERCIAL STRAWBERRY PRODUCTION

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SONIA G. SCHLOEMANN, B.S., UNIVERSITY OF MASSACHUSETTS

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by Associate Professor Wesley Autio

Key words: Strawberries, black root rot, *Rhizoctonia solani*, *Rhizoctonia fragariae*, weed control, fumigation, soil solarization, cover crops, biological control, *Trichoderma harzianum*, compost, suppressiveness.

The ability of soil solarization, cover crop rotations, anti-fungal biological control agents, and selected compost amendments to replace soil fumigation partially or fully in commercial strawberry production was studied. Strawberry plants, cv 'Honeyoye' and 'Kent' were grown in field and greenhouse studies where they were challenged either naturally or artificially with weed and disease pressure. Measurements of weed density and biomass were taken at specific intervals in the field studies. Measurements of strawberry plant survival, runner production, shoot and root fresh and dry weight, and visual evaluation root lesion density and plant vigor were taken in field and greenhouse studies.

Soil solarization had minimal effect on weed or strawberry growth. Weed biomass was lower and visual root root ratings were higher following solarization in one experiment compared to the control treatment. Buckwheat/winter rye and Sudex/winter rye cover crops suppressed weeds and improved strawberry growth with and without inoculation with *Rhizoctonia solani*. Strawberry plants grown in soil inoculated with the pathogen

Rhizoctonia spp. and also with the biological control agent *Trichoderma harzianum* were larger than those not treated with *T. harzianum*. Results differed somewhat depending on the species of *Rhizoctonia* (binucleate, multinucleate, or a mixture) with which the pots were inoculated. And, treatment of *Rhizoctonia*-inoculated soil with various composts did not suppress strawberry root infection effectively. One compost appeared to have a detrimental effect on strawberry plant growth.

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INTRODUCTION

1.1 Botanical Description of the Strawberry

The cultivated strawberry, *Fragaria x ananassa* Duch., is an herbaceous perennial plant obtained from the hybridization of the wild species *F. chiloensis* Duch. and *F. virginiana* Duch. *Fragaria* spp. are members of the family *Rosaceae* and the family tribe *Roseae* (or *Potentilleae*) and therefore botanically related to the genera *Potentilla* (cinquefoil), *Rubus* (raspberry and blackberry), and *Rosa* (rose) (Galleta and Bringhurst, 1990). Many cultivars of *F. x ananassa* have been produced that exhibit specific properties such as large fruit size, intense color, firmness, good flavor, suitability to a certain climate or production systems, or resistance to insects, mites, or pathogens.

The strawberry fruit is an enlarged fleshy receptacle bearing multiple seed-like achenes (true fruits) on its surface. While flowers of wild species of strawberry may be pistillate (female), staminate (male), or perfect, flowers of most modern cultivated strawberry varieties are perfect and are borne on a dichasial cyme. Leaves are compound pinnate and trifoliate comprised of three leaflets with serrate margins (Galletta and Bringhurst, 1990). The three leaflets come together basally at the end of a long petiole. Leaves, flower trusses, and stolons emerge from a fleshy crown. At the end of each year existing crowns undergo a process of lignification and lateral expansion producing 'new' crowns above the old crown. Two types of roots are produced, fleshy perennial or primary roots and fibrous transient or secondary roots. Primary roots arise adventitiously from the base of new leaves produced by the crown. These are soil-penetrating roots. Secondary roots arise from the primary roots and are the feeder roots. New primary and secondary roots are produced continuously by the expanding crown. The old crowns and roots remain attached to the plant throughout its life but decay over time. Under commercial management, this habit of producing new crowns and roots necessitates the

addition of new soil or organic matter over the plants each year to prevent the plants from growing themselves out of the ground.

Strawberries are propagated asexually from runner plants (daughters) produced at nodes on stolons emerging from the crowns of the mother plants. Single plants can produce multiple stolons, each with several runners. Modern commercial nurseries produce the mother plants through tissue culture propagation for its efficiency and speed, and to mitigate the risk of virus propagation. Mother plants initially are grown in greenhouses in soilless rooting media and then transplanted into the field. Once in the field, they are allowed to produce daughter plants. These daughters are harvested in large quantities for sale. Once planted, the daughter plants become mother plants which produce new runners.

1.2 Commercial Production in the Northeast

Strawberries are grown commercially in several regions of the United States. They grow best in a well drained, but not droughty, sandy loam with a pH between 5.5 and 6.5, a moderate to high organic matter content, and high cation exchange capacity (Galletta and Bringhurst, 1990). Different planting systems are used in different regions of the United States. In the West and South, the most common is the annual hill system, where mother plants are planted 20 to 30 cm apart in multiple rows on raised beds that are covered with plastic. Runners are not allowed to root, and the mother plants are fruited once and then removed from the field. Cultivars are bred specifically for this system of production.

In the North, strawberries are grown as perennials in beds planted as either wide matted rows (20 - 40 cm) where the mother plants are spaced such that the runners fill in the row more or less randomly, or as ribbon rows, where the mother plants are planted 10 to 15 cm apart and all runners are removed. Sometimes narrow matted or ribbon rows are set on a raised bed to improve air and water drainage.

Strawberries grown in the northern perennial system are fruited for multiple years. They are mulched in the winter for protection against sudden freezing, desiccation from winter winds, and heaving from the freeze/thaw cycle in the spring. Bed longevity usually is determined by productivity, and productivity is influenced by pest pressure, plant nutrition, and stress or damage resulting from winter injury, drought, or mechanical injury.

Competition from weeds and predation by disease organisms, nematodes, and insects can cause significant reductions in the profitability of commercial strawberry beds (Chandler, 1979). Soil-borne diseases especially can be devastating because they can destroy a planting rapidly with few if any control measures available once the plants are in the ground. The problem must be anticipated and treated prior to planting.

1.2.1 Weed Management

Successful weed control in strawberries is necessary to maintain bed productivity, bed longevity, and fruit quality (Chandler, 1979). Weeds are difficult to control in a perennial crop and may trigger the premature removal of a planting if not controlled adequately (Himelrick and Galletta, 1990). Weeds compete with strawberries for light, soil resources and provide a habitat for insects and diseases. Additionally, a dense weed canopy can inhibit spray penetration, reducing pesticide effectiveness (Himelrick and Galletta, 1990). Controlling weeds is crucial to success in commercial strawberry production (Himelrick and Galletta, 1990). Among the most common weeds found in strawberries grown in the Northeast are perennial grass and broadleaf species such as quackgrass (*Agropyron repens* (L.) Beauv.), goldenrods (*Solidago canadensis* L.),and dandelion (*Taraxacum officinale* Weber); annual grass species such as crabgrasses (*Digitaria sanguinalis* (L.) Scop. and *D. ischanaemum* (Schreb.) Muhl.), fall panicum (*Panicum dichotomiflorum* Michx.), and barnyard grass (*Echinochola crusgalli* (L.) Beauv.); annual broadleaf species such as, common lamb's quarters (*Chenopodium album* L.), purslane speedwell (*Veronica peregrina* L.), yellow wood sorrel (*Oxalis stricta* L.),

and horseweed (*Conyza canadensis* (L.) Cronq.), and winter annual species such as chickweed (*Stellaria media* (L.) Cyr.), and shepherd's purse (*Capsella bursa-pastoris* (L.) Medic. (Hemphill, 1980; Schloemann, 1992).

Weed control in strawberries is achieved in four ways: soil fumigation, herbicide application, mulching, and cultivation (Himelrick and Galletta, 1990). Soil fumigation is done primarily to suppress soil-borne diseases. Some weed control is an added benefit of fumigation. This benefit, however, does not continue beyond the first year because fields are recolonized easily by weed seeds through wind dispersal or importation on farm equipment. Since fumigation is expensive ($2,000 to $2,500 per hectare), it is not recommended for weed control alone.

Herbicides constitute a primary method of weed management in strawberries. Several pre- and post-emergence herbicides are used commonly in the northern perennial strawberry system; however, post-emergence broadleaf-weed management is difficult, because these materials can affect the strawberry plant also (Himelrick and Galletta, 1990). If weeds, especially broadleaf weeds, become established in the strawberry row, it is difficult to control them, and the bed longevity is usually shortened due to competition and other reasons described earlier. Since strawberries have only minor-crop status, there are relatively few herbicides registered for use on them compared with major crops. Herbicides and other pesticides are likely to become less available in the future owing to the high cost of reregistration for these materials. Therefore, relying on herbicides alone to control weeds in strawberries may be risky.

Strawberries are mulched for winter protection (Galletta and Bringhurst, 1990). In the spring, the mulch material is raked off the rows and deposited in the alleys between rows. The mulch provides an added benefit by suppressing weed growth between the rows and by providing a "clean" surface for pickers in the field (Himelrick and Galletta, 1990). In addition, it reduces splashing from rains or irrigation which can spread some

kinds of disease inoculum, particularly *Phytophthora cactorum* (Lebert & Cohn) Schröt (Grove *et al.*, 1985).

In the northern perennial strawberry system, a process of bed renovation is undertaken after harvesting. At this time, mature foliage is removed by mowing, stimulating growth of new foliage and rejuvenating the plants. The mulch material remaining in the alleys is tilled into the soil. A pre-emergence herbicide may be applied at this time, a cultivation schedule commenced, or both. Cultivation brings new weed seeds to the surface, and therefore, must be done regularly to control newly germinating weeds.

There is some evidence that herbicide applications may result in a higher disease incidence in a crop (Altman and Campbell, 1977; Papavizas and Lewis, 1979). This situation is referred to as herbicide-induced microbial invasion of plants (Greaves and Seargent, 1986). Curry and Teem (1976) list nine classes of herbicides and three unclassified herbicides that have activity that significantly affects roots. The effects range from inhibition of primary or lateral root growth to root cell disorganization and multi-nucleation. 2,4-dichlorophenoxyacetic acid (2,4-D) and other members of the phenoxy acid group have been studied more extensively than other herbicide groups (Greaves and Seargent, 1986). Members of this group can cause decreased root length and production of large numbers of short lateral roots (Totman and Davies, 1978). Since the lateral roots are often host to large numbers of rhizosphere microorganisms, stimulation of lateral root growth may enhance the environment for pathogen proliferation and eventual cortical penetration. Thus, herbicide application may predispose plants to pathogen infection. Katan and Eshel (1974) suggest four mechanisms by which herbicides can affect plant disease: direct effect on pathogens, effects on pathogen virulence, effects on host susceptibility, and effects on non-target microorganisms and their relationship with the host or pathogen.

Weed management requires significant inputs of labor and materials in order to be successful; approximately $5,000 per hectare (including fumigation) to bring a planting to fruiting and $1,250 per hectare per year thereafter, for the life of the planting (Goulart,

1991). And, weed control failure can lead to decreased productivity and shortened bed longevity (Hemphill, 1980; Schrocth and Monaco, 1980). When weed pressure is high, inputs are greater and the chance of failure is greater. When weed pressure is low, inputs can be reduced, the chance of success is greater, and profits likely will be increased. Additionally, herbicide application to strawberries may play a role in disease incidence predisposing the roots to pathogen infection. Therefore, suppressing weed pressure prior to planting strawberries and maintaining it at a low level with reduced herbicide inputs may make the enterprise more profitable and sustainable.

1.2.2 Black Root Rot: A Disease Complex of Strawberry

As mentioned in the botanical description above, strawberries have two types of roots: fleshy, structural, perennial roots and fibrous, secondary, feeder roots. Under good growing conditions and in the absence of pathogen infection, growth of both types of roots can be extensive. Healthy roots of both types generally are creamy white or light brown in color. In a porous friable soil, individual perennial roots may reach 100 cm in length (Galletta and Bringhurst, 1990). In a heavier soil, root growth is restricted. Most of the strawberry root system (50 - 90%) is found in the upper 15 cm of soil (Galletta and Bringhurst, 1990). An example of a healthy strawberry root system compared with roots affected by different diseases is shown in Figure 1.1.

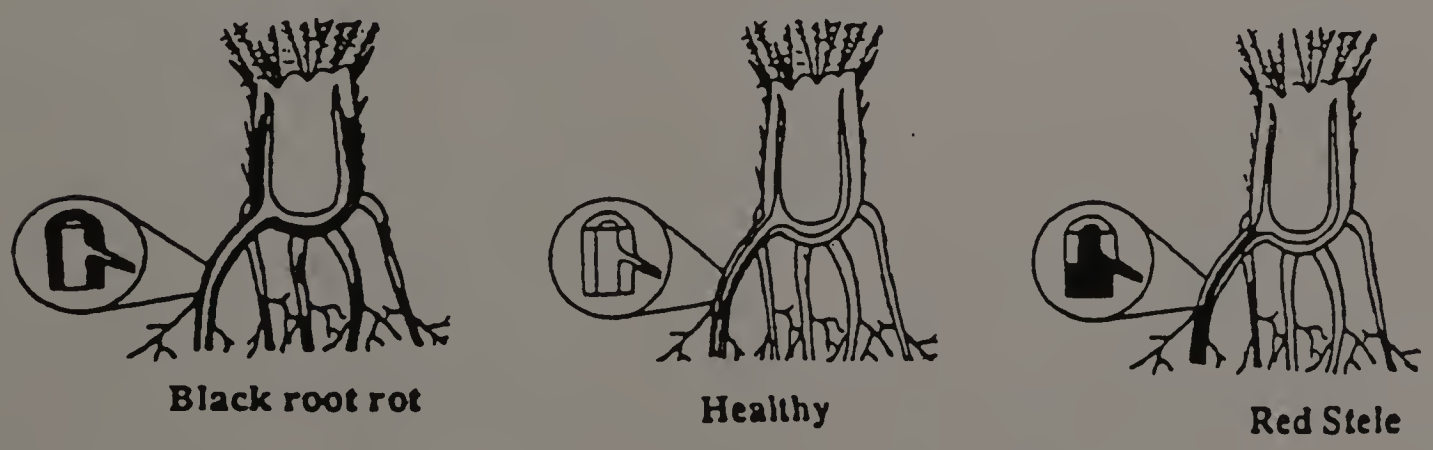

Figure 1.1. Typical appearance of strawberry roots showing black root rot (left) and red stele (right) diseases compared to a healthy plant (center) (after J. L. Maas, 1984.)

Perennial roots are structural, soil-penetrating organs which provide conduits for movement of water and nutrients, and tissue for storing food reserves. These roots are long-lived, persisting for months before eventually deteriorating and being replaced by new perennial roots which arise adventitiously from the crown. Dunne and Fritter (1989) found evidence that even decaying roots may still play a significant role in nutrient uptake of the plant. Since the stele remains intact for a while, even when the epidermal and cortical layers have decayed, it can still function in transporting water and nutrients.

The secondary roots are the finer feeder roots and are more transient than the perennial roots. They originate from the perennial roots and persist up to two weeks, after which they deteriorate, die and are replaced by new feeder roots (Galletta and Bringhurst, 1990). The cycle of root initiation, growth, deterioration, and replacement is important to the health of the plant, and disruption of this cycle can affect plant health significantly.

Secondary roots are more disposed by their composition and structure to be susceptible to infection by pathogens than perennial roots. Secretions of soluble root exudates from secondary roots generate a rhizosphere environment that promotes colonization by microorganisms, some of which are pathogenic (Greaves and Darbyshire, 1972). Cells of the epidermal layer of these secondary roots are thin walled and thus vulnerable to penetration. Very young perennial roots are similarly vulnerable. As these roots age, they undergo changes in structure and differentiation of tissues (Mann, 1927; 1930). These changes affect the ability of soil-borne fungi to penetrate the root epidermis and cortex, making older perennial roots less vulnerable to pathogen attack. On the other hand, if the endodermis has not sufficiently suberized to prevent vascular entry of a pathogen, decaying perennial roots may become infection courts as the epidermal and cortical cells break down (Kolattukudy, 1980).

Strawberries are affected by several soil-borne fungal pathogens including *Phytophthora* spp., *Rhizoctonia* spp., *Pythium* spp., *Verticillium* spp., and the root-lesion nematode *Pratylenchus penetrans* (Cobb) Filip & Stek.. These organisms may be present

singly or in combination with other organisms to produce diseases or disease complexes. *Phytophthora fragariae* Hick. causes a common disease known as red stele, named for its characteristic appearance. *Phytophthora cactorum* causes other crown- and root-rot diseases. Verticillium wilt is caused by *Verticillium albo-atrum* Reinke & Berthier. Black root rot is a disease complex in which several organisms have a role. Constituents of this complex include *Rhizoctonia solani* Künh *R. fragariae* Hussain & McKeen, *Pratylenchus penetrans*, and possibly *Verticillium* spp., and *Pythium* spp. (Maas, 1984). These organisms all are common inhabitants of soil and may be present without causing disease symptoms. Symptoms usually arise as a consequence of stress on the plant from sources including low winter temperatures, excessive or deficient water, nutrient stress, or wounding injury caused by insects feeding on the roots.

Symptoms of black root rot of strawberries include a general failure to thrive, poor runner production, stunted growth, small bluish leaves, small fruit , wilting or collapse of the plant canopy during water stress, or plant death. All these above-ground symptoms arise from a weakened, poorly functioning root system. Black root rot infection of the roots first causes blackening and death of fibrous secondary roots, and then deterioration and blackening of the cortex of the fleshy perennial roots. It is the infection of the perennial roots that is most significant, because they form the main conduits between the root system and the crown and canopy of the plant. At first, the core or stele of the root remains white but eventually the entire root becomes blackened and decayed. Once a lesion has girdled the root and the stele has become necrotic, the rest of the root below the lesion, including all its branches, dies (Zeller, 1932). The initial lesions are reddish but melanize and darken with age. The disease progression is distinct from that of red stele root rot which discolors the stele of the root first. Above-ground symptoms of the two diseases, however, are similar.

The extent of damage from black root rot in any given field is variable. In light infestations, a general loss of vigor may be observed in patches within the field. Rows

may appear thin or patchy in spots where runners are sparse. In more severe infestations, larger areas may exhibit decline, poor berry production, or wilting in hot weather. In very severe cases, portions of the planting die, sometimes within the first year of planting. More typically, the disease progresses over several years becoming more severe with time.

Black root rot is often difficult to recognize, especially in early stages of a field epidemic, because the symptoms are not always obvious or distinct. When a field simply shows poor growth and yield, it may be attributed to another cause such as poor fertility, winter injury, poor cultivar performance, or natural aging. These factors may contribute to the decline, but may do so in the context of black root rot pathogens. In order for a disease to be expressed in plants, three conditions must be satisfied as illustrated in Figure 1.2; i.e., a susceptible host and virulent pathogen must be coupled in an environment that is suitable for disease development.

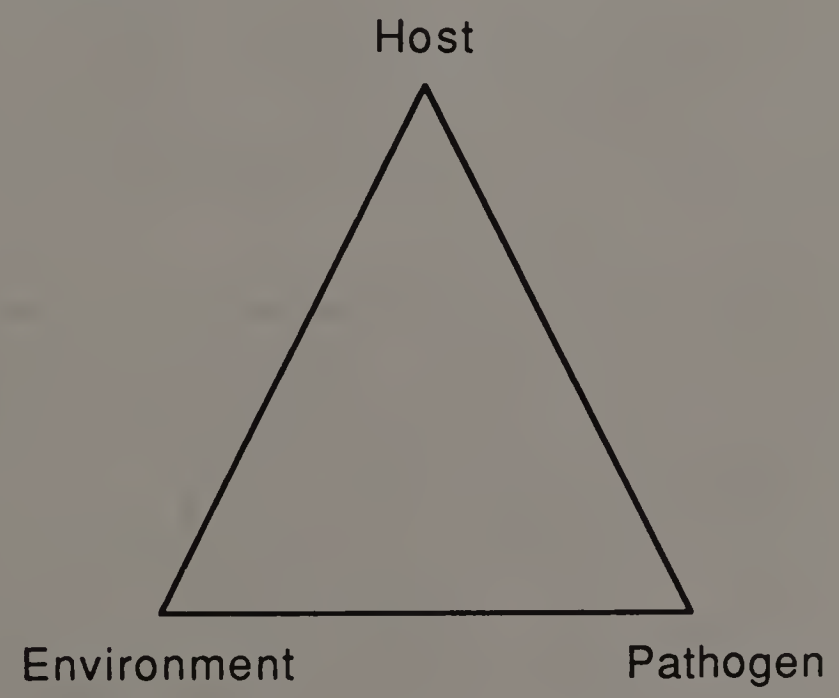

Figure 1.2. The 'Disease Triangle'.

Rhizoctonia spp. are considered to be primary pathogens in the black root rot complex in Massachusetts (Drosdowski, 1987), Connecticut (Martin, 1987; LaMondia and Elmer, 1992), Canada (Husain and McKeen, 1963), and Israel (Razik, *et al*, 1989). In fact, the relationship between *Rhizoctonia* spp. and black root rot of strawberry has been described for decades (Heald, 1920; Zeller, 1932; Boyd, 1933). In each of these studies,

the majority of isolations made from root lesions of plants exhibiting black root rot symptoms were identified to be *Rhizoctonia* spp., and *Rhizoctonia* spp. were the only organisms able to reproduce the original symptoms, thereby confirming Koch's postulate. Other organisms were also found and identified, but their role in the disease complex remains unclear. It is thought that *Pratylenchus penetrans* may be a key to the disease by producing entry sites for the pathogen (Raski, 1956; Townsend, 1962; LaMondia and Martin, 1989). Other organisms may play a role in weakening the plant so that it is more susceptible to infection by *Rhizoctonia*. Similar symptoms may be produced by other pathogens, such as *Pythium* spp. (Wilcox, pers. comm.) leading to some confusion.

The genus *Rhizoctonia* belongs to the Class Fungi Imperfecti, Order Mycelia Sterilia. It is a highly diversified genus. Species of this genus are soil-borne and ubiquitous. Defining attributes include primarily vegetative mycelium (rarely producing a perfect stage) characterized by hyphal branching at right or acute angles, hyphal constriction at the point of branching, and presence of septa near the point of branching (Husain and McKeen, 1963). Morphological characteristics, including the number of nuclei in cells of the mycelium, are used to differentiate among species (Ogoshi, 1987; Parmeter *et al.*, 1967). Further differentiation within species is made according to anastomosis groupings (AGs), i.e., isolates of individual species are grouped according to their ability to anastomose or fuse hyphae. If hyphae of different isolates are unable to fuse, they belong to different AG groups.

Rhizoctonia spreads by proliferation of mycelia in the soil growing saprophytically on soil organic matter. Species rarely form a perfect stage and therefore do not produce spores; however, resting structures of highly condensed mycelia called sclerotia are produced. These structures fulfill a role similar to spores. Sclerotia, 0.1 - 10 mm across, are resistant to environmental stress such as freezing or drought and can remain viable in the soil or plant residue for up to five years (Cook and Baker, 1983). This persistence adds to the difficulty in controlling diseases caused by *Rhizoctonia* spp. Even if the more

susceptible mycelia of the organisms are suppressed effectively with a biocidal treatment, sclerotia may survive, germinate, and recolonize the soil rapidly.

When mycelia of *Rhizoctonia* contact a host, they form infection cushions or appresoria with infection pegs. Root exudates given off by susceptible hosts stimulate hyphae of *Rhizoctonia* to proliferate. Husain and McKeen (1963) found that root exudates given off by strawberry plants grown in cool soil (5° and 10°C) stimulated mycelial proliferation in *Rhizoctonia fragariae,* while exudates from plants grown in warm soils (20° and 30°C) did not. Using chromatographic analysis of exudates, they were able to determine that greater amounts of the amino acids alanine, serine, glutamine, glycine, and threonine were present in exudates from roots in the cool soil and were, in fact, responsible for the enhanced growth of *Rhizoctonia.* Others have observed that black root rot infections occur primarily in cool soils of the spring and fall (Martin, 1988; LaMondia and Elmer, 1991).

There are two species of *Rhizoctonia* associated with black root rot of strawberries: *R. solani*, which is multinucleate, and *R. fragariae*, which is binucleate. Anastomosis groups of *R. solani* and *R. fragariae* are labeled AG 1-5 and AG A-O, respectively. Further delineation is made within some AG groups and are labeled intra-specific groups (ISG). This complicated taxonomy reflects the diversity in this genus.

Rhizoctonia solani (perfect state *Thanatephorus cucumeris*) is a ubiquitous and cosmopolitan pathogen with one of the widest host ranges known (Farr *et al.*, 1990). Baker (1970) describes *R. solani* as causing " ... more different types of disease to a wider variety of plants, over a larger part of the world, and under more diverse environmental conditions, than any other plant pathogenic species." In one of the earliest and most comprehensive descriptions of black root rot, Zeller (1932) found that in 78.5% of the 5,715 black-root-rot-related strawberry root lesions from which he isolated, *R. solani* was the causal agent. It has been associated with serious losses in strawberries in different parts of the world. In Israel, Elad and Chet (1981) and Razik *et al.* (1989) found *R. solani*

to be the major element of root rot disease in commercial fruiting and nursery fields. Van Adrichem and Bosher (1962) identified *R. solani* as a component of the strawberry root rot complex in British Columbia. Italian researchers found *R. solani* and *R. fragariae* to be the major constituents of the black root rot complex there (D'Ercole *et al.*, 1989).

Rhizoctonia fragariae (perfect state *Ceratobasidium* spp.) is also associated widely with the black root rot complex. Unlike *R. solani*, the perfect state of *R. fragariae* typically is found and commonly produces spores. Sclerotia are not produced by *R. fragariae*. Wilhelm *et al.* (1962) isolated *R. fragariae* from plants collected from the major strawberry producing areas of California and found it to be ubiquitous. In West Virginia, *R. fragariae* was isolated consistently from plants collected from declining and healthy fields as well as from nursery stock (Ribeiro and Black, 1971). Martin (1988) determined that *R. fragariae* associated with black root rot of strawberry belonged primarily to groups AG A, AG G, and AG I, with the former being the most common but the latter being the most virulent.

Drosdowski (1987), in a survey of fungi associated with black root rot in Massachusetts, found that *R. fragariae* was the dominant species isolated from plants collected from declining and healthy fields (as in West Virginia) and from wild strawberries. In laboratory tests, Husain and McKeen (1963) determined that root infection by *R. fragariae* occurred almost invariably and most severely at low soil temperatures (5° and 10°C) and rarely at higher soil temperatures (20° and 30°C). When plants were collected from the field in July, none of the root isolations yielded *Rhizoctonia*. *Pythium* spp. (35%), *Fusarium* spp. (29%), and *Trichoderma* spp. (20%) were the organisms most commonly isolated from roots collected at that time. Roots collected in March, however, yielded primarily *R. fragariae* (58%) with the other species found in lesser amounts (36% combined).

1.2.3 Soil Fumigation: Risks and Benefits

Chemical soil fumigation with compounds like methyl bromide, metam sodium (Vapam™), chloropicrin, and methyl isothiocyanate (Vorlex™) is the most common means of combating soil-borne diseases (Himelrick and Dozier, 1991). Methyl isothiocyanate has been the most commonly used fumigant for strawberries in the Northeast but has recently been voluntarily withdrawn from the market by the manufacturer. Methyl bromide is under special review by the EPA as a potential ozone-depleting chemical and may also be withdrawn from the market.

Benefits obtained from fumigation include suppression or elimination of weeds, soil-borne pathogens, nematodes, and some soil-inhabiting insects. In addition, it has been shown that fumigation can, in some cases, stimulate plant growth and vigor beyond the benefit obtained by the elimination of identifiable pests (Altman, 1970). Enhanced growth response after fumigation may be due to increased amounts of plant nutrients released by killed microorganisms (Kreutzer, 1965), temporary disruption of nitrification causing a build-up of NH_4^+ (Altman, 1963; Altman and Tsue, 1965), or other mechanisms. Altman and Tsue (1965) observed an increase of nitrogenous compounds in soil after fumigation and an increase of certain saprophytic microorganisms (*Pseudomonas* spp. and *Arthrobacter* spp.), some of which may be plant-growth-promoting rhizobacteria (PGPR).

PGPR belong primarily to the genus *Pseudomonas* and are associated with growth-promotion and greater yields in crops like soybean and canola (Zablotowitz *et al.*, 1991). They metabolize seed or root exudates and colonize the rhizosphere. The mechanisms of growth promotion are not understood but may include the production of antibiotics or siderophores and the resulting displacement of pathogens (Kloepper, 1988).

Altman and Lawlor (1966) further observed a fundamental ecological shift in soil treated with a biocide. They noted the destruction or disruption of soil parasites and non-spore-forming saprophytes and the subsequent colonization of the soil by spore-forming or sclerotia-forming microflora. This change resulted in niche replacement by organisms like

Trichoderma spp.(spore-forming beneficial fungus) or *Rhizoctonia* spp. (sclerotia-forming pathogen) after fumigation. They observed the development of a new constellation of climax species in treated soil that maintained a new dynamic equilibrium. Kreutzer (1960) described this equilibrium as being defined by soil biophase sustainers and inhibitors. Following biocidal treatment, the resulting biophase established a new set of influences on the other soil constituents including the crop. This concept is illustrated in Figure 1.3. Soil biophase constituents may be beneficial, benign, or pathogenic to the crop. And, successful fumigation (or any soil treatment) may require tools for predicting or influencing the constituents of the resulting soil biophase.

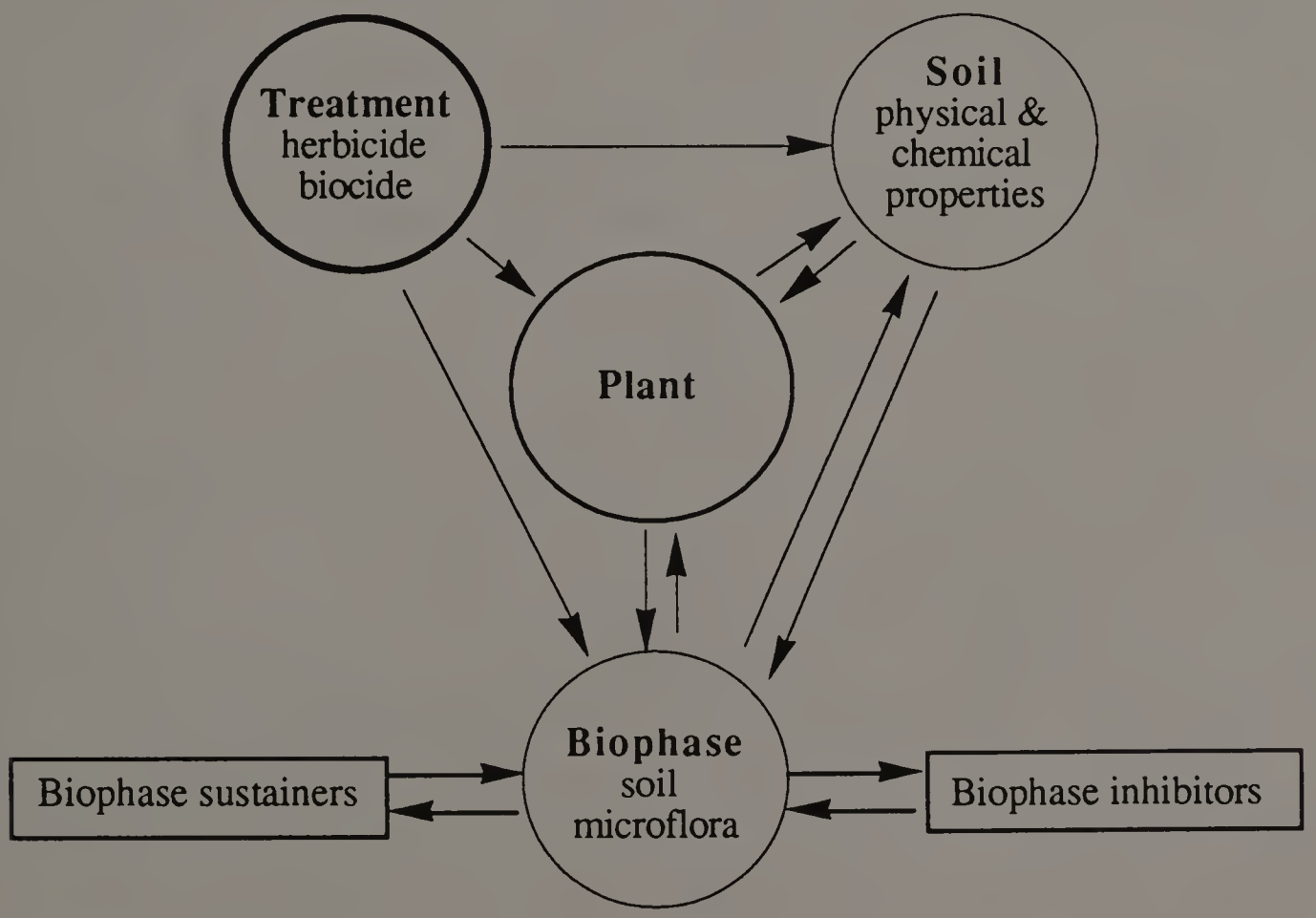

Figure 1.3. The influence of soil-applied treatments on soil, soil biophase, plant, and their interactions (after Altman, 1970).

While soil fumigation can improve crop growth and yield through various mechanisms, these benefits also carry three major economic and ecological risks. Three major risks predominate. First is an environmental risk. Since soil fumigants are applied directly into the soil (usually by injection), the risk of leaching of the active ingredient or

breakdown products is present. Relatively large quantities of these materials are applied to the soil (e.g., 280-375 liters per hectare for Vorlex™) and remain in place undisturbed for several weeks before the soil is ventilated. Guns (1989) found a five-fold increase in the bromine concentration in groundwater and a 15-fold increase in soil bromine in the 50 - 75 cm depth range one month after fumigation with methyl bromide in greenhouse culture in Belgium. Elevated levels of bromine lasted for up to two years. He also found that under certain conditions for up to two years after treatment, bromine accumulated in crops (especially lettuce and tomato) grown on treated soil. Methyl bromide has been identified by the U. S. Environmental Protection Agency (EPA) as a potential ozone-depleting chemical. As such, it is under review and may be subject to removal from the market.

The second risk is economic. Fumigation is an expensive practice, costing about $2,500 per hectare for material and application (Schloemann *et al.*, 1992). A grower must be sure that the potential benefits exceed the cost of treatment, an *a priori* judgement which is difficult and imprecise. For example, if a field is fumigated to control black root rot, the grower must infer a need to fumigate based on field history; i.e., a previous planting succumbed to symptoms that resembled black root rot. This inference may be incorrect. The previous planting may have declined owing to an abiotic stress such as winter cold, poor fertilization, drought, or renovation, completely independent of any pathogen. Less costly practices may remedy the problem, such as better winter protection, crop rotation with a 'stale seed bed' phase, resistant cultivar selection, a nutrient management plan, and beneficial soil amendments.

The third risk is the potential for treatment failure. Soil fumigation is not always effective against the target problem. For example, when transplants, especially those grown in field soil, are planted into fumigated soil, microorganisms (including pathogens) on the roots of the runners, even in small quantities, can colonize the soil rapidly where fumigation has destroyed the soil ecology and no biological checks and balances exist (Altman, 1970; Haasis, 1952). This response is known as 'the boomerang effect'

(Kreutzer, 1960). Kreutzer observed more disease in sugar beets after fumigation with chloropicrin due to the rapid recolonization of soil by *Rhizoctonia* and *Pythium*. Haasis (1952) described the control of bulb rot of iris caused by *Sclerotium rolfsii* Sacc.by soil fumigation but the concurrent increased infection from bulb-borne *Fusarium* transplanted into the fumigated soil. Huber *et al.* (1965) observed an increase in *Rhizoctonia* infection of potato following Telone™ fumigation for Verticillium wilt. In this case, the increase in NH_4^+ following fumigation both stimulated *Rhizoctonia* pathogenicity and made potato roots more susceptible to infection.

Anecdotal evidence exists that the boomerang phenomenon has occurred in fumigated strawberry plantings. The partial biological vacuum established by soil fumigation can be colonized rapidly by organisms inhabiting the roots of field-grown strawberry runner plants which are transplanted into the fumigated production field. These organisms may only be present in small quantities on the transplants but once placed in the fumigated soil, they may colonize it freely. If pathogens are present among these root inhabitants, disease severity may be worse with fumigation than without it (Yuen *et al.*, 1991; Elad *et al.*, 1981) Additionally, soil fumigation may activate resting structures of pathogens inhabiting the soil. Husain and McKeen (1963) described this phenomenon where sclerotia of *Rhizoctonia fragariae* did not germinate in non sterilized soil but germinated in sterilized soil.

Additionally, fumigation may exacerbate weed problems such as *Oxalis stricta* (yellow wood sorrel) by increasing germination by seed coat scarification (R. Bonnano, pers. comm.). The result may be trading one problem for another.

1.3 Thesis Rationale

Strawberries are a high value crop ($12,500-$60,000/ha gross value) in New England grown primarily as a component of diversified farms providing early cash flow and attracting customers to other profitable on-farm enterprises like road-side stands. As

such, they provide a key element to the profitability of some farm enterprises. Still others rely on revenues generated by sale (wholesale and retail) of strawberries as their primary crop and depend on their profitability for success. Successful strawberry production requires significant pest management inputs. Commercial strawberry producers rely mainly on crop protection chemicals for pest management.

In United States agriculture today, there is a trend toward reduction in the amount of crop protection chemicals used on farmland. This trend is motivated, in large part, by public concern over food and groundwater safety. The Alar™ scare in apples in 1989 served to focus public attention on chemicals applied to crops and the hazards associated with residues on food and potential groundwater contamination. Integrated pest management (IPM) is an approach to crop production that seeks to minimize the use of crop protection chemicals by emphasizing knowledge-based pest management rather than calendar-based or prophylactic pest control. The IPM practitioner is skilled in crop production methods (crop rotation, use of resistant cultivars, etc.) that help mitigate pest pressure and in pest monitoring techniques that indicate when pest management action is necessary. When pest management action is indicated, the emphasis is on management of the pest within the context of the entire pest complex and crop ecology rather than on controlling a single pest species in isolation. An example of this strategy in strawberries comes into play when field scouting indicates that the population of strawberry bud weevil (*Anthonomus signatus* Say) exceeds the action threshold and also indicates the presence of two-spotted mite (*Tetranychus urticae* L.). In this case, the IPM recommendation would be to use an insecticide for suppressing strawberry bud weevil that is not detrimental to the two-spotted mite predator, *Amblysieus fallacis*, thereby preserving the naturally occurring pest control agent of two-spotted mite. IPM actively involves the use of biological or biorational pest control agents in place of synthetic chemical pesticides when available, affordable, and effective.

The Council on Environmental Quality (Bottrell, 1979) defined IPM as "... the selection, integration, and implementation of pest control based on predicted economic, ecological, and sociological consequences. IPM seeks maximum use of naturally occurring pest controls, including weather, disease agents, predators, and parasites." Since that time, IPM has become part of a larger movement toward sustainable agriculture, concerned with more than just pest management. Just as one pest cannot be managed successfully in isolation of the whole pest complex, pest management practices cannot be viewed in isolation from the whole crop production system. The United States Department of Agriculture (USDA) defines sustainable agriculture as "...an integrated system of plant and animal production practices having a site-specific application that will, over the long term: satisfy human food and fiber needs, enhance environmental quality and the natural resource base upon which the agricultural economy depends, make most efficient use of non-renewable resources and on-farm resources and integrate, where possible, natural biological cycles, and complies with community norms and meets social needs. (Farm Bill, 1990)." As such, sustainable agriculture is a broad socio-political goal, not a prescribed set of practices, methods, or inputs to be applied broadly to farming. Efforts toward sustainability in agriculture, however, are guided by the scientific principles of ecology, i.e. agroecology, which have broad application and can be adapted to different farm settings. Agroecology provides a new area of agricultural research and powerful tools for generating new methods of pest management that do not rely exclusively on crop protection chemicals.

These broad trends within agriculture, fueled by public attitudes toward pesticides, provide powerful incentives for strawberry growers in the Northeast to adopt IPM methods in their production system, and many of them have. In a recent survey of Massachusetts strawberry growers, 83% identified themselves as IPM practitioners (Schloemann *et al.*, 1992). This high level of grower identification with IPM may reflect less actual pest management practices than a general attitude that IPM is something positive. Current IPM

methods in strawberries address key arthropod pests, fruit rot and leaf spot pathogens, and, to a lesser extent, weeds (Schloemann *et al.*, 1993). As such, growers cooperating with the university-sponsored IPM program in Massachusetts have reduced their overall pesticide inputs by 30-40% (Schloemann *et al.*, 1992). One of the most significant pest problems faced by New England strawberry growers, however, is black root rot, a disease problem for which, as yet, there is no IPM strategy. Additionally, the current conventional strategy of soil fumigation has diminished appeal due to high cost, potential treatment failure, potential environmental hazards, public pressure against fumigation, and loss of registered materials as discussed above.

For these reasons, it is necessary to investigate alternative practices to soil fumigation so that commercial strawberry production may remain a viable and sustainable element of successful farming in New England and so that a more comprehensive IPM system is available to strawberry growers. Furthermore, principles discovered through the investigation of these alternatives may have broad application to crop production and soil management in general since some of the pathogen and weed problems mitigated by the methods investigated are common among several crops and farming systems.

Investigating alternatives to soil fumigation requires that the focus, at first, remain narrow and specific. New building blocks are needed for an alternative strategy for soil-borne pest management. This strategy will be developed on a fundamentally different premise (i.e., enhancing the biological life and diversity in the soil rather than striving to achieve a biological vacuum through biocidal fumigation) and may achieve a more balanced and resilient agro-ecosystem that favors plant health over disease. These building blocks must first be constructed separately and later used in combination for a comprehensive approach to crop management.

When considering a new premise for management of soil-borne pests, it is useful to reconsider the disease triangle (Figure 1.2). In that depiction, the integral role of the soil biophase separate from the pathogen is not represented. The environment is simply another

leg of the triangle that is presented as a distinct entity. Burpee (1990) offers an alternative model (Figure 1.4) of the disease triangle that places the environment at the center of the relationship among plants, pathogens, and soil microflora (including biocontrol agents). When viewed this way, the true complexity of the relationships is represented and the key elements in management of disease are discernible. Plant genotypes can be manipulated to confer genetic resistance, pesticidal action can be taken against the pathogen, beneficial action can be taken to increase the effect of biocontrol agents, and the environment can be manipulated to affect the plant, pathogen, or biocontrol agents.

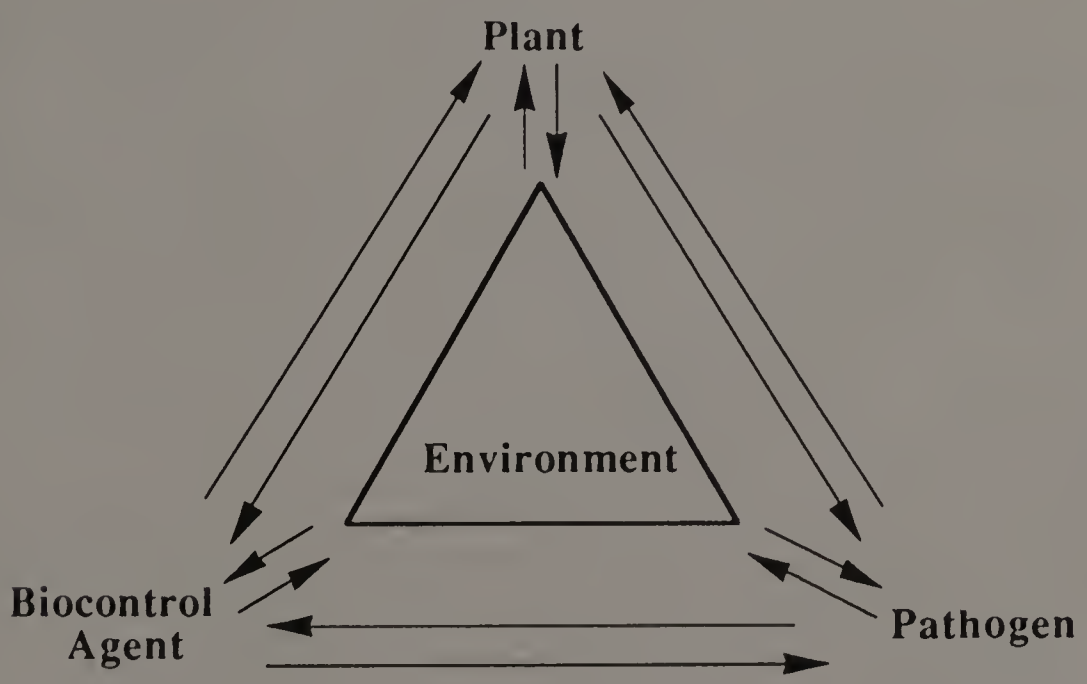

Figure 1.4. Disease triangle (after Burpee, 1990) illustrating the role of the environment on the interaction among plants, pathogens, and biocontrol agents in the development of plant disease.

1.4 Thesis Objectives

This thesis seeks to investigate four possible alternatives to soil fumigation for managing *Rhizoctonia*-induced black root rot and weeds in strawberries: soil solarization, cover crop rotations, microbial antagonists, and selected compost amendments to induce or enhance *Rhizoctonia* suppressiveness of soil. Each of these techniques has been studied extensively in other cropping systems and some have been studied in strawberries. None

individually is likely to substitute fully for soil fumigation, but in combination may provide viable or perhaps better and more sustainable alternatives to biocidal treatments.

Soil solarization has been used as a method for partial pasteurization of the top layer (15-20cm) of soil, changing the ecology to favor growth of beneficial or benign microorganisms, reducing disease potential, and reducing viability of weed seeds (Katan, 1980). Researchers have met with success using this method for suppressing a variety of pathogens including some which are detrimental to strawberries (*Rhizoctonia, Pythium, Verticillium*). Cover crop rotations have been used successfully for reducing weed pressure. Different mechanisms like interspacific competition and allelopathy may explain how cover crops suppress weeds. To a lesser extent, cover crops have been studied for disease suppressing properties. Solarization and cover crop rotations for suppressing weeds and black root rot in strawberries are discussed in Chapter II of this thesis.

The use of microbial antagonists for suppressing plant pathogens has been studied widely. One of the most commonly studied genera of antagonists is *Trichoderma* (Cook and Baker, 1983). Members of this genus have been studied extensively as biocontrol agents for *Rhizoctonia*, *Phytophthora*, *Pythium*, *Sclerotium*, and many other fungal pathogens. Many of these organisms are significant pathogens of strawberries. *Trichoderma* spp. are also often identified as soil biophase constituents in disease suppressive soil (Cook and Baker, 1983). Cook (1991) asserted that no other area of research offers more benefit to improving crop production and advancing agricultural sustainability than that of rhizosphere microbiology, and that understanding this field is the key to managing root, and therefore plant, health and plant nutrition through efficient nutrient uptake by roots. Mechanisms involved in disease suppressiveness and biological control of plant pathogens are not understood fully but include competition, antibiosis, and predation/parasitism (Liu and Baker, 1980). The use of microbial antagonists for suppressing *Rhizoctonia*-induced black root rot of strawberries is discussed in Chapter III of this thesis.

Disease suppression through the use of compost amendments is an area of study which is relatively new. While horticultural benefits of compost amendments have been long recognized, the role of compost in generating or enhancing disease suppressiveness in soil or growing media is an area of intense research and study. Selected composts have been identified which, when used to substitute for peat moss in container mixes, suppress damping off pathogens, e.g., *Rhizoctonia, Pythium, Phytophthora* (Hoitink, 1986). This new aspect of compost utilization is stimulating great interest, but the corollary aspect of compost engineering, with the aim of generating disease suppressive properties, is also compelling. A further societal benefit may be achieved if otherwise problematic organic residuals (e.g., municipal yard or solid waste or food processing waste) can be used to produce valuable compost. The use of selected composts for suppressing *Rhizoctonia*-induced black root rot in strawberries is discussed in Chapter IV of this thesis.

Finally, in the conclusion (Chapter V) of this thesis, the potential of each of these techniques individually and in combination as alternatives to soil fumigation for managing black root rot in strawberries and as a component of a comprehensive IPM strategy for growing strawberries in the Northeast will be discussed.

CHAPTER II
SOIL SOLARIZATION AND COVER-CROP ROTATION STUDY

2.1 Literature Review

2.1.1 Soil Solarization

2.1.1.1 Description and History

Solarization is the solar heating of soil using an unventilated clear plastic covering. In this technique, soil is covered for several weeks when the solar radiation potential is high. A "greenhouse effect" develops under the plastic, heating the soil. Elevated temperatures, either directly or indirectly, affect the physical, biochemical, and microbial composition of the soil and can result in the destruction of weed seeds and plant pathogens (De Vay, 1991a). Soil temperature is elevated to the greatest extent close to the surface of the soil, but for solarization to be effective, soil temperature must be raised at depths of 10 to 30 cm (Katan, 1987; Stapleton and De Vay, 1986). High soil moisture improves heat conductivity. Seventy percent of field capacity within the top 15 cm of soil and moist to 60 cm depth is optimal (De Vay, 1991b).

Solar heating of the soil for pest control originated from the use of black plastic film as mulch (De Vay, 1991a). It was noted that black plastic heated the soil and prevented weed growth by blocking sunlight. Katan *et al.* (1976) were the first to publish work on the heating of soil with transparent plastic film as a method of partial pasteurization to suppress soilborne diseases. In their studies, they buried inoculum of *Fusarium oxysporum* Schlech.and *Verticillium dahliae* Kleb.at 3 soil depths; 5 cm, 15 cm, and 25 cm. Soil was moistened and covered with clear polyethylene film for 14 days. The same inocula were buried in non-solarized soil for comparison. *V. dahliae* was eliminated by solarization at all three soil depths. At the 5 cm soil depth, *F. oxysporum* viability was reduced by 94 - 100%. At 15 cm and 25 cm, viability was reduced by 67 - 100% and 54 -

74%, respectively. This was the first of many studies which has led to the development of soil solarization as a viable pest management technique in some parts of the world. Katan *et al*, (1987) wrote a chronological bibliography of the first decade (1976-1986) of soil solarization research and cite 173 articles from over 20 countries.

The basis for soil solarization (or any kind of pasteurization process) is that many organisms, including many weeds and plant pathogens cannot survive or grow at very high temperatures. Some are inhibited or killed directly by temperature extremes, i.e., slowed or halted metabolism at low temperatures (Paul and Clark, 1989), or phase change of lipids in membranes at high temperatures (Brock, 1978). Some are killed or weakened as the result of biochemical changes in the soil environment or by the stimulation of thermophilic microbial antagonists following solarization (DeVay, 1991b).

Lethal temperatures differ among organisms and are expressed as LD_{90}. That is, the lethal dose (time) at a specified temperature that kills 90% of the target population. A certain organism may have an LD_{90} at 37°C of 2 weeks and an LD_{90} at 47°C of 1 hour (Pullman *et al.*, 1981). It may also be true that organisms have an LD_{90} that relates to accumulated time at a certain temperature (like degree days) rather than constant exposure at or exceeding a temperature threshold (DeVay, 1991a). This concept of thermal accumulation may be germane to the process of soil solarization since soil temperatures are generally not sustained for long periods of time, but rather fluctuate during the solarization process.

Soil solarization may also have a general effect of changing the soil biophase in the same way that fumigation does (see Chapter I). By partially sterilizing the soil, the balance and composition of biophase sustainers and inhibitors are altered. As the soil is recolonized, a different microbial community may be established than was present before (DeVay, 1991a). This new climax community may favor plant health or plant disease (Katan, 1980; Stapleton, 1991). Generally, thermophilic (or thermotolerant) and competitive soil organisms are not plant pathogens, and many are beneficial organisms

such as mycoparasites, mycorrhizae, and plant-growth-promoting rhizobacteria (DeVay, 1991a).

2.1.1.2 Plant-Growth Enhancement by Soil Solarization

As with fumigation, researchers have found that soil solarization can enhance plant growth (biomass and yield) beyond that expected from pathogen suppression. For example, availability of mineral nutrients can be increased after solarization providing an improved growing medium for plants (Stapleton, 1991). Katan (1980) reported that solar heating of the soil increased yields of various crops, including peanuts (*Arachis hypogae*), eggplant (*Solanum melogena* L.), tomato (*Lycopersicon esculentum* Mill.), onion (*Allium cepa* L.), carrot (*Daucus carota* L.) , and cotton (*Gossypium* spp.), from 35% to 215%. He suggested that these results may be due to several mechanisms including release of minerals into the soil, stimulation of beneficial microorganisms, or control of minor pathogens.

In Texas trials, Hartz *et al.* (1985) observed yield increases after soil solarization in peppers (*Capsicum frutescens* L.) and muskmelons (*Cucumis melo* L.). They found a 20% increase in yield of fall-grown peppers in plots solarized for one month when compared to conventionally grown peppers. They reported a 53% increase in yield if solarization plastic was pigmented and left in place as mulch when compared to conventionally grown peppers. They further reported a residual benefit from solarization the following year when muskmelons were grown in the same plots. They attributed the yield response to a selective shift in soil microflora (fungi, actimomycetes, and bacteria) resulting from repeated exposure to modest temperature increases rather than heat sterilization and elimination of plant pathogens. They suggested that solarization favors the survival and growth of selected beneficial organisms, including mycoparasites and mycorrhizae.

Also, Grinstein *et al.* (1979) observed an overall yield increase of peanuts of 53% and an increase in Grade A yield of 124% in plots solarized for six weeks prior to planting when compared to non-solarized plots. The resulting overall crop value was increased by 73% by pre-plant soil solarization. Stevens *et al.* (1990) reported a 178% yield increase of 'Allstar' strawberries grown in solarized soil over those grown in non-solarized soil.

2.1.1.3 Effect of Soil Solarization on Weeds

Soil solarization for weed control has been demonstrated in various cropping systems (Bell and Elmore, 1983; Egley, 1983; Standifer, 1984; Horowitz *et al.*, 1983). Katan *et al.* (1976) found almost complete control of four major weed species (*Alhagi maurorum* L., *Cyperus rotundus* L., *Notobasis syriaca* L., and *Prosopis farcata* Torr.) following soil solarization with clear 0.03 mm polyethylene plastic for four to five weeks in three experiments using eggplant (*Solanum melogena*) and tomato (*Lycopersicon esculentum*). Stapleton *et al.* (1989) found an 82% reduction in groundcover of both winter and summer weeds (species not identified) following mulching with clear or black plastic in established apple (*Malus* spp.) and pecan (*Carya illinoensis* (Wangenh.) Koch) orchards and in established vineyards with no detrimental effects on the crop plants. Daelemans (1989) found no measurable weed growth of *Imperata cylindrica* Cyr., *Amaranthus* spp., *Portulaca* spp., *Setaria* spp., *Digitaria* spp., *Ageratum* spp. in solarized vegetable plots even 23 days after removal of the plastic compared to non-solarized plots which were overgrown with these species.

Weeds under unventilated plastic are primarily destroyed by high temperatures (Elmore, 1991). High temperatures are lethal to many dormant or germinating seeds (Rubin and Benjamin, 1984). Additionally, weed seeds that are not killed directly by the heat may germinate under plastic and seedlings then are destroyed by the high temperatures (Rubin and Benjamin, 1984). Weed seeds, like disease propagules, differ in their

sensitivity to heat and have different LD_{90}s (Elmore, 1991). Not all are equally controlled by solarization.

One of the difficulties in maintaining the beneficial effects of solarization for weed control is in not disturbing the soil by bringing untreated soil to the surface (Elmore, 1991). The seed bank of weeds may extend significantly deeper into the soil profile than the effects of solarization. Thus, cultivation or bed preparation may negate the solarization effect. For this reason, it may be necessary to prepare the field for planting prior to the solarization treatment and plant the crop soon after removing the plastic with minimal soil disruption. Pigmenting and planting through the plastic may be an option for some crops.

2.1.1.4 Plant Pathogens and Soil Solarization

Soil temperatures in the range of 40° to 50°C are effective in controlling many soil-borne plant pathogens (Cook and Baker, 1983). Since Katan's early investigations (1976), soil solarization has been reported to destroy propagules of or suppress disease caused by *Verticillium dahliae* (Ashworth *et al.*, 1979; Grinstein *et al.*, 1979; Davis and Sorensen, 1986; Jimenez-Diaz, 1991), *Fusarium solani* (Mart.) Sacc. (Sarhan, 1991), *Pythium* spp. (Pullman *et al.*, 1981), *Rhizoctonia solani* (Pullman *et al.*, 1981), and *Sclerotium rolfsii* (Grinstein, 1979), to cite a few examples.

Control (or suppression) of soilborne plant pathogens by solarization results from direct and indirect effects. Lethal temperatures affect pathogen propagules directly by breaking down membrane stability and function and by the sustained inactivation of respiratory enzymes (Sandarum, 1986). In addition to destruction of soilborne pathogens by solarization, Katan (1980) offered three indirect biological mechanisms that may occur: 1) a partial or complete suspension of fungistasis which exposes germinating propagules to action by antagonists; 2) weakening of resting structures which also exposes them to action by antagonists; and 3) biochemical or physical stimulation of antagonists making them more active.

Stapleton *et al.* (1989) found a 55 - 97% decrease in natural populations of *Pythium ultimum* Trow and *Verticillium dahliae* at a soil depth of 0-23 cm in California soils when mulched with either clear or black plastic. Soil temperatures were raised 10° - 18°C and 8° - 12°C under clear and black plastics, respectively, when compared to bare soil. Pullman *et al.* (1981) found that *Pythium ultimum, Rhizoctonia solani, Verticillium dahliae, and Thielaviopsis basicola* (Berk. & Broome) Ferraris were suppressed in soil exposed to sublethal temperatures (37°C and 39°C) for long periods of time. They suggested that heat damage accumulates over time to a point where the propagules are unable to germinate and grow. In one study, sclerotia of *Sclerotium rolfsii* were weakened by exposure to elevated but sublethal temperatures (Lifschitz *et al.*, 1983). The outer layer of the sclerotia became cracked, and they were subsequently colonized heavily by *Trichoderma harzianum* Rifai, a fungal mycoparasite.

Most studies of solarization techniques and efficacy have been carried out in regions of the world with high solar radiation potential (e.g., Egypt, Israel, California, Texas). Garibaldi and Tamietti (1989), however, found significant suppression of *Rhizoctonia solani* and *Phytophthora nicotianae* Breda de Haan var. *parasitica* (Dastur) following solarization with single or double-layered polyethylene during the summer months in northern Italy. They reported that the effectiveness of solarization lasted for two to four successive bean crops (*Vicia fabia* Moench.) grown in the same plots. LaMondia and Brodie (1984) studied the effects of soil solarization on populations of *Globodera rostochiensis* nematodes in New York. They found a 96-98% reduction in natural soil populations of *G. rostochiensis* at a 10 cm soil depth following solarization, compared to control treatments.

Several researchers have studied the effects of soil solarization compared to soil fumigation in strawberry production. In Texas, Patten *et al.* (1991) found that solarization and fumigation increased yields of 'Chandler' by 21% and 32%, respectively, compared to bare soil. Benefits from solarization and fumigation were reported to last for 2 years.

Razik *et al.* (1989) found that yields from solarized strawberry plots were increased by 33% and from fumigated plots by 36% compared to control plots. Solarization and fumigation controlled weeds equally in their study.

In Japan, Horiuchi (1991) reported that over 1,300 hectares of strawberry land are treated with soil solarization to control Fusarium wilt, nematodes, crown rot, Verticillium wilt, and red stele. In Japan, a large portion of the strawberry crop is produced under plastic tunnels to increase production on marginal lands. As such, the Japanese system is well suited for soil solarization. Horiuchi (1991) reported a grower satisfaction index of 2.0 to 2.7 to (on a scale of 1 to 3) for this practice in achieving the desired disease-control results.

2.1.2 Cover Crop Rotations

2.1.2.1 General Benefits

Benefits of cover crops have been described previously (Lal *et al.*, 1991). They include reduction or prevention of wind and water erosion, soil moisture conservation, nutrient leaching reduction, increased soil nitrogen (by legumes), increased soil organic matter content and improved soil structure. Cover crops also provide some protection of surface waters from silt-accumulation and nitrate contamination resulting from excessive runoff from agricultural lands (Meisinger *et al.*, 1991). Roots of cover crops hold the soil in place, retain moisture, and absorb nutrients. They also contribute to a complex biochemical exchange that influences the soil ecology. Exudates from roots of cover crops influence the rhizosphere and rhizoplane microflora (beneficial and pathogenic), soil pH, and energy and nutrient cycling in the soil (Lal *et al.*, 1991). Thus, the distribution of microorganisms and the chemical composition of a cover cropped soil will differ from that of a bare soil even if other physical parameters are the same (Richards, 1978). The effects of plant roots on soil properties subsequently may influence the macroflora (including weeds) and fauna (earthworms, etc.) in the soil and likely will affect the conditions under

which a subsequent commercial crop is grown. Benefits of cover crops are summarized in Figure 2.1.

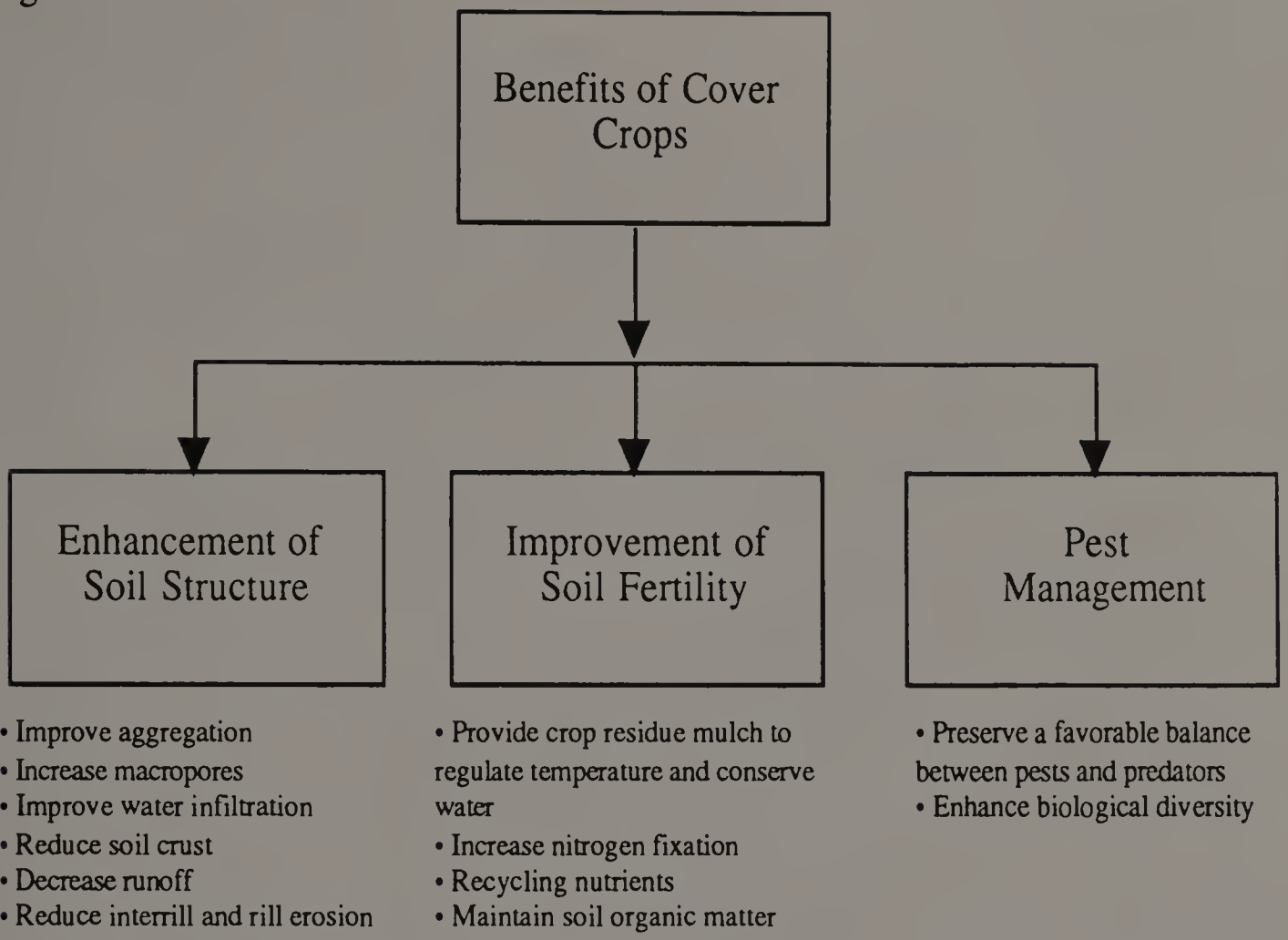

Figure 2.1. Potential benefits of cover crops (after Lal *et al.*, 1991).

Even though herbicides will continue to be a primary weed-management tool in the foreseeable future, alleviating weed pressure by incorporating cover-crop rotations and other strategies into the system will benefit the environment and agricultural sustainability. Currently, herbicides constitute 65% of all pesticide sales in the United States costing $3.6 billion (Worsham, 1991). Herbicides have played a key role in modern agriculture by protecting against significant crop losses due to weeds; however, the benefits of chemical control of weeds come at a cost that must be considered carefully. Persistence of herbicides in soil and groundwater (Williams *et al.*, 1988) and development of herbicide resistance in weed species (Worsham, 1991) are some of the costs of long-term use of these crop protection chemicals.

Management of soilborne diseases generates similar problems. As described in Chapter I, soil fumigation is a tool used for managing soilborne diseases in many crops but carries with it significant risks: high cost, potential environmental contamination, possible treatment failure, and declining availability of registered fumigation materials. If cover-crop use alone or in combination with other strategies can suppress disease caused by soilborne pathogens, these fumigant-related problems may be solved.

2.1.2.2 Weed Suppression by Cover Crops

Certain cover crop species have been shown to reduce weed growth (Putnam *et al.*, 1983; Worsham, 1991). Two mechanisms may contribute to this reduction: competition for available light and soil resources and allelopathy (Putnam, 1988). Allelopathy is the effect of one plant or group of plants on another through the production of chemical compounds released into the soil by living roots or decaying plant residues (Putnam and Tang, 1986). The allelopathic chemicals may act directly or indirectly on seed germination or seedling growth (Putnam *et al.*, 1983).

Allelopathic processes are common in natural plant communities (Putnam and Tang, 1986) and may be common in agricultural plant communities (Fay and Duke, 1977). Allelopathy was first thought to be a detrimental phenomenon in agricultural systems linked to weed effects on cultivated crops. Many weeds were identified as having allelopathic properties which added to their negative impact on crops (Putnam and Weston, 1986). Weed scientist subsequently considered the possibility of using allelopathy to their advantage in weed control.

Putnam and DeFrank (1983), Barnes and Putnam (1983), and Worsham (1984) identified the phytotoxicity of rye (*Secale cereale* L.) residues as a means of suppressing weed growth, especially in no-till or conservation till cropping systems. Barnes and Putnam (1983) found that a winter rye crop reduced the biomass of common lamb's quarters (*Chenopodium album* L.) by 98%, large crabgrass (*Digitaria sanguinalis*) by

42%, and common ragweed (*Ambrosia artemisiifolia* L.) by 90%, compared to a control. They identified the inhibitory compounds as root exudates that were taken up by weeds. Aqueous extracts of rye were studied and found effective in inhibiting growth of several species (Barnes and Putnam, 1986). Allelochemicals in sorghum (*Sorghum bicolor* (L.) Moench.) were isolated and identified by Lehle and Putnam (1982) and Weston *et al.* (1989), and aqueous extracts of these chemicals were found to inhibit germination of several species. The greatest inhibition was from extracts of top-growth that was less than four weeks old. Older plant tissues contained lower concentration of the allelochemicals (Weston *et al.*, 1989).

Growth of seedlings of *Brassica campestris* L., *Cyperus rotundus*, and *Digitaria sanguinalis* planted in pots containing excised roots of wild buckwheat (*Fagopyrum cymosum* Gaertn.) was restricted compared to those planted in buckwheat-free soil (Tsuzuki *et al.*, 1987). From this is was concluded that wild buckwheat exudes toxins inhibitory to the growth of these other species. Some plants may release compounds that inhibit the colonization of roots of neighboring plants by mycorrhizae. Crowell and Boerner (1988) found that growth of vescular-arbuscular mycorrizae- (VAM) colonized *Ambrosia artemisiifolia* was inhibited by the presence of black mustard (*Brassica nigra* (L.) Koch.).

Competition is another mechanism by which a cover crop affects weed growth. Smother crops are crops that are highly competitive with weeds for light, nutrients, and water. Some grain crops such as rye (*Secale cereale*), barley (*Hordeum vulagare* L.), millet (*Panicum miliaceum* L.), and sorghum (*Sorghum bicolor*), legumes such as alfalfa (*Medicago sativa* L.), clovers (*Trifolium* spp.), and vetch (*Vicia* spp.), or others such as buckwheat (*Fagopyrum esculentum* Gaertn.), rape (*Brassica napus* L.), black mustard (*B. nigra* (L.) Koch), and sesbania (*Sesbania exaltata* (Raf.) Cory) are used as smother crops (Anderson, 1983).

Cover crops can be grown singly or combined to exploit their individual properties. In combination, winter rye and hairy vetch (*Vicia villosa* Roth.) possess a high potential for competitiveness and biomass production, as well as nitrogen fixation (Hoffman and Regnier, 1991). Crops can also be grown in succession. Buckwheat (*Fagopyrum esculatum*) is a highly competitive, fast-growing summer cover crop that can reach maturity when planted after harvesting a summer cash crop (Martin, *et al.* 1976; Oplinger, 1975). It can add up to seven metric tons per hectare of organic matter to the soil when used as a green manure crop (Oplinger, 1975). Once incorporated in the soil, it decays rapidly making nutrients quickly available to succeeding crops (Robinson, 1980). It can be followed by a winter cover crop or crop mix like winter rye or winter rye plus hairy vetch.

2.1.2.3 Disease Suppression by Cover Crops

In a monoculture system of farming, where a single crop is grown over a large acreage for successive years, disease inoculum can often increase to a high density (Cook and Baker, 1983). High inoculum density makes disease management more difficult and the threat of disease outbreak more likely (Cook and Weller, 1987). Crop rotation is one way to reduce the inoculum density of plant pathogens (Cook and Baker, 1983; Cook and Weller, 1987). Many pathogens have a limited host range so employing a rotation that avoids alternate hosts for a pathogen, a farmer can avoid increasing inoculum density of that pathogen (Cook and Baker, 1983). The rotation scheme becomes more complicated when several significant soil-borne pathogens comprise the disease complex of a crop, as is the case in strawberries. Then a longer, more sophisticated rotation may be needed in order to gain a reliable benefit from rotation.

Cook and Baker (1983) state that "... a disease outbreak can commonly be traced to some ecological shock causing biological imbalance. Disease itself is an ecological force and will eventually restore balance within the ecosystem." Ecological stability is characterized by a diversity of organisms existing in a cycling environment (Baker and

Scher, 1987). In modern agriculture, there are many sources of ecological shock, including monoculture, genetic uniformity of crops, cultivation, and pesticide applications (Cook and Baker, 1983). Cover crop rotations can aid in increasing microbial diversity in soil simply by increasing its organic matter content (Cook and Baker, 1983; Cook and Weller, 1987). Even if the cover crop is harvested for grain, straw, or silage, the root biomass alone can add significant organic matter to the soil (Brady, 1984). The resulting increase in biological diversity in the soil ecosystem may promote antagonistic soil microorganisms and suppress disease potential.

Phatak *et al.* (1991) studied disease incidence of *Pythium* spp., *Rhizoctonia solani,* binucleate-*Rhizoctonia* spp., and *Laetisaria arvalis* Burdsall. in cucumber (*Cucumis sativus* L.) following nineteen overwintering cover crop treatments. Propagule densities of *Pythium* spp. were highest following Cahaba white vetch (*Vicia sativa* x *cordata* L.) and ryegrass (*Lolium multiflorum* Lam.), and lowest after canola (*Brassica napus*), the control, and subterranean clover (*Trifolium subterraneum* L.). There were no differences in propagule density for *Rhizoctonia solani,* but binucleate-*Rhizoctonia* density was found to be greatest following subterranean clover and least following canola. Rothrock and Kendig (1991) found the soil population and disease incidence caused by *Thielaviopsis basicola* in cotton to be significantly less following cover crops of hairy vetch or hairy vetch plus rye than in the fallow control plots.

The mechanisms involved in cover crop effects on disease are not well understood. In the case of black mustard, canola, and other members of the *Brassicaceae*, production of allelochemicals has been studied (Dhoesin and Boerner, 1991). *Brassica* spp. are known to produce mustard oils which hydrolize to form isothiocyanate compounds. These compounds are closely related to the active ingredient of the soil fumigant Vorlex™, methyl isothiocyanate. Thus, *Brassica* spp. may provide a powerful tool for suppressing plant pathogens in soils where they have accumulated. For other cover crop species, different mechanisms may be responsible for disease suppression.

2.1.3 Objectives

Two experiments in this study sought to evaluate solarization, fumigation, and several cover crop rotations for effects on weed density and distribution, strawberry plant growth, and root-disease incidence in strawberry.

2.2 Materials and Methods

2.2.1 Experiment I

A preliminary field experiment to evaluate soil solarization and cover crop rotations was conducted in 1989 on a Winooski very fine sandy loam (Aquic Udifluvent) at the University of Massachusetts Research Farm in South Deerfield, Massachusetts. The soil properties included: 1.07g/cm^3 bulk density, pH 5.5, 5.75% organic matter content, and 10.0 meq/100g cation exchange capacity. Strawberries were growing on the site of the experiment for the two years prior to 1989. Prior planting was done to simulate a commercial situation where strawberries are replanted on old strawberry land possibly causing soilborne disease inoculum potential to accumulate. No herbicides were used during that time so that normal to acute weed pressure was also present.

The experiment was a randomized complete block split plot design with six treatments and six replications with main plots receiving the treatments and subplots either receiving normal tillage before planting strawberries or not tilled. Plots were 6 meters long and 1.5 meters wide. The treatments consisted of:

1) Solarization with an ultrathin 0.8 mm, clear, 70-day photodegradable plastic film (manufactured by Lecklers, Inc. of LaSalle Michigan) and applied to the soil on 12 Aug. 1989,
2) Sudex (*Sorghum bicolor* x *sudanese* Piper) summer cover crop seeded at 28 kg ha^{-1} and followed by Austrian winter field pea (*Pisum sativum* L.) winter cover crop seeded at 78 kg ha^{-1},

3) Sudex summer cover crop seeded at 28 kg ha^{-1} and followed by black mustard (*Brassica nigra*) cover crop seeded at 22 kg ha^{-1},

4) buckwheat (*Fagopyrum esculatum*) summer cover crop seeded at 84 kg ha^{-1} and followed by winter rye (*Secale cereale*) winter cover crop seeded at 84 kg ha^{-1},

5) fumigation w/ Vorlex™ (methyl isothiocyanate) using a Fumigun™ 470-2A small plot soil injector (Neil A. Maclean, Co., Belmont, CA) calibrated to 375 L ha^{-1} (applied on 12 Sept 1989), and

6) a control where no treatment was applied.

Summer cover crops were all seeded on 12 Aug. 1989. Summer cover crops were incorporated to a depth of 20 cm with a rototiller on 3 Sept. 1989. Winter cover crops were seeded on 15 Sept. 1989. The rototiller was cleaned carefully between each treatment to avoid contamination between plots.

In 1990, plots were split and half (3 meters) of each plot (randomly chosen) was tilled normally prior to planting strawberries and the other half was treated as a 'no-till' planting system. Again, the rototiller was cleaned carefully between each treatment to avoid contamination. In the 'no-till' subplots, holes were punched (10 cm x 15 cm) in a row down the center with a bulb planter and strawberries were planted in these holes. Strawberries cv Honeoye of normal nursery stock (field grown runner plants supplied by Nourse Farms, Inc. of Whately, Massachusetts) were planted, 10/subplot, on 1 May 1990. Normal irrigation and fertilization regimes were followed for the season. No cultivation or pesticide treatments were made.

During the summer of 1989, soil temperatures were recorded using REOTEMP® M640 15 cm dial thermometers (REOTEMP Instrument Corp., San Diego, CA) at 48-hr intervals for 18 days starting 15 August. Temperature readings were taken in the early afternoon when the soil temperature was likely to be at its highest. Thermometers were set at a 10 cm soil depth in the solarization and buckwheat cover crop plots and in cultivated

bare soil with no weed cover. Temperature readings were taken in solarized, cover cropped and bare soil in four plots of each treatment.

In 1990, weed data were collected by visual assesment of percent of soil surface covered by weed canopy per m^2 on two sampling dates (15 May and 11 July), and density and distribution of monocot and dicot weed species as determined by counting and identifying weeds within a square-meter frame on 15 May 1990. Visual ratings of strawberry plant status on a scale of 1 to 5 where 1=dead with no green tissue, 2=dying with some green tissue, 3=stunted, 4=no dark tissue but somewhat stunted, 5=healthy, were conducted 11 July, ten weeks after planting. The experiment was terminated on 25 July, 12 weeks after planting when weed cover reached 100% in most of the experiment.

2.2.2 Experiment II

A second field experiment was initiated in 1990 at a different location at the same site as Experiment I except that it had not previously been planted with strawberries. Soil properties did not differ from Experiment I. Prior to the application of the treatments, plots were split and half (randomly chosen) were inoculated with *Rhizoctonia solani* (AG 1, B43) at a rate of 0.5 kg per m^2 of inoculum (30-50 $x10^4$ cfu's per gram of inoculum) on 16 July 1990. The inoculum was sprinkled evenly on the surface of tilled soil, raked in, irrigated, and allowed to remain undisturbed for two weeks prior to applying the treatments. Inoculum was prepared according to standard operating procedures (SOP): T101, T106, and T109 (provided in the appendices).

The second experiment was also a randomized complete block split plot design with six treatments and six replications with main plots receiving the treatments and subplots either receiving inoculation with *Rhizoctonia solani* prior to applying the main plot treatments or not inoculated. Plots were 6 meters long and 1.5 meters wide. Treatments were:

1) Solarization with non-photodegradeable 4 mm clear plastic film (commonly available) applied to the soil on 31 July 1990,

2) Sudex (*Sorghum bicolor* x *sudanese*) summer cover crop seeded at 28 kg ha^{-1} and followed with winter rye (*Secale cereale*) winter cover crop seeded at 84 kg ha^{-1} ,

3) buckwheat (*Fagopyrum esculentum*) summer cover crop seeded at 84 kg ha^{-1} and followed with winter rye winter cover crop seeded at 84 kg ha^{-1} ,

4) Japanese millet (*Echinochloa frumentacea* (L.) Beauv.) summer cover crop seeded at 45 kg ha^{-1} and allowed to 'winter-kill' instead of planting a winter cover crop,

5) fumigation w/ Vorlex™ (methyl isothiocyanate) using a Fumigun™ 470-2A small plot soil injector (Neil A. Maclean, Co., Belmont, CA) calibrated to 375 L ha^{-1} (applied on 15 Sept. 1990) and,

6) a control where no treatment was applied.

Soil was loosened with a spading fork at the time of treatment application. Summer cover crops were seeded on 31 July 1990 and winter cover crops on 15 Sept. 1990. Summer cover crops were incorporated with a rototiller on 5 Sept 1990. The rototiller was cleaned carefully between each treatment to avoid contamination between treatments. Winter cover crops were evenly broadcast over the plots and raked in. Tasks were always completed on non-inoculated subplots first and tools cleaned carefully between treatments to avoid contamination.

During the summer of 1990, soil temperatures were recorded using REOTEMP® M640 15 cm dial thermometers as in Experiment 1, except continuing for 18 days starting 15 August. Temperature readings were taken in the early afternoon when the soil temperature was likely to be at its highest. Thermometers were set at 2.5 cm and 10 cm soil depths in solarized and bare soil in six plots of each treatment.

During the spring of 1991, all plots were tilled to a depth of 20 cm on 18 April with a rototiller with care taken to avoid contamination between treatments. Strawberries cv

Honeoye from normal nursery stock (field grown runner plants supplied by Nourse Farms, Inc. of Whately, Massachusetts) were planted as in Experiment 1 on 1 May 1991.

In 1991, weed data was collected as in Experiment 1 on 13 May and 10 July. Density and distribution of monocot and dicot weeds was determined on 13 May by counting weeds within a square meter frame. Weeds were harvested by severing tops at the soil level on 10 July and weighed (fresh and dry). In addidtion, weed specied distribution was measured by ranking weed species found in each plot from 1-4 where 1=most abundant, 2=second most abundant, 3=third most abundant, and 4=fourth most abundant.

Strawberry plant survival (no. per plot) was evaluated eight weeks after planting. On 15 September, strawberry plants were harvested by severing plants at soil surface. The number of runners produced by each plant was recorded and plants were dried and weighed. Five root systems per subplot were collected randomly and evaluated visually on a scale of 1 to 5 where 1=dead and 5=excellent.

2.2.3 Determination of Inoculum Density

For both experiments in this study, inoculum density, as expressed as colony forming units (cfu), of *Rhizoctonia* inoculum were determined using serial dilution plating on a *Rhizoctonia* selective medium, RSM, as described in SOP T102 and T117 included in Appendix B.

2.2.4 Statistical Analysis

Statistical analyses were performed using SYSTAT for the Macintosh© (Statistics, Version 5.2 Edition. Evanston IL). Square root and arcsine transformations were performed percentage and count data as needed to stabilize variances or normalize the sample population (Damon and Harvey, 1987). Original data are presented in all tables and figures. Differences between treatments were analyzed by analysis of variance (ANOVA)

using the general linear model (GLM) procedure, with means separated by least significant difference (LSD) or single-degree-of-freedom contrasts (Damon and Harvey, 1987; Steel and Torrie, 1980; Gomez and Gomez, 1984). Ranked data (weed distrribution) was analyzed using Kruskal-Wallis one-way analysis of variance for non-parametric data (Steel and Torrie, 1980).

2.3 Results

2.3.1 Experiment I

From 15 Aug. 1989 to 2 Sept 1989, soil temperatures ranged from 25° to 43°C at 10 cm under the ultrathin photodegradeable plastic, from 21° to 29°C at 10 cm in bare soil, and from 21° to 26°C at 10 cm in the buckwheat cover crop plots (Figure 2.2). The average temperature difference between solarized and bare soil was 9.1°C, and between solarized and cover cropped soil was 11.0°C. These differences were highly significant ($p \leq 0.001$). The average difference between bare and cover cropped soil was 1.9°C, which also was highly significant.

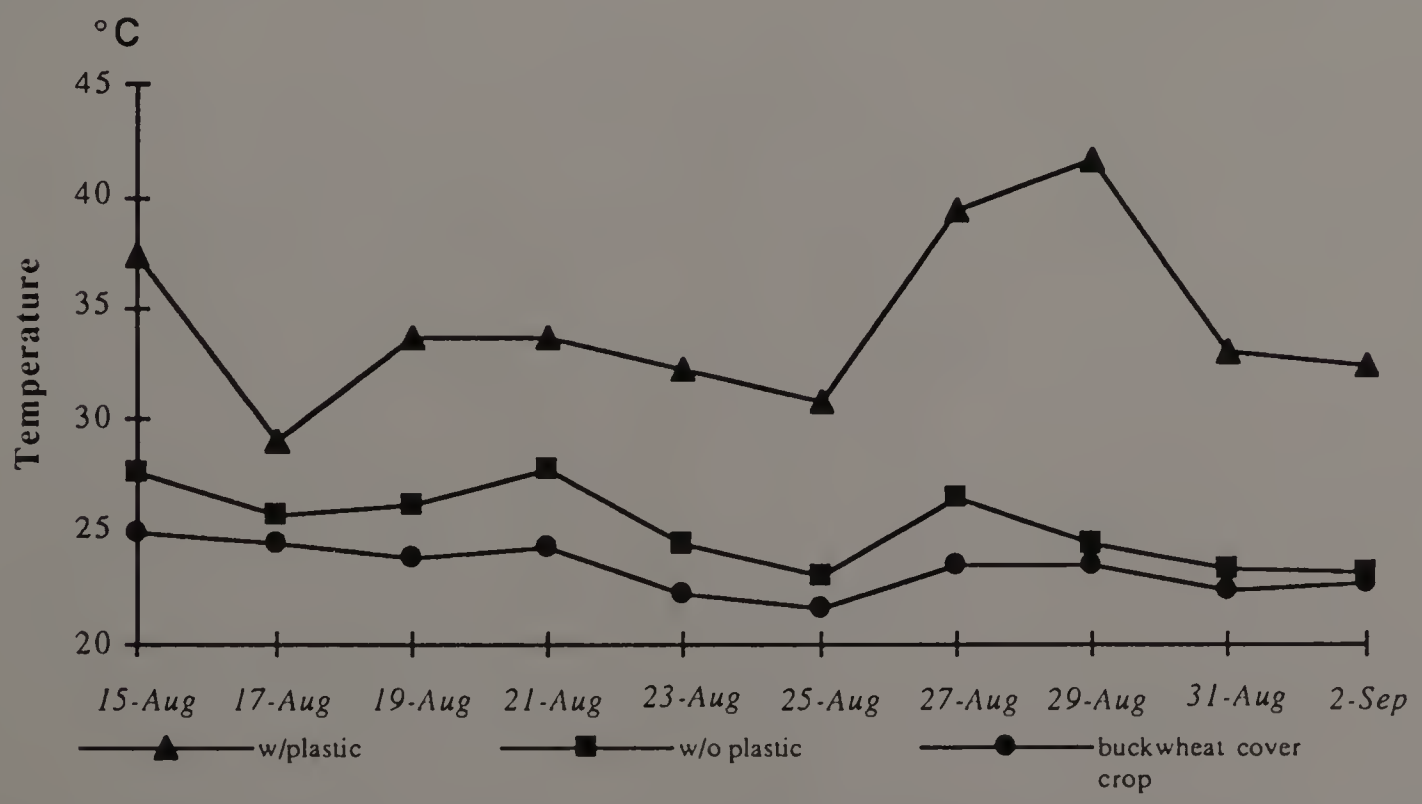

Figure 2.2. Mean mid-day soil temperature at 48-hour intervals from 8/15/89 to 9/2/89 at 10-cm soil depth with or without solarizing plastic mulch or in a buckwheat cover crop.

Percent weed cover was significantly greater in tilled subplots compared to no-till subplots (Table 2.1) and at the two data collection dates, three and ten weeks after planting strawberries ($p \leq 0.001$; data not shown). In the tilled subplots, the buckwheat/winter rye rotation plots had less weed cover than any other treatment at both three and ten weeks. And, fumigated plots had less weed cover than control plots at both dates while all remaining treatments were not different from the control plots.

At three weeks after planting, there were no differences in percent weed cover in the no-till subplots but, at ten weeks, fumigated plots had less weed cover than the sudex/black mustard rotation plots while all other plots were not significantly different (Table 2.1).

Table 2.1. Percent weed cover (% of soil surface covered by weed canopy) one year after six pre-plant treatments at two dates and two planting systems (till and no till) for strawberries[z].

	Percent Weed Cover[y]			
	Till		No-till	
Treatments	3 Weeks[w]	10 Weeks	3 Weeks	10 Weeks
Fumigation	11.0 b[x]	53.7 b	0.2 a	2.3 a
Soil solarization	12.6 bc	79.6 bc	0.9 a	7.0 ab
Buckwheat/winter rye	2.5 a	10.4 a	0.1 a	3.0 ab
Sudex/black mustard	10.5 bc	83.8 cd	0.3 a	8.2 b
Sudex/Austrian pea	16.7 c	95.8 d	0.3 a	5.6 ab
Control	17.1 c	94.6 cd	0.3 a	6.1 ab
Mean		69.6 ***		5.3

[z]Analysis of variance performed on transformed data (arcsine $(x + 0.5)^{1/2}$); ANOVA table A.1 in Appendix A.

[y] Data presented backtransformed from analyzed data.

[x] Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.

[w] Time after planting strawberries on 1 May 1990.

***Till and no-till significantly different at $p \leq 0.001$

Monocot weed species present primarily included large crabgrass (*Digitaria sanguinalis*) and fall panicum (*Panicum dichotomiflorum*) and dicot weed species included dandelion (*Taraxacum officinale*), chickweed (*Stellaria media*), shepherd's purse (*Capsella bursa-pastoris*), and purselane speedwell (*Veronica peregrina*). Mean density (no./m^2) of

monocot weed species and dicot weed species, and total weeds were significantly less in the no-till subplots than in the tilled subplots ($p \leq 0.01$). The effects of treatments were non-significant (Table 2.2).

Table 2.2. Weed density of monocot and dicot weed species and total weeds one year after six pre-plant treatments in two planting systems (till and no-till) for strawberries[z,y].

	Weed Density[x] (no./m^2)					
	Monocot		Dicot		Total	
Treatments	Till	No-till	Till	No-till	Till	No-till
Fumigation	0.3	0.0	9.4	0.1	9.7	0.1
Soil solarization	0.5	0.0	16.2	1.0	16.7	1.0
Buckwheat/winter rye	0.3	0.1	2.9	0.0	3.3	0.1
Sudex/mustard	1.3	0.1	12.4	0.1	13.7	0.2
Sudex/winter pea	0.4	0.1	8.9	0.1	9.4	0.1
Control	0.6	0.2	8.8	0.1	9.4	0.3
Mean	0.6 **	0.1	9.8 ***	0.2	10.4 ***	0.3

[z]Analysis of variances tables A.2, A.3, A.4 in Appendix A.

[y]Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.

[x] Weed density 3 weeks after planting strawberries on 1 May 1990.

** ,***Till and no-till significantly different at $p \leq 0.01$ and $p \leq 0.001$, respectively.

Visual rating of plant status (1=dead with no green tissue, 2=dying with some green tissue, 3=stunted, 4=no dark tissue but somewhat stunted, 5=healthy) showed a highly significant difference due to treatment and till effects. Plants in tilled subplots grew better than in the no-till subplots. Strawberry plants growing in the tilled buckwheat/rye treatment had the highest visual rating and were, together with the solarization and fumigation treatments, rated significantly higher than those growing in the control treatment (Table 2.3).

Table 2.3. Visual rating of strawberry plants eight weeks after planting (1990) in plots receiving six different pre-plant site preparation treatments (1989) and subplots which were tilled before planting strawberries[z].

Treatments	Visual rating[y,x]			
	Till		No-till	Mean[w]
Fumigation	4.33		3.00	3.67 b
Soil solarization	4.37		3.03	3.70 ab
Buckwheat/winter rye	4.60		3.50	4.05 a
Sudex/black mustard	4.17		2.87	3.52 bc
Sudex/Austrian winter field pea	4.13		2.63	3.38 bc
Control	3.87		2.70	3.28 c
Mean	4.24	***	2.95	

[z]Analysis of variance performed on transformed data ($(x + 0.5)^{1/2}$) ANOVA table A.5 in Appendix A.

[y]Data presented backtransformed from analyzed data.

[x]Visual rating of strawberry plants 10 weeks after planting on 1 May 1990.

[w]Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.

***Till and no-till significantly different at $p \leq 0.001$.

2.3.2 Experiment II

From 15 Aug. 1990 to 6 Sept 1990, mean soil temperatures at 2.5 cm under the transparent polyethylene plastic film ranged from 31.5° to 46.7°C and from 25.2° to 33.4°C 10 cm in bare soil. Mean soil temperatures at 10 cm under the transparent polyethylene plastic film ranged from 28.0° to 40.5°C and from 22.0° to 25.0°C in bare soil (Figure 2.3). The average temperature differences between solarized and bare soil at the 2.5 and 10 cm depths were 11.2° and 5.5°C, respectively. These differences were highly significant ($p \leq 0.001$, LSD).

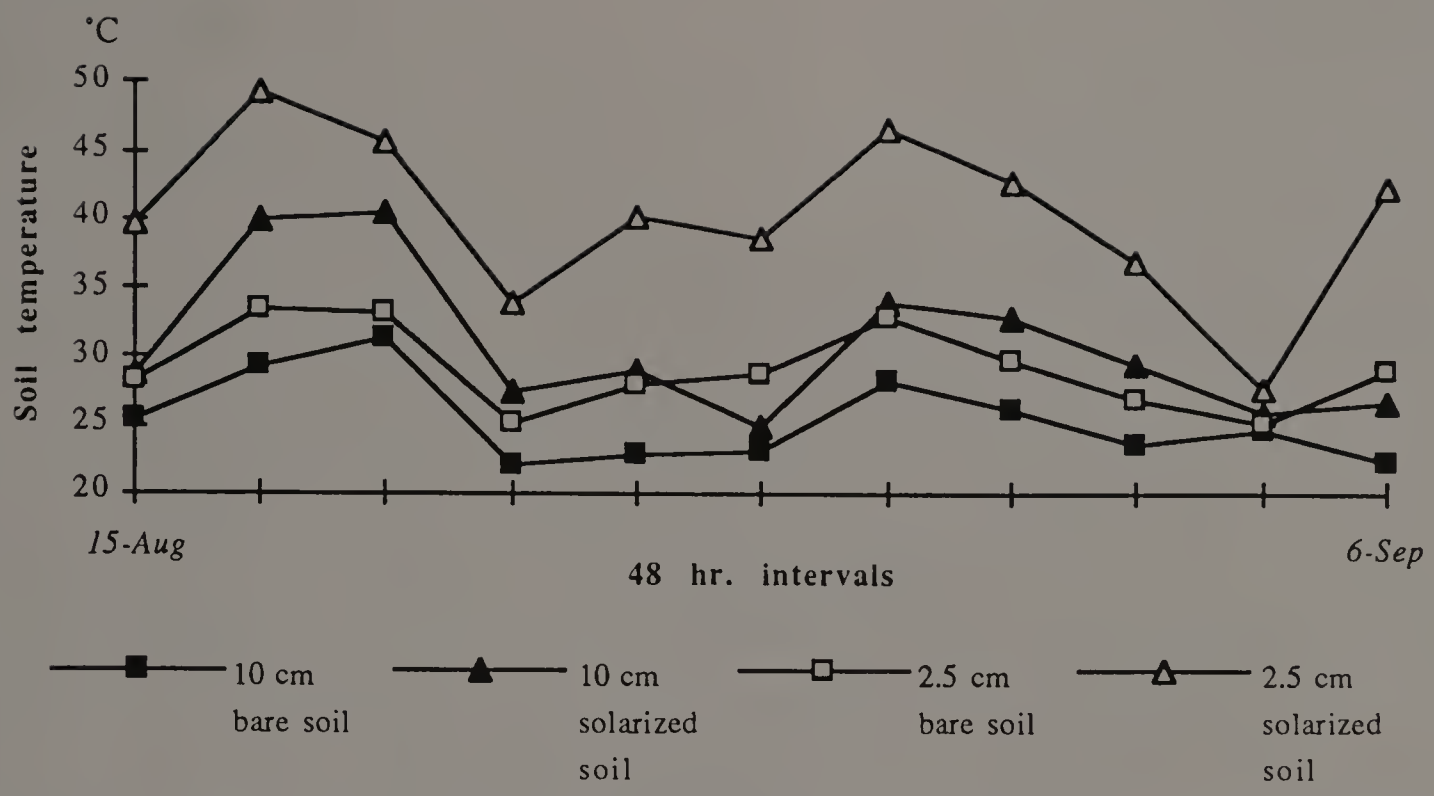

Figure 2.3. Mean mid-day soil temperatures at 48-hour intervals from 15 Aug. 1991 to 6 Sept 1991 at two soil depths in solarized and bare soil.

Percent weed cover was not significantly different in inoculated subplots compared to non-inoculated subplots at either date. On 31 May 1991, there were no significant differences among treatments. On 10 July 1991, buckwheat/winter rye was the only treatment that had significantly less weed cover than the control treatment. The Japanese millet treatment had the greatest amount of weed cover, significantly more than the buckwheat/winter rye, and fumigation (Table 2.4).

Table 2.4. Percent weed cover in strawberries one year after six pre-plant treatments with or without inoculation with *Rhizoctonia solani* (AG 1, B-43) at two dates[z].

Treatments	Percent Weed Cover[y,x]	
	4 Weeks[w]	8 Weeks[w]
Fumigation	2.4[y] a	58.3 b
Soil solarization	6.1 a	82.5 c
Buckwheat/winter rye	4.2 a	11.2 a
Sudex/rye	8.2 a	83.8 bc
Japanese millet	5.6 a	95.8 c
Control	7.0 a	79.6 bc
Mean	5.6 ***	68.5

[z]Analysis of variance performed on transformed data (arcsine(x)$^{1/2}$) ANOVA table A.6 in Appendix A.
[y]Data presented backtransformed from analyzed data.
[x]Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.
[w]4 and 8 weeks, respectively, after planting strawberries 1 May 1991.
***% weed cover at 4 weeks and 8 weeks significantly different at $p \leq 0.001$.

Weed species distribution was not affected by treatment or inoculation ($p \leq 0.5$; Kruskal-Wallis). Dominant weed species were purselane speedwell (*Veronica peregrina*) 36% , dandelion (*Taraxacum officionale*) 15% , and common chickweed (*Stellaria media*) 13% , with other species occurring in lesser amounts (Figure 2.4).

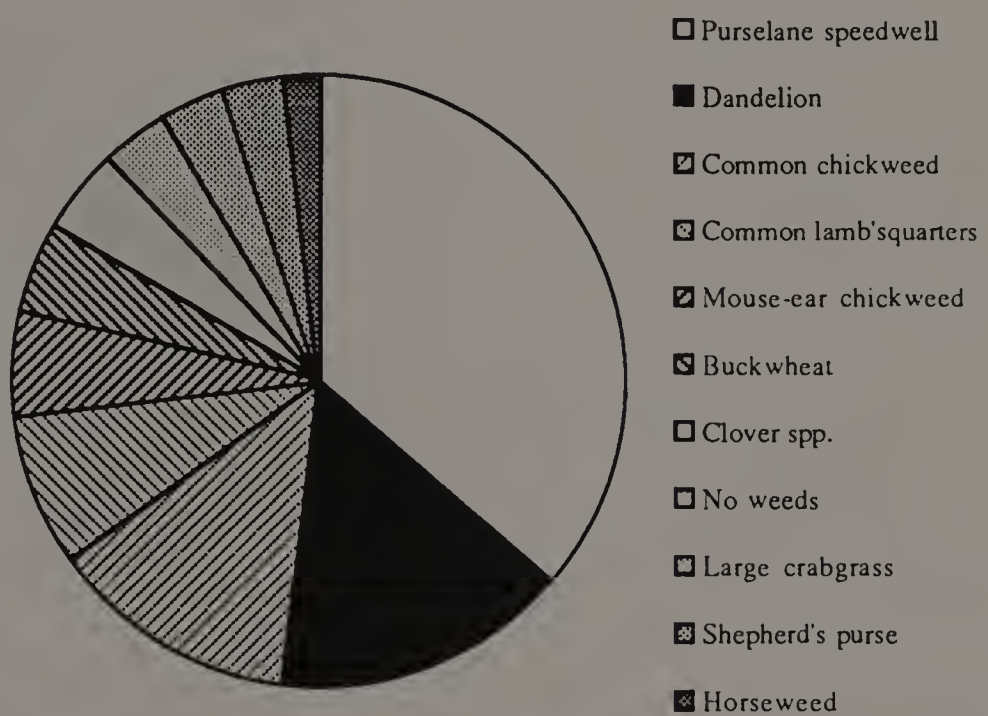

Figure 2.4. Relative frequency of weed species recorded in strawberries one year after six pre-plant treatments and 8 weeks after planting strawberries on 1 May 1991.

There was no effect of inoculation or treatment on monocot, dicot or total weed density (analysis of variance tables A.7, A.8 and A.9 in Appendix A). However, fresh and dry biomass of all weed species combined (top growth only) were significantly different due to inoculation and treatment. Sudex/winter rye, buckwheat/winter rye and solarization plots has less fresh and dry biomass than fumigation, Japanese millet and control plots (Table 2.5).

Table 2.5. Mean fresh and dry biomass (g/m^2 of top growth only) of weeds in strawberries one year after receiving six different pre-plant treatments and two inoculation treatments[z,y].

Treatment	Weed Fresh Weight[x]		Weed Dry Weight[x]	
Fumigation	1123.7	b	346.7	b
Soil solarization	485.3	a	210.2	a
Buckwheat/winter rye	313.6	a	92.7	a
Sudex/winter rye	216.5	a	102.9	a
Japanese millet	1362.7	b	485.4	c
Control	2202.7	c	614.3	c

[z]Analysis of variance table A.10 and A.11, respectively, in Appendix A.
[y]Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.
[x]Weed weights 8 weeks after planting strawberries on 1 May 1991.

Percent survival of strawberry plants was not affected by treatment or inoculation, but there was a significant interaction between treatment and inoculation. With inoculation with *Rhizoctonia solani*, solarization, fumigation, the buckwheat and winter rye, and Sudex and winter rye plots were not different from the non-inoculated plots, while the Japanese millet and control plots were different (Figure 2.5).

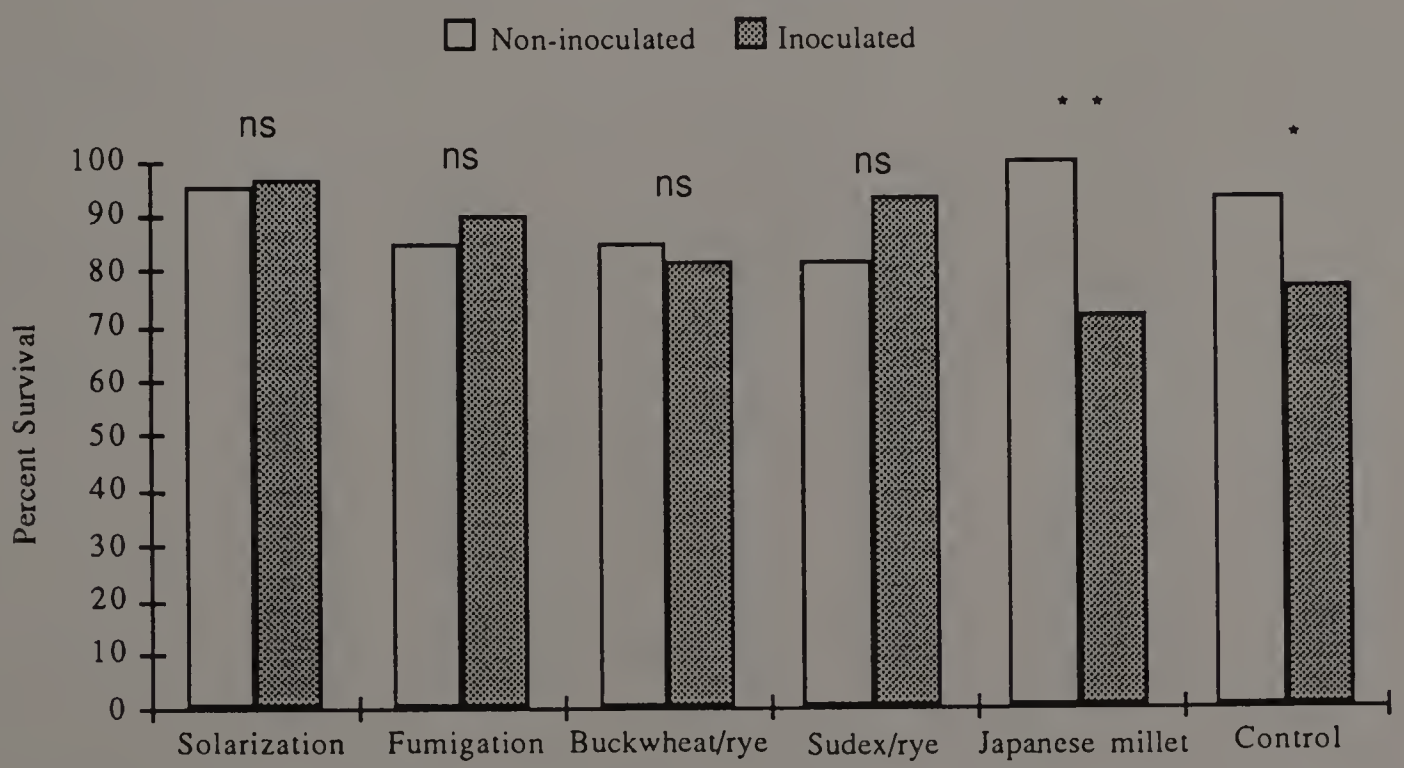

Figure 2.5. Survival percentage of strawberry plants (no. surviving/no. planted) after six pre-plant treatments grown in soil with or without inoculation with *Rhizoctonia solani* (**,*, ns inoculated and non-inoculated plots significantly different at $p \leq 0.01$ and 0.05 or not significant, respectively; analysis of variance table A.12 in Appendix A.)

Biomass of strawberry shoots per plot and per plant, as well as the number of strawberry runners per plant were not significantly affected by inoculation with *R. solani*., but were significantly affected by treatment ($p \leq 0.01$). Shoot weight per plot and per plant were highest in the Sudex plus winter rye plots (Table 2.6). All other treatments except Japanese millet were higher than the control plots. Plants grown in the Sudex plus winter rye and buckwheat plus winter rye plots produced the most runners and were the only treatments that produced more runners than the control plots (Table 2.6).

Visual ratings of root lesion density indicated no significant differences among treatments, but a highly significant difference due to inoculation with *R. solani* ($p \leq 0.001$) (Table 2.7).

Table 2.6. Top dry weight (g) of strawberry plants per plot and per plant and number of strawberry runners per plant 12 weeks after planting following six pre-plant soil treatments[z,y].

Treatments	Shoot Weight of Plants per Plot (g)	Shoot Weight per Plant (g)	No. Runners per Plant
Fumigation	58.7 b	6.4 b	0.8 bc
Soil solarization	50.2 bc	5.2 bc	0.5 c
Buckwheat/winter rye	59.1 b	6.8 b	1.3 ab
Sudex/winter rye	85.1 a	9.6 a	1.6 a
Japanese millet	50.3 bc	5.5 bc	0.7 bc
Control	28.9 c	3.3 c	0.2 c

[z]Analysis of variance table A.14, A.15, and A.13, respectively, in Appendix A.
[y]Means in columns followed by same letters are not significantly different at $p \leq 0.05$; LSD.

Table 2.7. Visual ratings (where 1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) of strawberry roots grown in soil receiving 6 pre-plant treatments and with or without inoculation with *Rhizoctonia solani*.[z].

	Root ratings[y]	
Treatments	Inoculated	Non-inoculated
Fumigation	2.8	3.7
Soil solarization	3.5	3.9
Buckwheat/winter rye	3.3	3.8
Sudex/winter rye	3.4	3.7
Japanese millet	3.0	3.3
Control	3.2	3.7
Mean	3.2 ***	3.7

[z]Analysis of variance of root ratings done on data after square root transformation; ANOVA table A.16 in Appendix A.

[y]Data presented backtransformed from analyzed data.

***Inoculated and non-inoculated significantly different at $p \leq 0.001$

2.4 Discussion

2.4.1 Tillage Effects

While, in Experiment I, the no-till plots showed significantly less weed growth both in terms of percent weed cover and weed density, the growth of strawberry plants in the no-till plots also was adversely affected. Pritts *et al.* (1992) found that strawberry yield was reduced in the no-till killed sod plots compared to the conventionally treated plots. They also reported weed suppression in plots where strawberries were planted into non-tilled killed sods but the standard herbicide treatments provided better weed suppression over time. Poor plant growth and yield may be due, in part, to restricted root growth and runner establishment in a non-tilled soil. An improvement in planting technology for a no-till system of strawberry culture may alleviate this problem. It is, never-the-less, not a desirable method of growing strawberries based on the results of this experiment.

2.4.2 Cover Crop Effects

In Experiment I, under normal tillage practices, buckwheat followed by rye as a pre-plant cover crop rotation suppressed weed growth more than fumigation for up to 10 weeks in the establishment year and both suppressed weed growth more than control plots where no treatment was applied. In Experiment II, the buckwheat and winter rye rotation plots exhibited notable weed suppression by two measures: percent weed cover and weed biomass.

Sudex and winter rye rotation plots were not different from the control plots in terms of percent weed cover but were significanly less than the control in terms of weed biomass. This may be explained by the higher (though not significanly) density of weeds in the Sudex/rye plots. Weeds in the Sudex/rye plots may have germinated later resulting in a higher percentage cover but low biomass production. Weed species distribution was not affected by the cover crop treatments in this study.

Strawberry plant growth (visual ratings, runner production, and top growth) in both experiments was better in the Sudex and winter rye and the buckwheat and winter rye plots than in control plots suggesting that these rotation confers not only weed suppression but enhances strawberry plant growth significantly.

These results are unlike those reported by Pritts *et al.* (1991) where no significant differences were found between preplant cover crops (including buckwheat and sudangrass) and a fallow control treatment in strawberry yield, mean berry weight, plant fresh biomass, or runner production nor any significant weed suppression within or between rows. However, in their study sudangrass appeared to suppress broadleaf weed species and buckwheat suppressed grasses but neither suppresses yellow nutsedge (*Cyperus esculentus* L.) which was a dominant weed. This may explain the overall lack of weed suppression from the cover crops. Results differing from those in our study may be due to a difference in the composition and intensity of weed pressure as well as different soil characteristics. Further evaluation may be needed on a variety of soil types and

conditions over longer periods of time and possibly in larger plots to determine the value of cover crops for weed suppression in strawberries.

Disease suppressive effects were not directly evident in Experiment II since the inoculation effect on plant growth was insignificant. Visual evaluation of root lesion densities did show an effect from inoculation but it was not shown in other plant measurements. However, as mentioned above, growth parameters were enhanced significantly in buckwheat plus winter rye and Sudex plus winter rye treatments and may have been related to some low level of disease suppression. Additionally, measurement of survival percentage showed that plots inoculated with *Rhizoctonia solani* were not different from non-inoculated plots in the fumigation, solarization, buckwheat plus rye and Sudex plus rye treatments while reduced in the control and Japanese millet treatments. Micro-plot studies may help to clarify the role of cover crops in disease suppression.

2.4.3 Solarization Effects

Soil solarization with clear polyethylene plastic succeeded in elevating temperatures significantly to a depth of 10 cm, even under late summer conditions in the Northeast. Maximal temperatures in the 35° - 45°C range at 10 cm and 45° - 50°C range at 2.5 cm were reached. However, solarization only suppressed weeds significantly in Experiment II in terms of weed biomass. Percent weed cover and density were unaffected by solarization in both experiments. Strawberry growth was enhanced by soil solarization in Experiment I in terms of visual rating of the plants but that result was not repeated in Experiment II.

Hartz *et al.* (1993) reported significant weed suppression and strawberry yield increase from soil solarization compared to untreated control plots under California conditions. Additionally, soil solarization was equally lethal to *Phytophthora cactorum* and *Verticillium dahliae* (in terms of % pathogen survival) as fumigation with either methyl bromide or metam sodium. It is unclear whether or not the effect of solarization is strong enough to reliably suppress weeds or pathogens under Northeastern conditions. Further

studies on the effect of different temperature regimes (e.g., repeated exposure to sublethal temperatures) on weed seed germination and pathogen propagule viability will help determine if soil solarization is a dependable management tool in this region.

Additionally, modification of solarization timing and subsequent planting system could enhance the benefits of this technique for northern latitudes. As mentioned earlier, for solarization to be effective, soil must not be disturbed following treatment. This would require a modification in current planting practices. For example, soil solarization in strips for several weeks between a low-growing harvested crop during the peak solar radiation period of the summer (beginning at the summer solstice) and high-density fall planting of strawberries without removing the plastic. Strawberry runners would not be allowed to root (as in a ribbon row) and plastic could be pigmented as in Hartz *et al.* (1985). This system may have higher labor requirements and shortened longevity, but lower pesticide inputs and higher yield may enhance profitability.

2.4.4 *Rhizoctonia* Inoculation Effects

Inoculation with *Rhizoctonia solani* (AG 1-, B43) had no effect on weed growth and only affected plants as indicated by visual root evaluation of lesion densities. Visual rating of root lesions were significantly lower (i.e., more lesions) for plants grown in soil inoculated with the pathogen than non-inoculated soil but none of the treatments had an effect. Further studies with more precise measures of pathogen viability, growth and infectivity may be needed to determine whether or not cover crop rotations or soil solarization have a measurable, repeatable effect on strawberry diseases.

CHAPTER III
BLACK ROOT ROT BIOLOGICAL CONTROL STUDY

3.1 Literature Review

3.1.1 Biological Control of Plant Pathogens and Disease Suppression

The potential for biological control of plant pathogens by other microorganisms is well recognized in natural and agricultural ecosystems (Baker and Cook, 1974; Cook and Baker, 1983; Baker and Scher, 1987). Biological control of soil-borne plant pathogens often is referred to as disease suppressiveness and is described as a condition in which the pathogen does not establish or persist, is present but causes little or no damage, or causes damage for a short period of time but ceases to be damaging while still present in the soil (Baker and Cook, 1974). Baker (1991) asserted that disease-suppressiveness attributable to indigenous microorganisms is present in most cropping systems and that biocidal treatments to soils are likely to disrupt suppressiveness and result in the recolonization of the soil by plant pathogens. This assertion supports the concept of the "boomerang effect" described by Kreutzer (1960) and Altman (1970) which is discussed in Chapter I.

Soils can be categorized as either generally or specifically suppressive (Gerlagh, 1968). General suppression is considered to be related directly to the total amount of microbial biomass in the soil and likely is the result of intense competition among organisms in the soil or rhizosphere for carbon, oxygen, and other soil ecosystem resources (Baker and Cook, 1974; Cook and Baker, 1983). Mechanisms of specific suppression include antibiosis and parasitism involving the presence of specific microorganisms antagonistic to a specific pathogen during a susceptible stage in the pathogen's life cycle such as propagule germination or host penetration (Benson and Baker, 1970). Specific suppression often occurs together with general suppression (Cook and Baker, 1983).

3.1.2 *Trichoderma* spp. as Biological Control Agents

Descriptions of many types of parasitism, antagonism, and fungistasis between soil-borne plant-pathogenic organisms and other soil microorganisms have been reported (Knudsen and Bin, 1990; Lumsden and Locke, 1989; Liu and Baker; 1980). Cases of disease suppressiveness have been reported to occur naturally (Lumsden *et al.*, 1990) or be induced by inoculation with specific antagonists (Harman *et al.*, 1989).

Members of the fungal genus *Trichoderma* are among the most investigated agents of naturally occurring and induced biological control (Baker, 1991; Adams, 1990; Cook and Baker, 1983). Weindling and Emerson (1936) were the first to isolate antifungal compounds from cultures of *Trichoderma* spp. and were among early researchers who suggested the potential of *Trichoderma* spp. for use as a biological control agent for plant pathogenic fungi (Weindling, 1934; Weindling and Fawcett, 1939; Weindling and Emerson, 1936). Dennis and Webster (1971 a-c) described the antibiotic and parasitic mechanisms of antagonism of *Trichoderma* spp. against target organisms. And, Wells *et al.* (1972) first reported field control of *Sclerotium rolfsii* by *T. harzianum.* Since then, *Trichoderma* spp. have been reported to reduce disease caused by *Armillaria mellea* (Vahl:Fr.) Kumm. (Cook and Baker, 1983), *Rhizoctonia solani* (Davet, 1986; Harman *et al.*, 1980, 1981; Elad *et al.*, 1980a,b; Liu and Baker, 1980; Hadar *et al.*, 1979; Bell and Wells, 1977), *Phytophthora* spp. (Smith et at., 1990), and *Pythium* spp. (Harman *et al.*, 1980, 1981; Wolffhechel, 1989). Elad *et al.* (1980b) also found that following soil solarization or fumigation, soil inoculation with *Trichoderma harzianum* enhanced control of *R. solani* over that achieved by either treatments alone and also resulted in the control of *Sclerotium rolfsii.*

The use of *T. harzianum* has been investigated for controlling *Rhizoctonia solani* in strawberries (Elad *et al.*, 1981). In this study, two field experiments were conducted where nursery beds were planted in fumigated soil and inoculated twice with *T. harzianum*; once at planting and again one month later. A 18-46% reduction in disease severity was

achieved in *T. harzianum*-treated nursery plots compared to control plots. A third field experiment was conducted where plants from the treated and control plots were transplanted to fruiting fields for yield evaluation. Plots in the fruiting field were split, and half were treated again with *T. harzianum.* Plants from *T. harzianum*-treated plots that did not receive additional inoculation when transplanted resulted in a 21-37% yield increase over untreated transplants. Additional *T. harzianum* inoculation did not enhance yield compared to plants treated only in the nursery beds.

3.1.2.2 Taxonomy and Morphology of *Trichoderma* spp.

Trichoderma spp. are spore forming fungi belonging to the order Hyphomycetes, family Moniliaceae (Farr *et al.*, 1989). The genus is comprised of 20 species groups of which five or six are associated with biological control of plant pathogens (Cook and Baker, 1983). Many species of *Trichoderma*, including *T. harzianum* Rifai (teleomorph, *Hypocrea* spp.) are cosmopolitan in distribution (Farr *et al.*, 1989) and are found commonly in soil (Cook and Baker, 1983). Hyphae of *Trichoderma* spp. produce conidiophores bearing flask shaped phialides singly or in clusters shown in (Cook and Baker, 1983). Chlamydospores commonly are formed as resting structures. In culture, colonies usually grow rapidly with aerial mycelia tufted white or green (Cook and Baker, 1983).

3.1.2.3 Biocontrol Modes of Action of *Trichoderma* spp.

The primary modes of action of *Trichoderma* spp. have been described as mycoparasitism of host fungi and aggressive competition with host and non-host fungi for soil resources (Webster and Lomas, 1964; Cook and Baker, 1983). As illustrated in Figure 3.2, mycoparasitism takes place by hyphae of *Trichoderma* spp. coiling around the hyphae of host fungi, lysing membranes and causing hyphae to collapse and disintegrate (Cook and Baker,1983). Once the host cell integrity is destroyed, the mycoparasite

absorbs the hyphal contents (Chet, 1987). The mechanism of cell destruction by *T. harzianum* involves the release of ß-(1,3) glucanase and chitinase, lysing walls of *R. solani* (Hadar *et al.*, 1979). Chet *et al.* (1981) and Chet and Elad (1983) described hyphae of *Trichoderma* spp. growing directly toward host mycelia, chemotropically stimulated in the presence of hyphal exudates produced by the host species. Elad *et al.* (1983) reported evidence that *Trichoderma* hyphae may bind to *R. solani* hyphae as a precursor to lysis. Additionally, *Trichoderma* spp. are aggressive saprophytes able to utilize a range of polymers including cellulose and hemicelluloses as growth substrates (Deacon, 1983).

3.1.2.4 Growth Parameters of *Trichoderma* spp.

Soil characteristics affect the growth and biological control effectiveness of *Trichoderma* spp. (Cook and Baker, 1983). For example, efficacy of *Trichoderma* spp. in disease suppression was greater in acid soil, i.e. pH 3.5 to 6.5 (Chet and Baker, 1980; Roiger *et al.*, 1991, Harman, 1992). *Trichoderma* spp. also were favored in moist soil (> -.135 MPa) and at soil temperatures below 25°C (Liu and Baker, 1980; Elad *et al.*, 1980a).

3.1.2.5 Rhizosphere Competence of *Trichoderma* spp.

In addition to environmental parameters (e.g., the soil characteristics described above) that affect the biocontrol activity of *Trichoderma* spp., the isolate must be suited to inhabit the target zone required for conveying crop protection against pathogen infection (Chet, 1987; Harman, 1992). Protection of subterranean plant parts is best accomplished with rhizosphere-competent isolates (Harman, 1992). Such isolates are those capable of colonizing the root surface or rhizosphere of the target crop (Harman, 1990). Rhizosphere-competent isolates can be obtained from crop roots grown in naturally suppressive soils or through a process of genetic manipulation or mutation of candidate isolates (Ahmad and Baker, 1987; 1988a,b; Harman, 1990).

3.1.2.6 Delivery Methods for *Trichoderma* spp.

Biocontrol inocula must contain a carrier capable of providing a substrate for the initial growth of the organism when released in the target zone (Cook and Baker, 1983). Harman *et al.* (1981) reported a significant benefit to amending conidial suspensions of *T. hammatum* (Bonord.) Bainier with chitin or dried mycelium from a non-pathogenic *Rhizoctonia* as a food base for protecting seedlings from damping-off diseases. Backman and Rodriguez-Kabana (1975) used molasses-enriched clay granules plus diatomaceous earth as a carrier for *T. harzianum* in controlling *Sclerotium rolfsii*. Other methods for inoculating soil with *Trichoderma* include conidia-impregnated alginate and wheat bran pellets (Lumsden and Locke, 1989), a wheat bran and sawdust inoculum (Elad *et al.* 1980a), and a wheat bran and peat inoculum (Sivan *et al.*, 1984; Paulits *et al.*, 1986; Maplestone *et al.*, 1991).

3.1.3 Role of Biocontrol in Integrated Pest Management

Effective mycoparasitism and saprophytic competitiveness indicate that *Trichoderma* spp. play a role in both general and specific disease suppression in soil (Chet, 1987). Elad *et al.* (1980b) observed that natural populations of *Trichoderma* spp. increased following soil fumigation or solarization (sub-lethal biocidal treatments), that *Rhizoctonia solani* failed to recolonize the soil effectively, and that a combination of soil solarization or fumigation and inoculation with *T. harzianum* provided the best control of *R. solani* and *S. rolfsii*. Integration of cultural or chemical controls with biological controls may provide powerful and long-lasting disease-management options in integrated pest management programs for different crops (Baker and Scher, 1987).

3.1.4 Objectives

The objective of this study was to test isolates of *Trichoderma harzianum* as potential biological control agents of *Rhizoctonia solani* and *R. fragariae* in strawberries.

3.2 Materials and Methods

Two candidate isolates of *Trichoderma harzianum* were obtained 1) from the collection of G. Harman (Cornell University, Ithaca New York) and 2) by isolation from strawberry roots collected from ten locations in Massachusetts, following procedures described in Roiger *et al.* (1991). *Trichoderma harzianum* inoculum was prepared according to Smith *et al.* (1990).

3.2.1 Greenhouse Experiment I

The first greenhouse experiment evaluated two *Trichoderma harzianum* isolates for biological control of *Rhizoctonia solani* infection of strawberry roots. The experimental design was a fully factorial randomized complete block with two main classifications and 10 replications. The first classification contained two treatments: inoculation with three grams of *R. solani* (AG1, B43) inoculum at 20 x 10^5 colony forming units (cfu)/gram, prepared according to standard operating procedures SOP: T101, T106, and T109; provided in the appendices and an untreated control. The second classification contained four treatments: inoculation with four grams of either *T. harzianum* isolate 'Y' or '11' inoculum at 30 x 10^6 and 25 x 10^5 cfu/gram, respectively, prepared according to Smith *et al.* (1990), four grams of double autoclaved, non-inoculated wheatbran/peat carrier, and an untreated control. There were a total of eight treatment combinations.

The growing medium was a pasteurized soil mix with soil, sand, and perlite in a ratio of 2:1:1. The soil mix was double heat sterilized at 180°F for five hours on two consecutive days and 350 ml (1.5 g/ml dryweight equivalent) decanted into 10 cm diameter clean plastic pots. Plants were greenhouse-grown, tissue-cultured plantlets cv Kent provided by Nourse Farms, Inc., Whately, MA.

R. solani inoculum was allowed to incubate in pots for one week at ambient greenhouse air temperature (22° to 26°C) after being incorporated into the soil mix. *T. harzianum* inoculum then was added and likewise allowed to incubate for one week at

ambient greenhouse air temperature prior to planting strawberries. Care was taken to prevent contamination by washing tools and hands with 20% bleach solution between treatments. The strawberry plants were allowed to grow for 12 weeks. Plants were fertilized with 50 ml of a 1% solution of Peter's® 20-20-20 All Purpose Plant Food (Grace Sierra Hort. Products Co., Milpitas, CA) weekly.

Plant survival and runner production were recorded at the end of the experiment. Roots and shoots were separated by dividing crowns where the roots emerged, dried and weighed. Visual evaluations of root lesion density on primary and secondary roots were made on a scale of 1 to 5 (where 1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) at the end of the experiment.

3.2.2 Greenhouse Experiment II

The second greenhouse experiment evaluated the same two isolates of *T. harzianum* for biological control of infection of strawberry roots by *R. fragariae* and *R. solani*. combined. The experiment was a fully factorial randomized complete block design with two main classifications and nine replications. The two treatments in the first classification included inoculation with two grams of *R. solani* (AG1, B43) inoculum at 35 x 10^5 cfus/gram and two grams of *R. fragariae* inoculum at 25 x 10^4 cfus/gram prepared according to SOP T101, T106, and T109 (provided in the appendices) mixed together and an untreated control. The four treatments in the second classification were: four grams of *T. harzianum* 'Y' (30 x 10^6 cfus/gram) or four grams of *T. harzianum* '11' (35 x 10^5 cfus/gram) prepared according to Smith *et al.* (1990), four grams double autoclaved wheat bran/peat carrier, and an untreated control. There were a total of eight treatment combinations.

The growing medium was a pasteurized sand. The sand was double heat sterilized at 83°C for five hours on two consecutive days and 350 ml (1.2 g/ml dryweight equivalent)

decanted into 10 cm diameter clean plastic pots. Plants were dormant greenhouse-grown tissue-cultured plantlets cv. Kent provided by Nourse Farms, Inc., Whately, MA that had been stored at -2 °C for two months.

Fresh weight of each strawberry plant was recorded at the beginning and end of the experiment in order to evaluate plant growth and for use in analysis of covariance. *R. solani* plus *R. fragariae* inoculum was allowed to incubate in pots for one week at ambient greenhouse air temperature (22° to 26°C) after being incorporated in the soil mix. *T. harzianum* inoculum then was added and likewise allowed to incubate for one week at ambient temperature prior to planting strawberries. Care was taken to prevent contamination by washing tools and hands with 20% bleach solution between treatments. The strawberry plants were allowed to grow for 10 weeks. Plants were fertilized with 50 ml of a 1% solution of Peter's® 20-20-20 Al l Purpose Plant Food (Grace Sierra Hort. Products Co., Milpitas, CA) or Hoagland's nutrient solution (Hoagland and Arnon, 1938) on alternating weeks.

Plant survival, fresh weight, and runner production were recorded at the end of the experiment. Roots and shoots were separated by dividing crowns where the roots emerged, then dried and weighed. Visual evaluations of plant health and root lesion density on primary and secondary roots were made on a scale of 1 to 5 (where, root ratings were done as in Greenhouse Experiment I and, for plant ratings, 1=dead with no green tissue, 2=dying with some green tissue, 3=sick with mostly green tissue, 4=no dark tissue but somewhat stunted, 5=healthy, no stunting, and all green tissue) at the end of the experiment.

3.2.3 Greenhouse Experiment III

The third greenhouse experiment evaluated the same two isolates of *T. harzianum* for biological control of infection of strawberry roots by *R. fragariae* and *R. solani.*, separately. It was a fully factorial randomized complete block design with two main

classifications and seven replications. The first classification contained four treatments: three grams of *R. solani* inoculum (AG1, B43) at 40 x 10^5 cfus/gram, three grams or *R. fragariae* inoculum (AGI, 1005) at 25 x 10^5 cfus/gram, three grams of sterile oat carrier, and an untreated control. The second classification contained four treatments: four grams of *T. harzianum* 'Y' (32 x 10^6 cfus/gram), four grams of t. *harzianum* '11'(25 x 10^5 cfus/gram) prepared according to Smith *et al.* (1990), four grams of double autoclaved, non-inoculated wheatbran/peat carrier, and an untreated control. There were a total of 16 treatment combinations.

Planting medium was pasteurized sand which was heat sterilized at 83°C for five hours on two consecutive days and 350 ml (1.2 g/ml dryweight equivalent) decanted into 10 cm diameter clean plastic pots. Strawberry plants cv Honeoye were obtained from field harvested runners and rooted in soilless mix in the greenhouse. Runners were rooted for one week under mist and then grown for three weeks in the greenhouse in order to produce root systems. Plants were washed, weighed, and grouped according to weight prior to planting. Treatments were assigned randomly among plants within weight groups.

Inoculum of *Trichoderma* and *Rhizoctonia* were added to the pots at the same time and allowed to incubate in the sand one week at ambient greenhouse temperatures (24°C) prior to planting strawberries. Strawberries were grown for 12 weeks. Plants were watered with deionized water only (pH 5.8 to 6.2) and fertilized with 50 ml of a 1% solution of Peter's® 20-20-20 Al l Purpose Plant Food (Grace Sierra Hort. Products Co., Milpitas, CA) or Hoagland's nutrient solution (Hoagland and Arnon, 1938) on alternating weeks.

Plant growth and health variables were measured as in Greenhouse Experiment II except that plants were not divided into shoots and roots but were weighed as whole plants and no visual root evaluations were made.

3.2.5 Determination of Inoculum Density

For all experiments in this study, inoculum density, as expressed as colony forming units (cfu), of *Trichoderma* and *Rhizoctonia* inoculum were determined using serial dilution plating on *Trichoderma* selective media, TSM, (Smith *et al.*, 1990) or *Rhizoctonia* selective media, RSM, (Martin, 1988), respectively, according to SOP T102, included in the appendices.

3.2.6 Statistical Analysis

Statistical analyses were performed using SYSTAT for the Macintosh© (Statistics, Version 5.2 Edition, Evanston, IL). Square root and arcsine transformations were performed on percentage and count data, as needed, to stabilize variances or normalize the sample population (Damon and Harvey, 1987; Steel and Torie, 1980). Back-transformed data from transformations are presented in all tables and figures. Differences between treatments were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure, with means separated by least significant difference (LSD) or single-degree-of-freedom contrasts (Damon and Harvey, 1987; Gomez and Gomez,1984).

3.3 Results

3.3.1 Greenhouse Experiment I

Whole-plant weight was affected by treatment and inoculation, where plants grown with either of the *T. harzianum* isolates weighed more ($p \leq 0.05$) than control plants and plants inoculated with *R. solani* weighed less ($p \leq 0.01$) than non-inoculated plants (Table 3.1). Similarly, shoot weights were affected by treatment and inoculation with those of plants grown with either of the *T. harzianum* isolates greater ($p \leq 0.05$) than control plants, and those of plants inoculated with *R. solani* less ($p \leq 0.001$) than those of non-inoculated plants (Table 3.2). Root weight were affected by treatments but not inoculation. Root

weights of plants grown with either of the *T. harzianum* isolates were greater ($p \leq 0.05$) than those grown with the wheat bran and peat carrier or control plants.

Table 3.1. Dry weight (g) of whole strawberry plants grown in soil with or without inoculation with *Rhizoctonia solani* and treated with one of two isolates of *Trichoderma harzianum*, a sterile wheat bran and peat carrier, or not treated[z].

	Whole-plant Weight (g)		
Treatments	Inoculated[x]	Non-inoculated	Mean[y]
T. harzianum 'Y'[w]	3.79	5.41	4.60 ab
T. harzianum '11'[w]	4.47	5.35	4.90 a
Wheat bran/peat[v]	2.92	4.78	3.85 bc
Control	3.25	4.05	3.65 c
Mean	3.61 **	4.90	

[z]Analysis of variance table A.19 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

[x]Inoculated with 3 g *R. solani* inoculum (20×10^5 cfu/g) grown on double-autoclaved oat seed carrier.

[w]Treated with 4 g *T. harzianum* isolate 'Y' or '11' inoculum (25 and 30×10^5 cfu/g, respectively) grown on double-autoclaved wheatbran and peat plus H_2O carrier (1:1 v/v).

[v]Sterile double-autoclaved wheatbran and peat (1:1, v/v).

**Inoculated significantly different from non-inoculated at $p \leq 0.01$.

Table 3.2. Dry weight (g) of roots and shoots of strawberry plants grown in soil with or without inoculation with *Rhizoctonia solani* and treated with one of two isolates of *Trichoderma harzianum*, a sterile wheat bran and peat carrier, or not treated[z].

	Strawberry Root Weight (g)		
Treatments	Inoculated[x]	Non-inoculated	Mean[y]
T. harzianum 'Y'[w]	0.75	0.86	0.81 a
T. harzianum '11'[w]	0.82	0.78	0.80 a
Wheat bran/peat[v]	0.79	0.56	0.67 b
Control	0.73	0.54	0.64 b
Mean	0.77 ns	0.69	
	Strawberry Shoot Weight (g)		
T. harzianum 'Y'	3.03	4.56	3.79 ab
T. harzianum '11'	3.64	4.57	4.11 a
Wheat bran/peat	2.13	4.22	3.18 bc
Control	2.52	3.50	3.01 c
Mean	2.83 ***	4.21	

[z]Analysis of variance tables A.17 and A.18 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

[x]Inoculated with 3 g *R. solani* inoculum (20×10^5 cfu/g) grown on double-autoclaved oat seed carrier.

[w]Treated with 4 g *T. harzianum* isolate 'Y' or '11' inoculum (25 and 30×10^5 cfu/g, respectively) grown on double-autoclaved wheatbran and peat plus H_2O carrier (1:1 v/v).

[v]Sterile double-autoclaved wheatbran and peat (1:1, v/v).

***,ns Inoculated significantly different from non-inoculated at $p \leq 0.001$ or not significantly different, respectively.

The number of runners produced by each strawberry plant was affected by treatment and inoculation. Untreated control plants produced more runners ($p \leq 0.05$) than those treated with either isolate of *T. harzianum*, but plants inoculated with *R. solani* produced fewer runners ($p \leq 0.001$) than non-inoculated plants (Table 3.3).

Table 3.3. Number of runners produced by plants grown in soil with or without inoculation with *Rhizoctonia solani* and treated with one of two isolates of *Trichoderma harzianum*, sterile wheat bran and peat carrier, or not treated[z].

	Number of Runners per Plant		
Treatments	Inoculated	Non-inoculated	Mean[y]
T. harzianum 'Y'	0.4	1.9	1.1 b
T. harzianum '11'	0.4	1.7	1.0 b
Wheat bran/peat	0.5	2.0	1.2 ab
Control	1.1	2.2	1.6 a
Mean	0.6 ***	2.0	

[z]Analysis of variance table A.20 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

*** Inoculated significantly different from non-inoculated at $p \leq 0.001$.

Inoculation with *R. solani* reduced ($p \leq 0.001$) visual rating scores of primary and secondary roots . Treatments affected ($p \leq 0.05$) visual ratings of secondary roots only, with plants grown with *T. harzianum* 'Y' resulting in healthier appearing roots than plants grown with wheat bran/ peat or the control (Table 3.4).

Table 3.4. Visual ratings (where 1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) of primary and secondary strawberry roots from plants grown in soil with or without inoculation with *R. solani* and treated with one of two isolates of *T. harzianum*, sterile wheat bran and peat carrier or not treated[z,y].

	Visual rating of strawberry roots[x]				
	Primary roots		Secondary roots		
Treatments	Inoculated[w]	Non-inoculated	Inoculated[w]	Non-inoculated	Mean
T. harzianum 'Y'[v]	2.4	3.9	2.4	4.1	3.2 a
T. harzianum '11'[v]	2.5	3.4	2.2	3.7	3.0 ab
Wheat bran/peat [u]	2.0	3.0	1.7	3.3	2.5 b
Control	2.1	2.9	2.2	2.9	2.5 b
Mean	2.2 ***	3.3	2.1 ***	3.5	

[z]Analysis of variance per formed on transformed data $((x + .05)^{1/2})$; ANOVA tables A.21 and A.22 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

[x]Data presented backtransformed from analyzed data.

[w]Inoculated with 3 g *R. solani* inoculum (20×10^5 cfu/g) grown on double-autoclaved oat seed carrier.

[v]Treated with 4 g *T. harzianum* isolate 'Y' or '11' inoculum (25 and 30 x 10^5 cfu/g, respectively) grown on double-autoclaved wheatbran and peat plus H_2O carrier (1:1 v/v).

[u]Sterile double-autoclaved wheatbran and peat (1:1, v/v).

*** Inoculated significantly different from non-inoculated at $p \leq 0.001$.

3.3.2 Greenhouse Experiment II

Whole-plant weights of strawberry plants inoculated with *R. solani* and *R. fragariae* were less ($p \leq 0.001$) than non-inoculated plants (Table 3.5). Dry weight of *T. harzianum* 'Y'-treated plants inoculated with *Rhizoctonia* were not significantly different from non-inoculated plants. Root and shoot weights of inoculated strawberry plants were less ($p \leq 0.05$ and $p \leq 0.001$, respectively) than non-inoculated plants (Table 3.6).

Table 3.5. Fresh and dry weight (g) of whole strawberry plants grown in soil with or without inoculation with a combination of *Rhizoctonia solani* and *R. fragariae* and treated with one of two isolates of *Trichoderma harzianum* , a sterile wheat bran and peat carrier, or not treated[z,y].

Treatment	Whole-plant Fresh Weight (g)[x]			
	Inoculated[w]		Non-inoculated	
T. harzianum 'Y'[v]	6.74	ab	16.01	a
T. harzianum '11'[v]	8.64	a	13.73	b
Wheat bran/peat[u]	7.66	a	12.17	b
Control	5.40	b	12.95	b
Mean	7.11	***	13.71	

Treatment	Whole-plant Dry Weight (g)			
	Inoculated		Non-inoculated	
T. harzianum 'Y'	1.08	a ***	2.74	a
T. harzianum '11'	1.71	a ns	1.96	b
Wheat bran/peat	1.67	a **	2.62	a
Control	1.17	a **	2.22	ab
Mean	1.41	***	2.38	

[z]Analysis of covariance and analysis of variance tables A.25 and A.26 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

[x]Adjusted least squares means reported for data analyzed for covariance.

[w]Inoculated with 2 g each *R. solani* and *R. fragariae* inoculum (30 and 35 x 10^5 cfu/g, respectively).

[v]Treated with 4 g *T. harzianum* isolate 'Y' or '11' inoculum (30 and 35 x 10^5 cfu/g, respectively).

[u]Sterile double-autoclaved wheatbran and peat (1:1, v/v).

,*, ns Inoculated significantly different from non-inoculated at $p \leq 0.01$, $p \leq 0.001$ or not significantly different, respectively.

Table 3.6. Dry weight (g) of roots and shoots of strawberry plants grown in soil with or without inoculation with a combination of *Rhizoctonia solani* and *R. fragariae* and treated with one of two isolates of *Trichoderma harzianum* or a sterile wheat bran and peat carrier, or untreated[z].

Treatments	Root Dry Weight (g)			
	Inoculated		Non-inoculated	
T. harzianum 'Y'	0.86		1.69	
T. harzianum '11'	1.25		1.21	
Wheat bran/peat	1.27		1.59	
Control	1.00		1.31	
Mean	1.09	*	1.45	

	Shoot Dry Weight (g)[y]			
T. harzianum 'Y'	0.22	ab	1.05	a
T. harzianum '11'	0.47	a	0.75	b
Wheat bran/peat	0.40	a	1.03	a
Control	0.17	b	0.91	ab
Mean	0.32	***	0.94	

[z]Analysis of variance tables A.23 and A.24 in Appendix A.

[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.

*,*** Inoculated significantly different from non-inoculated at $p \leq 0.05$, $p \leq 0.001$, respectively.

The number of runners produced by each strawberry plant was not affected by treatments, but plants inoculated with *R. solani* and *R. fragariae* produced fewer ($p \leq 0.001$) runners than non-inoculated plants (Table 3.7). Visual ratings of whole strawberry plants were lower ($p \leq 0.001$) for plants inoculated with a *R. solani* and *R. fragariae* than non-inoculated plants. Among inoculated strawberry plants, visual ratings of plants treated with either *T. harzianum* and wheat bran plus peat were higher than control plants (Table 3.8). Visual ratings of primary and secondary roots were lower ($p \leq 0.001$) for plants inoculated with a *R. solani* and *R. fragariae* than non-inoculated plants. Visual ratings of primary and secondary roots were unaffected by treatments.(Table 3.9).

Table 3.7. Number of runners produced by plants grown in soil with or without inoculation with a combination of *Rhizoctonia solani* and *R. fragariae* and treated with one of two isolates of *Trichoderma harzianum*, sterile wheat bran and peat carrier or not treated[z].

	Number of runners		
Treatments	Inoculated		Non-inoculated
T. harzianum 'Y'	1.0		1.7
T. harzianum '11'	1.0		1.9
Wheat bran/peat	1.0		1.7
Control	0.7		1.9
Mean	1.0	***	1.8

[z]Analysis of variance table A.27 in Appendix A.
*** Inoculated significantly different from non-inoculated at $p \leq 0.001$.

Table 3.8. Visual ratings of strawberry plants (1=dead, 2=almost dead, 3=some discoloration, 4=slightly stunted, 5=healthy) grown in soil with or without inoculation with a combination of *Rhizoctonia solani* and *R. fragariae* and treated with one of two isolates of *Trichoderma harzianum*, sterile wheat bran and peat carrier, or not treated[z,y].

	Whole-plant Visual Rating[x]		
Treatment	Inoculated		Non-inoculated
T. harzianum 'Y'	2.2[u] b		5.0 a
T. harzianum '11'	3.1 a		5.0 a
Wheat bran/peat	2.4 ab		5.0 a
Control	1.4 c		5.0 a
Mean	2.3	***	5.0

[z]Analysis of variance per formed on transformed data $((x + .05)^{1/2})$; ANOVA table A.30 in Appendix A .
[y]Means in columns followed by different letters significantly different at $p \leq 0.05$; LSD.
[x]Data presented backtransformed from analyzed data.
*** Inoculated significantly different from non-inoculated at $p \leq 0.001$.

Table 3.9. Visual ratings (where 1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) of primary and secondary strawberry roots from plants grown in soil with or without inoculation with a combination of *Rhizoctonia solani* and *R. fragariae* and treated with one of two isolates of *Trichoderma harzianum*, sterile wheat bran and peat carrier or not treated.

	Visual Rating of Primary Roots[z]		
Treatments	Inoculated[y]		Non-inoculated
T. harzianum 'Y'[x]	2.6[v]		4.7
T. harzianum '11'[x]	3.2		4.3
Wheat bran/peat[w]	2.9		4.6
Control	2.4		4.4
Mean	2.8	***	4.5
	Visual Rating of Secondary roots		
T. harzianum 'Y'	2.6		4.3
T. harzianum '11'	2.1		4.4
Wheat bran/peat	2.3		4.3
Control	2.8		4.2
Mean	2.4	***	4.3

[z]Analysis of variance per formed on transformed data $((x + .05)^{1/2})$; ANOVA tables A.28 and A.29 in Appendix A .

[x]Data presented backtransformed from analyzed data.

*** Inoculated significantly different from non-inoculated at $p \leq 0.001$.

3.3.3 Greenhouse Experiment III

Ending fresh and dry weights were affected by inoculation with *Rhizoctonia* isolates but not by treatment (Table 3.10) Fresh weight of strawberry plants was greater ($p \leq 0.001$) for plants grown in sand amended with the sterile carrier or no amendment than those inoculated with either species of *Rhizoctonia.* Dry weight of strawberry plants was greater ($p \leq 0.01$) for plants grown in unamended sand than sand amended with either species of *Rhizoctonia* or with the sterile carrier.

Table 3.10. Whole plant fresh and dry weights of strawberry plants grown in sand inoculated with either *Rhizoctonia solani* or *R. fragariae*, amended with a sterile oat carrier or non-inoculated and treated with one of two isolates of *Trichoderma harzianum*, a sterile wheat bran and peat carrier, or not treated[z,y].

	Fresh Weight of Whole Plants (g)[x]			
Treatments	*R. fragariae* [w]	*R. solani* [w]	Sterile carrier[v]	Control
T. harzianum 'Y'[u]	2.32	4.24	3.88	3.73
T. harzianum '11'[u]	3.75	2.74	4.71	3.87
Sterile carrier[t]	3.05	1.66	4.33	4.06
Control	2.59	2.63	4.08	4.75
Mean	2.93 b	2.82 b	4.25 a	4.12 a
	Dry Weight of Whole Plants (g)			
T. harzianum 'Y'	0.86	1.44	0.93	1.23
T. harzianum '11'	1.12	0.95	1.01	1.18
Sterile carrier	0.84	0.58	1.00	1.44
Control	0.70	0.65	0.97	1.55
Mean	0.88 b	0.91 b	0.98 b	1.35 a

[z] Analysis of covariance and analysis of variance tables A.34 and A.35, respectively, in Appendix A.

[y]Means in rows followed by different letters significantly different at $p \leq 0.05$; LSD.

[x]Adjusted least squares means reported for data analyzed for covariance.

[w]Inoculated with 3 g of either *R. solani* or *R. fragariae* inoculum (40×10^5 and 25×10^4 cfu/g, respectively) grown on double-autoclaved oat seed carrier.

[v]Sterile double-autoclaved oat seed plus H_2O carrier (1:2 v/v).

[u]Treated with 4 g *T. harzianum* isolate 'Y' or '11' inoculum (32 and 25×10^5 cfu/g, respectively) grown on double-autoclaved wheat bran and peat plus H_2O carrier (1:1:1 v/v/v).

[t]Sterile double-autoclaved wheat bran and peat plus H_2O carrier (1:1:1 v/v/v).

Plants inoculated with *R. solani* or *R. fragariae* gained less weight than non-inoculated plants ($p \leq 0.01$) but were unaffected by treatment *T. harzianum* isolates (Table 3.11). Visual ratings of plant health and root lesion density were affected by inoculation with *Rhizoctonia* but not by treatment with *Trichoderma* (Table 3.12). Plant and root ratings were both lower for plants grown in sand inoculated with either species of *Rhizoctonia* than those grown in sand amended with sterile wheat bran and peat or not treated.

Table 3.11. Visual ratings of plants and roots from plants grown in sand inoculated with either *Rhizoctonia solani* or *R. fragariae*, amended with a sterile oat carrier or non-inoculated and treated with one of two isolates of *Trichoderma harzianum*, a sterile wheat bran and peat carrier, or not treated[z].

	Visual Plant Rating			
Treatments	*R. fragariae*	*R. solani*	Sterile carrier	Control
T. harzianum 'Y'	2.1	3.0	2.7	2.7
T. harzianum '11'	2.7	2.1	3.0	2.6
Sterile carrier[u]	2.4	1.6	3.1	3.7
Control	1.9	2.1	3.3	3.6
Mean	2.3 b	2.2 b	3.0 a	3.1 a
	Visual Root Rating			
T. harzianum 'Y'	2.1	3.0	3.0	3.4
T. harzianum '11'	2.7	2.3	2.9	3.4
Sterile carrier	2.6	1.7	3.4	4.1
Control	1.8	2.0	4.0	3.8
Mean	2.3 b	2.3 b	3.3 a	3.7 a

[z]Means in rows followed by different letters significantly different at $p \leq 0.05$; LSD.

3.4 Discussion

The three experiments in this study were similar in design and variable in result. It is usefull to review the representative effects briefly prior to discussion (Table 3.12). Experiment I involved inoculation with *Rhizoctonia solani*, Experiment II used *R. solani* and *R. fragariae* in combination, and Experiment II used *R. solani* and *R. fragariae* separately. All experiments used *Trichoderma harzianum* isolates 'Y' and '11' and a sterile carrier.

Table 3.12. Summary of effects on whole plant dry weight from three experiments on biological control of *Rhizoctonia* spp. with two*Trichoderma harzianum* isolates.

Effect	Experiment I	Experiment II	Experiment III
Rhizoctonia spp.			
R. solani	-[z]	*n/a*	-
R. fragariae	*n/a*[y]	*n/a*	-
combination	*n/a*	-	*n/a*
Trichoderma harzianum			
'Y'	+[x]	none	none
'11	+	none	none
Sterile carrier	none[w]	none	none
Interaction between *Trichoderma* and *Rhizoctonia* inoculation (p values)	0.418	0.006	0.063

[z]Negative effect.

[y]Treatment not applied in this experiment.

[x]Positive effect.

[w]Effect not significant different from control treatment at $p \leq 0.05$

3.4.1 Effect of *Rhizoctonia* Inoculation on Strawberry Plants

Inoculation with *Rhizoctonia* had an effect on all growth variables measured in this study except root weight in Experiment I. However, determining an effect on root and shoot weight was imprecise since the division of the crown was somewhat arbitrary and variation in location of the cut changed weight measurements significantly. In the future, determining the effect on root weights will require the excision of each of the perennial roots from the crown so that the roots, alone, can be weighed. Then, the whole crown could be weighed as part of the shoot of the plant. Since this was not the methodology of these experiments, root and shoot weights were combined to form whole plant weights. Effects on whole plant weights then were evaluated.

The effect of inoculation on whole plant weights was highly significant in Experiments I and II, where *R. solani* and a mixture of *R. solani* and *R. fragariae* were

used, respectively. Similarly, in Experiment III, inoculation with *R. solani* and *R. fragariae* as separate treatments, significantly reduced fresh and dry whole plant weights compared to controls. In the first two experiments, some of the difference in plant weights could have been the result of increased runner production in the non-inoculated plants. But, in Experiment III, plants did not produce any runners.

Visual evaluations of root lesions also showed the effect of inoculation in all three experiments. The scale used for these evaluations was somewhat limited and appeared insufficient for detecting fine differences between *Trichoderma* treatments discussed below. Greater precision in measuring lesion density on roots may be gained by using digital image analysis, and this technology will be tried in future studies.

The detrimental effect of *Rhizoctonia* on strawberry roots is well known and was described in Chapter I. Results of inoculation obtained in these experiments confirm the expected effect of disease pressure on the plants. Conditions of this study, however, were artificial since sterilized soil mix or sand were used to grow plants and "clean" tissue cultured plantlets or rooted runners were used as experimental subjects. These provisions had the effect of reducing the microbial population in the root zone to the organisms introduced as part of the experiments (plus some random contamination from the air and water). In the field, the microbial population is complex and conditions cannot be controlled, and plants received form the nursery are dormant field grown runners with roots that are populated by soil microorganisms. As a result, disease progression will differ from that in controlled greenhouse conditions. Conclusions cannot be drawn from controlled studies that predict what will happen in the field, or *vice-versa*. But, it is still necessary to confirm that the disease organism under investigation is producing the expected effect on plants, and this was proven in each of these experiments.

3.4.2 Effect of *Trichoderma harzianum* on Root Disease and Plant Growth

In Experiment I, whole-plant dry-weights were greater in both treatments receiving *Trichoderma harzianum* than in the controls. Since the *Rhizoctonia*-inoculation effect was also significant and there was no interaction effect, it is not possible to say that the *Trichoderma* treatments suppressed *Rhizoctonia* infections. Greater plant weights may instead have resulted from plant growth enhancement from *Trichoderma* as described in the literature review for this chapter. Interestingly, this plant growth enhancement cannot be attributed to increased runner production since runner production was reduced by both *Trichoderma* treatments when compared to controls.

In Experiment II, whole plant fresh weights among plants inoculated *Rhizoctonia* and treated with *T. harzianum* '11' were also greater than controls. And, while whole-plant dry weights of *Trichoderma*-treated plants were not greater than controls in Experiment II, dry weight of plants inoculated with *Rhizoctonia* and treated with *T. harzianum* '11' were not significantly different than non-inoculated plants. This suggests that the *Trichoderma* suppressed the detrimental effect of *Rhizoctonia* inoculation in this experiment. In Experiment III, treatment with *T. harzianum* did not have an effect on fresh or dry weights, but it appeared that plants treated with *T. harzianum* isolate '11' grew larger when inoculated with *R. fragariae* and plants treated with *T. harzianum* 'Y' grew larger when inoculated with *R. solani*. This would not be a surprising result since isolate '11' was obtained from strawberry roots that likely could have been exposed to *R. fragariae* and isolate 'Y' was obtained from a research collection that showed in vitro suppression of *R. solani*. The beneficial effects of treatment with *T. harzianum* '11' may relate to the issue of rhizosphere competence described by Ahmad and Baker (1987; 1988a; 1988b). Much more research on these and other isolates is needed before practical applications for the field, if any, are developed.

Numbers of runners in Experiments I and II were not affected by treatment with *Trichoderma*., nor were visual evaluations of the roots. However, visual plant evaluations

in Experiment II showed that with inoculation with *Rhizoctonia*, plants treated with *T. harzianum* '11' were rated higher than the controls.

No conclusions can be drawn from results obtained by treatment with the sterile wheat bran plus peat. This treatment was included as a carrier control to determine whether effects of *Trichoderma* addition were due to the organism or the carrier. However, Baker *et al.* (1984) suggest the fallacy of this reasoning in that, the carrier alone is not the same as the carrier colonized and partially digested by a living organism. They use the example of the wheat bran and peat carrier for *Trichoderma* inoculum in their argument. They say it cannot be considered a control but rather must be considered as a treatment unto itself and results from treatment with wheat bran and peat cannot be used to extrapolate meaning about inoculation with any organism grown on that substrate. In the case of *Trichoderma*, difficulty arises from its tendency to sporulate profusely, even on the surface of the potting medium during the course of an experiment. Dispersal of *Trichoderma* spores from watering or ventilation fans may cause pots containing the carrier substrate to be colonized by *Trichoderma* , inadvertently. Therefore, treatments with sterile carrier alone may not be sterile. In the future this treatment will be discontinued and watering methods may have to be modified to a capillary system with individual trays for each pot.

Overall, it appeared that treatment with isolates of *Trichoderma* had beneficial effects on strawberry plant growth in the presence of *Rhizoctonia solani* and *R. fragariae* but the benefits were variable. In order for more reliable benefits fot strawberry growth to be defined, more experiments must be conducted. Laboratory experiments should be conducted that evaluate the population kinetics of these organisms in field soil with and without the presence of strawberry roots to determine the important components of the system (i.e., pH, temperature, soil moisture and organic matter content, other compatible biocontrol organisms, water stress and nutritional status of the plant, plant cultivar differences, etc.). Also, field studies must be conducted under normal production conditions to determine the practical aspects of this method of disease management (i.e.,

cost, method of application, timing of application, duration of effects, etc.). It is likely that if biological management of soilborne diseases with introduced microorganisms becomes a viable option in commercial strawberry production, it will be as part of a package of practices that could include cultivar selection, crop rotation, possibly soil solarization and cover cropping, and organic matter amendment with compost to enhance the available substrate for sustaining the biocontrol organism(s) introduced into the system, plus other changes.

CHAPTER IV
DISEASE SUPPRESSION STUDY USING SELECTED COMPOSTS

4.1 Literature Review

4.1.1 General Benefits of Organic Matter Amendments

Soil organic matter (SOM) is the fraction of the soil made up of dead plant and animal tissue in various stages of decomposition (Brady, 1984). Per unit mass, this is the most chemically and biologically active fraction of the soil and contains an important reservoir of essential elements needed for plant growth (Bohn *et al.*, 1985). Organic matter in soil also helps promote the formation of soil aggregates improving soil structure, and promotes chelation of copper, zinc, and other polyvalent cations making them more available to higher plants (Bohn *et al.*, 1985). It contains a large reserve of carbon needed to support microbial life (protozoa, nematodes, fungi, bacteria, actinomycetes and some micro arthropods) which is needed to break down newly added organic matter into a relatively stable form known as humus, and are a food source for other microorganisms and micro- and macro- arthropods (Paul and Clark, 1989 ; Bohn *et al.*, 1985).

Benefits of adding organic matter to soil are known and include an increase in water holding capacity and cation exchange capacity, improved aeration and tilth, soil pH buffering, and the addition of plant nutrients through mineralization (Brady, 1984). The incorporation of organic matter into soil also stimulates microbial activity as microorganisms consume added carbon, nitrogen, and each other, in a complex food chain (Paul and Clark, 1989).

4.1.2 Compost Properties and Production

Compost is a form of organic matter commonly applied to soils. It can be described as a stable form of organic matter (i.e., no longer undergoing rapid degradation) which

results from a process of biological decomposition by microorganisms under controlled conditions (Golueke, 1972). The stipulation that it results from a controlled process distinguishes it from the organic humus which results from decomposition under natural conditions as in the forest floor (Rynk, 1992).

Composting is primarily an aerobic process of decomposition involving thermophilic and mesophilic microorganisms (Golueke, 1972). Hoitink and Fahy (1986) describe three phases that comprise the composting process: (1) the initial phase lasting one to two days where temperatures in the organic matter mass rise sharply and readily degradable compounds break down; (2) a thermophilic phase lasting months where temperatures in the center of the pile are sustained at high levels (45° to 65°C) and cellulose and other complex molecules are degraded; and (3) a stabilization phase lasting an undetermined amount of time where temperatures decline, the rate of decomposition decreases and mesophilic organisms recolonize the pile.

Conditions which must be controlled in order for composting to occur include proportioning of materials that make-up the mass of organic matter to be composted with regard to carbon and nitrogen content and ratio (C:N ratio), particle size, water holding capacity, and pH, and the maintenance of adequate moisture within and aeration of the organic matter mass (Rynk, 1992). Dimensions of the mass are also important in the composting process. In order for the decomposition process to accelerate, generating heat and sustaining a thermophilic phase, the pile must be of sufficient mass or else acceleration never occurs or is not sustained (Golueke, 1972). Dimensions vary with the materials used but are generally thought to be a minimum of 1 m^2 and up to 3-4 m high, 6-7 m wide and any length (Rynk, 1992). Upper limits are usually defined by the size of the equipment used to aerate or otherwise manipulate the mass. All of these conditions affect the biological activity in the mass, thereby affecting the process of composting. Desirable ranges of the above parameters are summarized in Table 4.1.

Table 4.1. Recommended conditions for rapid composting.

Condition	Reasonable Range [a]	Preferred Range
Carbon to nitrogen (C:N) ratio	20:1 - 40:1	24:1 - 30:1
Moisture content	40 - 65%	50 - 60%
Oxygen concentration	Greater than 5%	Much greater than 5%
Particle size (diameter)	0.3 - 1.25 cm	Varies [b]
pH	5.5 - 9.0	6.5 - 8.0
Temperature (°C)	44° - 66°	55° - 50°

[a]These recommendations are for *rapid* composting. Conditions outside these ranges can also yield successful results.
[b]Depends on the specific materials, pile size, and/or weather conditions.
(After Rynk, 1992)

Sources of raw materials for composting vary widely, ranging from common household kitchen scraps for back-yard composting to municipal mixed solid waste diverted from landfills and water treatment plants for large scale industrial composting (Rynk, 1992). Many industries and companies are finding composting an appealing option for disposing of organic residuals as other disposal options are restricted or increasingly expensive. Nynex Information Systems has initiated a project for composting outdated telephone directories by shredding them and using them first as animal bedding prior to composting (Logan, 1991; Gould, 1992). In 1990, Proctor & Gamble Co., makers of Pampers™ disposable diapers, began a $20 million research and education initiative for promoting mixed solid waste composting as an alternative to landfilling or incineration (Kunzler, 1992). Ocean Spray, Inc., which formerly disposed of cranberry waste from processing in local landfills, and is now prohibited from this practice, contracts with a company to compost the material at a profit (B. Page, pers. comm.). Indeed, composting is increasingly being viewed as an attractive strategy in integrated waste management; an equal partner with recycling and conservation (Gouin, 1989; Logsdon, 1990). End uses of compost also vary, ranging from use in potting mixes for greenhouse or container grown nursery crops, to use as landfill caps (Gouin, 1989).

The trend toward increased manufacturing of compost is relevant to agriculture because of increasing availability of this potentially beneficial soil amendment, but also because of increased pressure for land application of some composts that are an otherwise difficult disposal problem (Gouin, 1989; Logsdon, 1990; Richard and Chadsey, 1990). This is particularly true of sludge based composts which may have an increased risk of contamination by heavy metals or other toxins (Richard and Chadsey, 1990).

Compost quality standards are, as yet, ill-defined and not universally accepted (Gouin, 1989). Attempts are being made to develop scientifically based compost quality standards with highest end-use recommendations attached (Rynk, 1992). Such standards rate characteristics like particle size, color, soluble salt concentration, respiration rate , etc. with respect to various grades recommended for certain end uses (Rynk, 1992).

Discussion of the broad spectrum of compost properties relevant to plant growth or soil improvement is beyond the scope of this study; however, generalizations about compost quality are possible. For example, a high quality compost is one that would directly benefit plant growth or soil conditions for plant growth while a poor quality compost would be detrimental. A poor quality compost may also contain leachable contaminants (heavy metals), be unstable (high respiration rate), have noxious odors, contain viable weed seeds or plant pathogens, or have physical contaminants like plastic or glass. Desirable properties of compost may go beyond basic horticultural benefits of plant growth to include the ability of a compost to suppress plant disease (Hoitink and Fahy, 1986).

4.1.3 Disease Suppression by Compost

Increased microbial activity following addition of organic matter to soil has been shown to contribute to suppression of plant disease (Hoitink and Fahy, 1986). This suppression is thought to result primarily from increased competition among soil organisms for carbon and nitrogen and other soil resources (i.e., general suppression), and

secondarily, from specific suppression by direct predation or parasitism on pathogens by antagonists residing in, or stimulated by organic matter added to soil (Benson and Baker, 1970; Cook and Baker, 1983).

Compost made from various organic residues have been shown to suppress plant pathogens such as *Phytophthora cinnamomi* Rands, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum*, and *Pythium aphanidermatum* (Edson) Fitzp. (Hoitink, 1980; Hoitink and Fahy, 1986; Gorodecki and Hadar, 1990; Hadar and Mandelbaum, 1986; Spring *et al.*, 1980). Composted hardwood bark, when used in place of peat in potting media, suppressed damping off diseases of cucumber caused by *Pythium ultimum* (Chen *et al.*, 1983). Vaughn *et al.* (1954) reported control of red stele of strawberry (*Phytophthora fragariae*) following incorporation of composted Douglas Fir bark (@90-225 mt/ha). Incorporation of Douglas fir sawdust increased losses by the disease. Malek and Gartner (1975) reported suppression of plant parasitic nematodes, including *Pratylenchus penetrans,* following incorporation of composted hardwood bark, whereas incorporation of peat stimulated nematode populations. Lumsden *et al.*, (1982) reported long-term suppression of lettuce drop caused by *Sclerotinia minor* Jagger following soil incorporation of composted municipal sludge. Interestingly, this suppression was not correlated with a drop in the soil population of the pathogen, but rather with an increase in microbial activity (assessed as dehydrogenase activity) and an increase in the total organic matter content in the soil over four years.

Researchers have reported on suppression of *Rhizoctonia solani* following compost amendment of soil or potting media (Hoitink, 1980; Hoitink *et al.*, 1976; Hoitink and Kuter, 1985; Kuter *et al.*, 1983). Much of the documented suppression of *R. solani* has been from use of composted hardwood bark (CHB) (Kuter *et al.*, 1983; Nelson and Hoitink, 1982; 1983). Damping-off of *Celosia argentina* L. caused by *R. solani* was suppressed in planting medium containing 50% or more of CHB (Hoitink, 1980).

Increasing the ratio of peat in the medium reduced or eliminated the suppression and fresh bark did not suppress disease.

The properties of CHB responsible for disease suppression are not well understood, but are thought to include five factors:

(1) the particle size of CHB is larger than other organic amendments like peat and improve aeration of the root zone, especially in container grown crops (Hoitink and Fahy, 1986);

(2) high nitrogen content increases disease caused by *Phytophthora* and *Fusarium* (Engelhard and Woltz, 1973) and nitrogen content of CHB is often low because immobilization may be still taking place (Hoitink and Fahy, 1986);

(3) pH reduction suppresses certain pathogens such as *P. cinnamomi* but not *R. solani* which may be favored by low pH (Blaker and MacDonald, 1983);

(4) CHB supports high populations of antagonistic and phagous organisms (Kuter *et al.*, 1983), and

(5) water extracts prepared from bark contain toxic compounds (e.g., ethyl esters of hydroxy-oleic acids) with fungicidal properties (Hoitink, 1980; Hoitink and Fahy, 1983).

Antagonistic microorganisms commonly isolated form CHB include members of the genera *Trichoderma*, *Gliocladium*, *Penicillium*, *Morteierella*, *Paecilomyces*, *Geomyces*, and *Ophiostoma* with *T. hamatum* and *T. harzianum* the most abundant taxa (Nelson *et al.*, 1983). Composted hardwood bark is not commonly available in New England. However, composted municipal yard waste made from leaves of mixed hardwoods is abundant. Properties of the two composts are thought to be similar (H. Hoitink, pers. comm.).

4.1.4 Objective

The objective of this study was to test different composts for the ability to suppress *R. solani* infection of strawberry roots and to determine whether soil inhabiting microorganisms were important to disease development or suppression.

4.2 Methods and Materials

4.2.1 Greenhouse Experiment I

The first greenhouse experiment evaluated four composts for suppression of *Rhizoctonia solani* infection of strawberry roots. The experimental design was a fully factorial randomized complete block with three main classifications and 10 replications. The first classification contained two treatments: inoculation with three grams of *R. solani* (AG1, B43) inoculum at 25 x 10^5 colony forming units (cfu)/gram, prepared according to standard operating procedures SOP: T101, T106, and T109; provided in the appendices and an untreated control. The second classification contained five treatments: 50 ml3 of Municipal Yard Waste (MYW) compost primarily made from leaves of mixed hardwood and shade trees obtained from the City of Springfield Dept. of Public Works, Springfield, MA; 50 ml3 MYW compost primarily made from leaves of mixed hardwood and shade trees obtained from the City of Northampton Sanitary Landfill, Northampton, MA; 50 ml3 agricultural compost made from cranberry presscake mixed with chicken manure obtained from Mass Natural Fertilizer Co., Westminster, MA; 50 ml3 agricultural compost made from horse and cow manure obtained from Moody Hill, Inc., NY; and an untreated control where no compost was added. The third classification contained two treatments: pasteurized soil, sand, perlite (2:1:1) soil mix (double heat sterilized at 85°C for five hours on two consecutive days) and non-pasteurized soil mix of the same composition. There were a total of 20 treatment combinations.

Pasteurization of the soil mix was done prior to the other treatments. 300 ml (1.5 g/ml dryweight equivalent) of soil mix decanted into 10 cm diameter clean plastic pots. *R. solani* inoculum was allowed to incubate in pots for one week at ambient greenhouse air temperature (22° to 26°C) after being incorporated into the soil mix. Compost treatments then were added and likewise allowed to incubate for one week at ambient greenhouse air temperature prior to planting strawberries. Plants were greenhouse-grown, tissue-cultured plantlets cv. Kent provided by Nourse Farms, Inc., Whately, MA.

Care was taken to prevent contamination by washing tools and hands with 20% bleach solution between treatments. The strawberry plants were allowed to grow for 12 weeks. Plants were fertilized with 50 ml of a 1% solution of Peter's® 20-20-20 All Purpose Plant Food (Grace Sierra Hort.. Products Co., Milpitas, CA) weekly.

Plant survival and runner production were recorded at the end of the experiment. Roots and shoots were separated by dividing crowns where the roots emerged, dried and weighed. Visual evaluations of root lesion density on primary and secondary roots were made on a scale of 1 to 5 (where 1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) at the end of the experiment.

4.2.2 Greenhouse Experiment II

The second greenhouse experiment evaluated two composts before and after heat sterilization for suppression of *Rhizoctonia solani* infection of strawberry roots. The experimental design was a fully factorial randomized complete block with three main classifications and 10 replications. The first main classification contained three treatments: inoculation with three grams of *R. solani* (AG1, B43) inoculum at 30 x 10^5 colony forming units (cfu)/gram, prepared according to standard operating procedures SOP: T101, T105, and T109; provided in the appendices; inoculation with three grams of sterile oat seed carrier, and an untreated control. The second main classification contained five

treatments: 50 ml of Municipal Yard Waste (MYW) compost primarily made from leaves of mixed hardwood and shade trees obtained from the City of Springfield Dept. of Public Works, Springfield, MA; 50 ml of the same MYW compost autoclaved twice at 85°C for one hour each on two consecutive days; 50 ml agricultural compost made from cranberry presscake mixed with chicken manure obtained from Mass Natural Fertilizer Co., Westminster, MA; 50 ml of the same agricultural compost autoclaved twice at 85°C for one hour each on two consecutive days; and an untreated control where no compost was added. The third main classification contained two treatments: pasteurized soil, sand, perlite (2:1:1) soil mix (double heat sterilized at 85°C for five hours on two consecutive days) and non-pasteurized soil mix of the same composition. There were a total of 30 treatment combinations.

Pasteurization of the soil mix was done prior to the other treatments. 300 ml (1.5 g/ml dryweight equivalent) of soil mix decanted into 10 cm diameter clean plastic pots. *R. solani* inoculum was allowed to incubate in pots for one week at ambient greenhouse air temperature (22° to 26°C) after being incorporated into the soil mix. Compost treatments then were added and likewise allowed to incubate for one week at ambient greenhouse air temperature prior to planting strawberries. Plants were greenhouse-grown, tissue-cultured plantlets cv. Kent provided by Nourse Farms, Inc., Whately, MA.

Care was taken to prevent contamination by washing tools and hands with 20% bleach solution between treatments. The strawberry plants were allowed to grow for 16 weeks. Plants were watered with deionized water to suppress the pH. Plants were fertilized with 50 ml of 1% solution of Peter's® 20-20-20 All Purpose Plant Food (Grace Sierra Hort. Products Co., Milpitas, CA) or Hoagland's nutrient solution (Hoagland and Arnon, 1938) on alternating weeks.

4.2.3 Statistical Analysis

Statistical analyses were performed using SYSTAT for the Macintosh© (Statistics, Version 5.2 Edition, Evanston, IL). Square root transformations were performed on rating data to stabilize variances or normalize the sample population (Damon and Harvey, 1987). Back-transformed data from transformations are presented in all tables. Differences between treatments were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure, with means separated by least significant difference (LSD) (Damon and Harvey, 1987; Gomez and Gomez,1984).

4.3 Results

4.3.1 Experiment I

Whole dry weights of strawberry plants were not affected by compost treatments or by pasteurization of the soil mix. However, inoculation with *R. solani* reduced the dry weight of plants ($p \leq 0.001$). Interestingly, the amount of dry weight reduction due to inoculation was less for plants in non-pasteurized soil mix than in pasteurized soil mix (Table 4.2).

Dry weight of shoots and roots were also evaluated. Root dry weights were not affected by treatment, inoculation or soil mix pasteurization (data not shown; analysis of variance table A.35 in Appendix A). And, shoot dry weights were affected by inoculation similarly to whole plant dry weight (data not shown; analysis of variance table A.36 in Appendix A).

Table 4.2. Whole plant dry weights of strawberry plants grown in pasteurized or non-pasteurized soil mix with or without inoculation with *Rhizoctonia solani* and treated with one of four composts, or not treated[z].

	Dry Weight of Whole Plants (g)			
	Pasteurized soil[y]		Non-pasteurized soil	
Compost Treatments[w]	Inoculated[x]	Non-inoculated	Inoculated	Non-inoculated
Springfield MYW[v]	2.77	4.11	3.93	3.68
Northampton MYW	2.76	3.92	2.81	4.27
MassNatural™[u]	3.26	4.34	2.88	3.78
Moody Hill™[t]	2.57	4.20	2.51	3.03
Control (no compost)	3.35	4.69	3.46	3.39
Mean	2.94	4.26	3.12	3.65
Inoculation effect[s]	***		*	
Pasteurization effect[r]	ns	**		

[z] Analysis of variance table A.37 in Appendix A.

[y]Heat pasteurized at 83°C for five hours on two consecutive days.

[x]Inoculated with 3 g of *R. solani* inoculum (40 x 10^5 cfu/g).

[w]Potting soil mix amended with 50 ml of compost material from sources listed.

[v]MYW=municipal yard waste primarily comprised of leaves from mixed hardwoods.

[u]Agricultural compost made from cranberry waste and chicken manure.

[t]Agricultural compost made from horse and cow manure.

[s]Difference between plant weights from inoculated vs. non-inoculated soil within pasteurization.

[r]Difference between plant weights from pasteurized vs. non-pasteurized soil within inoculation.

*,**,***, ns Plant weights significantly different at p≤0.5, p≤0.01, p≤0.001, or not significant, respectively.

The number of strawberry runner plants produced was not affected by compost treatments or by soil pasteurization. Inoculation with *R. solani* reduced the number of runners compared to non-inoculated plants (Table 4.3).

Table 4.3. Number of runners produced by strawberry plants grown in pasteurized or non-pasteurized soil mix with or without inoculation with *Rhizoctonia solani* and treated with one of four composts, or not treated[z].

	Number of Runners	
Compost Treatments[x]	Inoculated[y]	Non-inoculated
Springfield MYW	0.6	1.3
Northampton MYW	0.5	1.4
MassNatural™	0.6	0.7
Moody Hill™	0.4	0.8
Control (no compost)	0.4	1.2
Mean	0.5 ***	1.1

[z] Analysis of variance table A.38 in Appendix A.

[y]Inoculated with 3 g of *R. solani* inoculum (40 x 10^5 cfu/g).

[x]Potting soil mix amended with 50 ml of compost material from sources listed.

*** Number of runners significantly different at p≤0.001.

Visual ratings of strawberry roots were affected by the compost treatments and by inoculation with *R. solani* (Table 4.4). Three of the four compost treatments plus the control were rated higher than the Moody Hill™ compost treatment. In the non-pasteurized soil mix, inoculation did not have an effect on visual ratings but, in pasteurized soil, non-inoculated plants received a higher rating (Table 4.4). And, among non-inoculated plants, roots from pots with non-pasteurized soil mix were not rated differently from pasteurized soil. However, among inoculated pots, roots from pasteurized soil mix were rated lower than those from non-pasteurized soil.

Table 4.4. Visual rating of strawberry roots (1=100% lesion coverage, 2=75% lesion coverage, 3=50% lesion coverage, 4=25% lesion coverage, 5=no lesions) grown in pasteurized or non-pasteurized soil mix with or without inoculation with *Rhizoctonia solani* and treated with one of four composts, or not treated[z].

	Strawberry Root Rating				
	Pasteurized soil[y]		Non-pasteurized soil		
Compost Treatments[w]	Inoc.[x]	Non-inoc.	Inoc.	Non-inoc.	Mean
Springfield MYW	2.9	3.8	3.4	3.3	3.35 a
Northampton MYW	3.1	3.8	3.1	3.2	3.30 a
MassNatural™	2.8	3.0	3.2	4.1	3.27 a
Moody Hill™	2.2	3.1	3.3	2.7	2.82 b
Control (no compost)	3.3	3.8	3.7	3.7	3.62 a
Mean	2.9	3.5	3.3	3.4	
Inoculation effect[v]	**		ns		
Pasteurization effect[u]	**	ns			

[z] Analysis of variance table A.39 in Appendix A.
[y]Heat pasteurized at 83°C for five hours on two consecutive days.
[x]Inoculated with 3 g of *R. solani* inoculum (40 x 10^5 cfu/g).
[w]Potting soil mix amended with 50 ml of compost material from sources listed.
[v]Difference between plant weights from inoculated vs. non-inoculated soil within pasteurization.
[u]Difference between plant weights from pasteurized vs. non-pasteurized soil within inoculation.
** , ns Root rating significantly different at $p \leq 0.001$, or not significant, respectively.

4.3.2 Experiment II

Fresh and dry weight of whole plants were not affected by compost treatment, pasteurization of soil mix, or *Rhizoctonia*-inoculation (Table 4.5). However, visual root rating of plants was higher in pasteurized soil compared to non-pasteurized ($p \leq 0.05$; data

not shown), and higher in non pasteurized Springfield MYW than either pasteurized or non-pasteurized MassNatural™ compost (Table 4.6), but inoculation did not affect the visual rating of the roots. Within the inoculation treatment, there were differences among compost treatments, but none was different from the control treatment where no compost was applied. Roots from plants inoculated with *R. solani* were rated higher in the pasteurized Springfield MYW treatment than either of the MassNatural™ compost treatment (Table 4.6). Unfortunately, a flaw in the experimental design makes it impossible to evaluate the effect of compost pasteurization on any of the experimental measures.

Table 4.5. Whole plant fresh and dry weights of strawberry plants grown in pasteurized or non-pasteurized soil mix with or without inoculation with *Rhizoctonia solani* and treated with one of four composts, or not treated[z].

	Fresh Weight of Whole Plants (g)					
	Pasteurized soil[y]			Non-pasteurized soil		
Compost Treatments[w]	Inoc.[x]	Oat Carrier	Non-inoc.	Inoc.	Oat Carrier	Non-inoc.
Springfield MYW[v]						
Pasteurized	6.47	5.95	6.58	7.13	4.17	8.32
Non-pasteurized	5.35	7.92	5.50	5.24	7.28	7.12
MassNatural™[u]						
Pasteurized	4.02	6.90	5.80	6.46	5.64	5.45
Non-pasteurized	2.32	5.60	7.16	4.19	4.01	5.77
Control (no compost)	4.61	6.41	3.82	5.30	4.84	5.85
	Dry Weight of Whole Plants (g)					
	Pasteurized soil			Non-pasteurized soil		
	Inoc.	Oat Carrier	Non-inoc.	Inoc.	Oat Carrier	Non-inoc.
Springfield MYW						
Pasteurized	1.00	0.84	1.17	1.15	0.88	1.71
Non-pasteurized	0.95	1.82	1.10	0.83	1.76	1.38
MassNatural™						
Pasteurized	0.91	1.26	0.86	0.88	0.71	0.97
Non-pasteurized	0.42	1.22	1.08	0.73	0.68	1.12
Control (no compost)	0.79	1.23	0.67	0.94	0.96	1.01

[z] Analysis of covariance and variance tables A.40 and A.41, respectively, in Appendix A.
[y]Heat pasteurized at 83°C for five hours on two consecutive days.
[x]Inoculated with 3 g of *R. solani* inoculum (40 x 10^5 cfu/g).
[w]Potting soil mix amended with 50 ml of compost material from sources listed.
[v]MYW=municipal yard waste primarily comprised of leaves from mixed hardwoods.
[u]Agricultural compost made from cranberry waste and chicken manure.

Table 4.6. Visual rating of strawberry roots from plants grown in pasteurized or non-pasteurized soil mix with or without inoculation with *Rhizoctonia solani* and treated with one of four composts, or not treated[z,y].

	Strawberry Root Rating[x]			
Compost Treatments[v]	Inoc.[w]	Oat Carrier	Non-inoc.	Mean
Springfield MYW				
Pasteurized	3.6	2.9	3.1	3.2 abc
Non-pasteurized	3.4	3.9	3.1	3.4 a
MassNatural™				
Pasteurized	2.5	3.1	2.9	2.8 bc
Non-pasteurized	2.2	2.6	3.1	2.7 c
Control (no compost)	3.4	3.5	3.3	3.3 ab
Pasteurized soil mix	2.9	3.8 a	3.2	3.3 a
Non-pasteurized soil mix	3.1	2.6 b	2.9	2.9 b

[z] Analysis of variance performed on transformed data $((x+0.5)^{1/2})$; ANOVA table A.42 in Appendix A.
[y]Means in columns followed by different letters are significantly different at $p \leq 0.05$.
[x]Data presented backtransformed from analyzed data.
[w]Inoculated with 3 g of *R. solani* inoculum (40×10^5 cfu/g).
[v]Potting soil mix amended with 50 ml of compost material from sources listed.

4.4 Discussion

4.4.1 Effect of Inoculation with *Rhizoctonia solani*

Plants inoculated with *R. solani* were smaller (dry weight) than those not inoculated, produced fewer runners, and had roots that were rated lower in visual evaluation than non-inoculated plants in Experiment I, but not in Experiment II. In Experiment II, inoculation did not affect any of the measurements of strawberry plant growth. The analyses of variance of these experiments suggest that the effect of inoculation was not strong and only marginally significant (effect on fresh weight $p=0.09$; effect on dry weight $p=0.06$). The marginal effect of inoculation may have been due to excessive variability in the plants at the beginning of the experiment or due to contamination

during the experiment. Whichever the case, this result compromised much of the value of Experiment II.

4.4.2 Effect of Soil Pasteurization

Soil pasteurization did not have an overall effect in either experiment except for on the visual root ratings in Experiment II where roots from pasteurized soil were rated higher than those from non-pasteurized soil. No statistical significance was found in the interaction between pasteurization and treatment, but with in Experiment I, the data appear to suggest that non-pasteurized soil contains some agent of *R. solani* suppression as evidenced by similar plant weights of inoculated and non-inoculated plants in control treatments. Additionally, the Springfield MYW compost appeared to have a similar effect. Also, visual ratings of roots were not different between inoculated and non-inoculated plants in the non-pasteurized soil, suggesting the possibility of a living factor in the soil which plays a role in suppression of *R. solani.*

4.4.3 Effect of Compost Treatments

Compost treatments did not have a consistent effect on strawberry plant growth or visual root evaluations. In Experiment I, Moody Hill agricultural compost appeared to have a detrimental effect on root ratings but no detectable effect on plant growth or runner production. None of the other composts had detectable effects. In Experiment II, no coherent treatment effects were evident from the data.

4.4.4 Summary

Clearly, no conclusions about compost effects on strawberry growth or suppression of *Rhizoctonia* can be drawn from the experiments in this study. Much additional work must be done to pursue this method of disease suppression. Experimental methods must insure that plant variability is minimized at the outset of the experiment and

that sources of contamination are eliminated. Additionally, the factor of soil sterilization must be sure to produce the desired result, i.e., eliminating the living fraction of the soil or compost and otherwise not altering it. Heat sterilization may not be the desired method for this since it changes the physical and chemical properties of the material (Sandler *et al.*, 1988). Gamma irradiation may be a preferred method for sterilizing these materials and will be considered for future experiments. Finally, evaluations of root lesion density may be done more accurately with the use of digital image analysis. This technology will be developed and used in future studies.

CHAPTER V
CONCLUSIONS

None of the strategies investigated in this thesis (soil solarization, cover crop rotations, application of biological control agents or compost amendments) will, alone, serve as alternatives to soil fumigation for controlling weeds or black root rot in strawberries. It is possible, however, that a combination of these strategies may provide a practical and affordable approach to these difficult problems. A review of benefits and drawbacks of the four strategies is valuable.

5.1 Soil Solarization

Advantages of soil solarization include the absence of toxic chemicals which makes it safe for the applicator as well as mitigating the risk of chemical contamination of soil or groundwater. Solarization can be an effective method of weed and disease suppression in strawberries (Hartz *et al.*, 1993). In our studies, solarization was often as good as fumigation and usually better than control plots for weed control and promoting subsequent strawberry plant growth. However, the effect of solarization was not consistent between the two experiments we conducted in two consecutive years. This points out one of the major drawbacks of this technique which is the unreliable levels of solar radiation in the Northeast. There may not be sufficient solar radiation each summer to depend on this technique of soil disinfestation. Furthermore, the timing of applying plastic to the soil is during the peak production period of the growing season. In order to use this method, a grower must be willing to remove land from production during this time. This may be acceptable for small plantings but too costly for large ones. Additionally, in order to sustain the benefits of solarization, the soil must be disturbed as little as possible after treatment so as not to mix treated and untreated soil. This may require a completely new planting system for strawberries. Finally, disposal of the plastic used for solarization is

problematic. Even photodegradable plastic film leaves strips in the field where edges were covered with soil. Collection of plastic residue is labor intensive and leaving it in the field creates a nuisance for cultivation and other field operations.

5.2 Cover Crop Rotations

Pre-plant cover crop rotations can also benefit strawberry culture by suppressing weeds and diseases. Buckwheat or Sudex followed by winter rye seeded the year prior to planting strawberries can suppress weed growth and promote strawberry plant growth in the establishment year. How long those benefits last is not known from these studies. The drawback of this method is in needing to commit most of the year prior to planting strawberries to growing a non-cash crop. Sudex may be used for mulch if harvested but it is unknown whether removing topgrowth from the field lessens the benefit compared to soil incorporation of the whole plant. Again, this may be more acceptable in small land areas than in large ones.

5.3 Use of the Biological Control Agent *Trichoderma harzianum*

The biological control agent *Trichoderma harzianum* can suppress *Rhizoctonia* infection of strawberry plants (Elad *et al.*, 1981). In our study, *T. harzianum* benefited strawberry plant growth with or without inoculation with *Rhizoctonia*, most of the time. However, of the two isolates used, results were not consistent as to which one was beneficial in each case. Issues of timing of field application, formulation and delivery system, and cost have not yet been addressed. Field studies must be conducted to assess whether predictable results can be achieved.

5.4 Use of Compost Amendments

The value of compost amendments for disease suppression in strawberries was not established in this study. However, the abundance of research results supporting this

method compels further study. It may be that compost already determined to be suppressive (e.g., composted hardwood bark) should have been included in the study, even though it is not a practical option for this region. Comparisons between CHB and municipal yard waste compost could then be made. Specific properties that confer suppressiveness to a compost should also be defined so that compost production technology can be refined in order to produce a reliably disease suppressive product. Inoculation of compost with beneficial organisms may also prove valuable in enhancing the value of the compost and providing a delivery substrate for biological control agents.

The primary drawback of using compost amendments for disease suppression is lack of reliable results, but a secondary drawback is handling and cost of the material. On-farm composting alleviates much of these drawbacks, but not all growers have the option to do on-farm composting. If cost and handling of municipally produced compost (e.g., municipal yard waste compost) proves affordable, and benefits to growers sufficient to offset those costs, an important link may be forged between agriculture and waste management concerns to form a system of organic matter cycling (recycling) with benefits to all parties.

APPENDIX A
ANALYSIS OF VARIANCE TABLES

Chapter II: Experiment I

Table A.1. Analysis of variance for percent weed cover, transformed by arcsine $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	0.039	5	0.007		
Treatment	2.525	5	0.505	15.18	0.0001
Tr*Rep	0.832	25	0.033		
Split Plot					
Till	11.230	1	11.230	424.43	0.0001
Till*Rep	0.052	5	0.010		
Tr*Till	1.709	5	0.342	12.92	0.0001
Tr*Till*Rep	0.661	25	0.026		
Split Split Plot					
Date	7.637	1	7.637	2545.67	0.0001
Date*Rep	0.015	5	0.003		
Tr*Date	0.714	5	0.143	11.84	0.0001
Tr*Date*Rep	0.302	25	0.012		
Till*Date	2.976	1	2.976	294.81	0.0001
Till*Date*Rep	0.265	5	0.005		
Tr*Till*Date	0.517	5	0.103	10.30	0.0001
Tr*Till*Date*Rep	0.252	25	0.010		

Table A.2. Analysis of variance for monocot weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	1.841	5	0.368	1.036	0.418
Treatment	2.867	5	0.573	2.065	0.104
Tr*Rep	6.944	25	0.278		
Split Plot					
Till	4.753	1	4.753	14.535	0.012
Till*Rep	1.635	5	0.327		
Treatment*Till	1.977	5	0.395	1.113	0.379
Tr*Till*R	8.883	25	0.355		

Table A.3. Analysis of variance for dicot weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	70.747	5	14.149	0.426	0.826
Treatment	329.625	5	65.925	2.001	0.113
Tr*Rep	823.644	25	32.946		
Split Plot					
Till	1651.592	1	1651.592	78.651	0.000
Till*Rep	104.995	5	20.999		
Treatment*Till	253.996	5	50.799	1.529	0.217
Tr*Till*Rep	830.785	25	33.231		

Table A.4. Analysis of variance for total weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	86.566	5	17.313	0.524	0.756
Treatment	350.783	5	70.157	2.134	0.094
Trt*Rep	821.925	25	32.877		
Split Plot					
Till	1833.555	1	1833.555	75.716	0.000
Till*Rep	121.080	5	24.216		
Treatment*Till	279.063	5	55.813	1.690	0.174
Trt*Till*Rep	825.606	25	33.024		

Table A.5. Analysis of variance for visual rating of strawberry plants; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	0.056	5	0.011	1.028	0.381
Treatment	0.275	5	0.055	4.582	0.004
Trt*Rep	0.300	25	0.012		
Split Plot					
Till	1.870	1	1.870	445.603	0.000
Till*Rep	0.021	5	0.004		
Treatment*Till	0.024	5	0.005	0.439	0.817
Trt*Till*Rep	0.274	25	0.011		

Chapter II
Experiment II

Table A.6. Analysis of variance for percent weed cover; transformed by arcsine $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	0.049	5	0.010		
Treatment	3.025	5	0.605	10.39	0.0001
Trt*Rep	1.456	25	0.058		
Split Plot					
Inoculation	0.012	1	0.012	2.18	0.199
Inoc*Rep	0.028	5	0.006		
Trt*Inoc	0.011	5	0.002	0.44	0.8183
Trt*Inoc*Rep	0.129	25	0.005		
Split Split Plot					
Date	14.269	1	14.269	927.92	0.0001
Date*Rep	0.076	5	0.015		
Trt*Date	2.615	5	0.523	8.98	0.0001
Trt*Date*Rep	1.456	25	0.058		
Inoc*Date	0.006	1	0.006	0.89	0.3881
Inoc*Date*Rep	0.036	5	0.007		
Trt*Inoc*Date	0.007	5	0.001	0.24	0.9432
Trt*Inoc*Date*Rep	0.140	25	0.006		

Table A.7. Analysis of variance for monocot weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	20.815	5	4.163	0.996	0.440
Treatment	16.644	5	3.329	0.749	0.594
Trt*Rep	111.062	25	4.442		
Split Plot					
Inoc	21.641	1	21.641	3.127	0.137
Inoc*Rep	34.603	5	6.921		
Treatment*Inoc	17.133	5	3.427	0.820	0.547
Trt*Inoc*Rep	104.487	25	4.179		

Table A.8. Analysis of variance for dicot weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	575.006	5	115.001	2.888	0.034
Treatment	1708.017	5	341.603	1.845	0.140
Trt*Rep	4628.086	25	185.123		
Split Plot					
Inoc	52.835	1	52.835	1.490	0.277
Inoc*Rep	177.272	5	35.454		
Treatment*Inoc	270.826	5	54.165	1.360	0.273
Trt*Inoc*Rep	995.358	25	39.814		

Table A.9. Analysis of variance for total weed density.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	605.664	5	121.133	3.041	0.028
Treatment	1809.799	5	361.960	2.073	0.103
Trt*Rep	4364.446	25	174.578		
Split Plot					
Inoc	142.105	1	142.105	2.343	0.186
Inoc*Rep	303.288	5	60.658		
Treatment*Inoc	33.0452	5	66.090	1.659	0.181
Trt*Inoc*Rep	995.696	25	39.828		

Table A.10. Analysis of variance for weed weight (fresh).

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	4028352.284	5	805670.457	10.722	0.000
Treatment	$.351425*10^8$	5	7028947.977	18.187	0.000
Trt*Rep	9661333.049	25	386453.322		
Split Plot					
Inoc	362270.720	1	362270.720	3.183	0.134
Inoc*Rep	568995.840	5	113799.168		
Treatment*Inoc	694435.840	5	138887.168	1.848	0.140
Trt*Inoc*Rep	1878589.440	25	75143.578		

Table A.11. Analysis of variance for weed weight (dry).

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	114715.442	5	22943.008	5.926	0.001
Treatment	2696955.579	5	539391.116	23.337	0.000
Trt*Rep	577825.213	25	23113.009		
Split Plot					
Inoc	18898.920	1	18898.920	2.480	0.176
Inoc*Rep	38096.489	5	7619.298		
Treatment*Inoc	29297.369	5	5859.474	1.514	0.221
Trt*Inoc*Rep	96786.617	25	3871.465		

Table A.12. Analysis of variance for percent survival of strawberry plants; transformed by arcsine.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	0.978	5	0.196	1.935	0.124
Treatment	0.470	5	0.094	1.076	0.397
Trt*Rep	2.183	25	0.087		
Split Plot					
Inoc	0.459	1	0.459	1.290	0.308
Inoc*Rep	1.778	5	0.356		
Treatment*Inoc	1.883	5	0.377	3.724	0.012
Trt*Inoc*Rep	2.528	25	0.101		

Table A.13. Analysis of variance for runners/plant.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	8.322	5	1.664	12.611	0.000
Treatment	15.712	5	3.142	5.117	0.002
Trt*Rep	15.352	25	0.614		
Split Plot					
Inoc	0.517	1	0.517	0.605	0.472
Inoc*Rep	4.273	5	0.855		
Treatment*Inoc	0.416	5	0.083	0.631	0.678
Trt*Inoc*Rep	3.299	25	0.132		

Table A.14. Analysis of variance for strawberry plants biomass/plot.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	17512.321	5	3502.464	13.517	0.000
Treatment	19923.274	5	3984.655	4.605	0.004
Trt*Rep	21631.340	25	865.254		
Split Plot					
Inoc	283.629	1	283.692	0.196	0.676
Inoc*Rep	7220.948	5	1444.190		
Treatment*Inoc	2840.194	5	568.039	0.604	0.697
Trt*Inoc*Rep	6478.046	25	259.122		

Table A.15. Analysis of variance for strawberry plant biomass/surviving plant.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	179.514	5	35.903	15.128	0.000
Treatment	266.364	5	53.273	22.447	0.000
Trt*Rep	194.343	25	7.774		
Split Plot					
Inoc	14.725	1	14.725	1.508	0.274
Inoc*Rep	7220.948	5	9.765		
Treatment*Inoc	7.167	5	1.433	0.604	0.697
Trt*Inoc*Rep	59.331	25	2.373		

Table A.16. Analysis of variance for root rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Whole Plot					
Replication	0.078	5	0.016	1.264	0.310
Treatment	0.152	5	0.030	2.323	0.073
Trt*Rep	0.328	25	0.013		
Split Plot					
Inoc	0.298	1	0.298	53.977	0.001
Inoc*Rep	0.028	5	0.006		
Treatment*Inoc	0.048	5	0.010	0.776	0.576
Trt*Inoc*Rep	0.307	25	0.012		

Table A.17. Analysis of variance of root weight.

Source	SS	DF	MS	F-ratio	P
Replication	0.699	9	0.078	1.962	0.085
Treatment	0.451	3	0.150	3.935	0.019
Inoculation	0.154	1	0.154	2.638	0.139
Treatment*Inoc	0.341	3	0.114	2.869	0.055
Treatment*Rep	1.032	27	0.038		
Inoc*Rep	0.524	9	0.058		
Treatment*Inoc*Rep	1.069	27	0.040		

Table A.18. Analysis of variance of top weight.

Source	SS	DF	MS	F-ratio	P
Replication	12.441	9	1.382	1.228	0.320
Treatment	15.868	3	5.289	4.849	0.008
Inoculation	38.135	1	38.135	27.336	0.001
Treatment*Inoc	4.509	3	1.503	1.335	0.284
Treatment*Rep	29.454	27	1.091		
Inoc*Rep	12.555	9	1.395		
Treatment*Inoc*Rep	30.396	27	1.126		

Table A.19. Analysis of variance of whole plant weight.

Source	SS	DF	MS	F-ratio	P
Replication	16.621	9	1.847	1.277	0.294
Treatment	21.477	3	7.159	5.152	0.006
Inoculation	33.449	1	33.449	17.709	0.002
Treatment*Inoc	4.235	3	1.412	0.976	0.418
Treatment*Rep	37.517	27	1.390		
Inoc*Rep	16.999	9	1.889		
Treatment*Inoc*Rep	39.042	27	1.446		

Table A.20. Analysis of variance of runners produced.

Source	SS	DF	MS	F-ratio	P
Replication	3.450	9	0.383	0.831	0.594
Treatment	4.150	3	1.383	3.291	0.036
Inoculation	36.450	1	36.450	211.645	0.000
Treatment*Inoc	0.550	3	0.183	0.398	0.756
Treatment*Rep	11.350	27	0.420		
Inoc*Rep	1.550	9	0.172		
Treatment*Inoc*Rep	12.450	27	0.461		

Table A.21. Analysis of variance of primary root visual rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.246	9	0.027	0.303	0.967
Treatment	0.450	3	0.150	2.305	0.099
Inoculation	1.910	1	1.910	28.785	0.000
Treatment*Inoc	0.105	3	0.035	0.386	0.764
Treatment*Rep	1.757	27	0.065		
Inoc*Rep	0.597	9	0.066		
Treatment*Inoc*Rep	2.443	27	0.090		

Table A.22. Analysis of variance of secondary root visual rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.546	9	0.061	1.155	0.361
Treatment	0.499	3	0.166	3.420	0.031
Inoculation	3.127	1	3.127	59.968	0.000
Treatment*Inoc	0.222	3	0.074	1.405	0.263
Treatment*Rep	1.312	27	0.049		
Inoc*Rep	0.469	9	0.052		
Treatment*Inoc*Rep	1.419	27	0.053		

Experiment II

Table A.23. Analysis of variance of strawberry root weight.

Source	SS	DF	MS	F-ratio	P
Replication	0.792	8	0.099	0.390	0.915
Treatment	0.757	3	0.252	0.978	0.420
Inoculation	2.258	1	2.258	6.444	0.035
Treatment*Inoc	1.706	3	0.569	2.239	0.110
Treatment*Rep	6.199	24	0.258		
Inoc*Rep	2.803	8	0.350		
Treatment*Inoc*Rep	6.096	24	0.254		

Table A.24. Analysis of variance of strawberry top weight.

Source	SS	DF	MS	F-ratio	P
Replication	0.623	8	0.078	1.037	0.436
Treatment	0.275	3	0.092	1.829	0.169
Inoculation	6.919	1	6.919	192.451	0.000
Treatment*Inoc	0.785	3	0.262	3.487	0.031
Treatment*Rep	1.203	24	0.050		
Inoc*Rep	0.288	8	0.036		
Treatment*Inoc*Rep	1.802	24	0.075		

Table A.25. Analysis of covariance of whole strawberry plant fresh weight.

Source	SS	DF	MS	F-ratio	P
Starting fresh wt.	328.436	1	328.436	68.371	0.000
Replication	20.662	8	2.577	0.536	0.001
Treatment	58.254	3	19.418	4.042	0.011
Inoculation	781.050	1	781.050	162.592	0.000
Treatment*Inoc	65.664	3	21.888	4.556	0.006
Error	302.635	55	4.804		

Table A.26. Analysis of variance of whole strawberry plant dry weight.

Source	SS	DF	MS	F-ratio	P
Replication	1.791	8	0.224	0.482	0.857
Treatment	1.935	3	0.645	1.470	0.248
Inoculation	17.082	1	17.082	35.803	0.000
Treatment*Inoc	4.546	3	1.515	3.260	0.039
Treatment*Rep	10.530	24	0.439		
Inoc*Rep	3.817	8	0.477		
Treatment*Inoc*Rep	11.157	24	0.465		

Table A.27. Analysis of variance of runners produced.

Source	SS	DF	MS	F-ratio	P
Replication	9.000	8	1.125	2.613	0.033
Treatment	0.819	3	0.273	0.291	0.832
Inoculation	11.681	1	11.681	64.692	0.000
Treatment*Inoc	1.042	3	0.347	0.806	0.503
Treatment*Rep	22.556	24	0.940		
Inoc*Rep	1.444	8	0.181		
Treatment*Inoc*Rep	10.333	24	0.431		

Table A.28. Analysis of variance of primary root visual rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.270	8	0.034	0.991	0.468
Treatment	0.091	3	0.030	0.901	0.455
Inoculation	3.455	1	3.455	103.973	0.000
Treatment*Inoc	0.187	3	0.062	1.827	0.169
Treatment*Rep	0.807	24	0.034		
Inoc*Rep	0.266	8	0.033		
Treatment*Inoc*Rep	0.818	24	0.034		

Table A.29. Analysis of variance of secondary root visual rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.545	8	0.068	1.282	0.299
Treatment	0.049	3	0.016	0.242	0.866
Inoculation	4.687	1	4.687	76.865	0.000
Treatment*Inoc	0.134	3	0.045	0.837	0.487
Treatment*Rep	1.620	24	0.067		
Inoc*Rep	0.488	8	0.061		
Treatment*Inoc*Rep	1.276	24	0.053		

Table A.30. Analysis of variance of whole plant visual rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.179	8	0.022	0.405	0.907
Treatment	0.551	3	0.184	3.325	0.037
Inoculation	8.937	1	8.937	399.900	0.000
Treatment*Inoc	0.551	3	0.184	3.325	0.037
Treatment*Rep	1.325	24	0.055		
Inoc*Rep	0.179	8	0.022		
Treatment*Inoc*Rep	1.325	24	0.055		

Experiment III

Table A.31. Analysis of covariance of ending fresh weight.

Source	SS	DF	MS	F-ratio	P
Starting fresh Wt.	83.866	1	83.866	31.353	0.000
Replication	17.312	6	2.885	1.078	0.043
Treatment	3.412	3	1.137	0.425	0.735
Inoculation	48.558	3	16.186	6.051	0.001
Treatment*Inoc	36.330	9	4.037	1.509	0.156
Error	254.118	95	2.675		

Table A.32. Analysis of variance of whole plant dry weight.

Source	SS	DF	MS	F-ratio	P
Replication	10.770	6	1.795	7.805	0.000
Treatment	0.476	3	0.159	0.421	0.740
Inoculation	4.007	3	1.336	5.221	0.009
Treatment*Inoc	4.054	9	0.450	1.958	0.063
Treatment*Rep	6.788	18	0.377		
Inoc*Rep	4.605	18	0.256		
Treatment*Inoc*Rep	12.419	54	0.230		

Table A.33. Analysis of variance of visual plant rating; transformed by $(x + 0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	1.500	6	0.250	2.644	0.025
Treatment	0.004	3	0.001	0.016	0.997
Inoculation	1.830	3	0.610	5.670	0.006
Treatment*Inoc	1.429	9	0.159	1.679	0.117
Treatment*Rep	1.549	18	0.086		
Inoc*Rep	1.936	18	0.108		
Treatment*Inoc*Rep	5.107	54	0.095		

Table A.34. Analysis of variance of visual root rating; transformed by $(x + 0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	2.030	6	0.338	3.567	0.005
Treatment	0.002	3	0.001	0.006	0.999
Inoculation	3.615	3	1.205	13.232	0.000
Treatment*Inoc	1.349	9	0.150	1.580	0.145
Treatment*Rep	2.009	18	0.112		
Inoc*Rep	1.639	18	0.091		
Treatment*Inoc*Rep	5.123	54	0.095		

Table A.35. Analysis of variance of root weight.

Source	SS	DF	MS	F-ratio	P
Replication	0.530	9	0.059	1.258	0.263
Treatment	0.237	4	0.059	1.266	0.285
Inoculation	0.114	1	0.114	2.429	0.121
Soil Pasteurization	0.022	1	0.022	0.460	0.499
Trt*Inoc	0.576	4	0.144	3.007	0.018
Trt*Soil Past.	0.284	4	0.071	1.516	0.200
Inoc*Soil Past.	0.152	1	0.152	3.251	0.073
Trt*Inoc*Soil Past	0.276	4	0.069	1.468	0.214
Error	8.002	171	0.047		

Table A.36. Analysis of variance of shoot weight.

Source	SS	DF	MS	F-ratio	P
Replication	19.646	9	2.183	2.380	0.015
Treatment	8.100	4	2.025	2.207	0.070
Inoculation	38.217	1	38.217	41.661	0.000
Soil Pasteurization	1.906	1	1.906	2.077	0.151
Trt*Inoc	2.785	4	0.696	0.759	0.553
Trt*Soil Past.	6.461	4	1.615	1.761	0.139
Inoc*Soil Past.	5.611	1	5.611	6.116	0.014
Trt*Inoc*Soil Past	4.039	4	1.010	1.101	0.358
Error	156.866	171	0.917		

Table A.37. Analysis of variance of whole plant weight.

Source	SS	DF	MS	F-ratio	P
Replication	24.071	9	2.675	2.113	0.031
Treatment	10.260	4	2.565	2.026	0.093
Inoculation	42.500	1	42.500	33.569	0.000
Soil Pasteurization	2.332	1	2.332	1.842	0.176
Trt*Inoc	3.772	4	0.943	0.745	0.563
Trt*Soil Past.	9.197	4	2.299	1.816	0.128
Inoc*Soil Past.	7.611	1	7.611	6.012	0.015
Trt*Inoc*Soil Past	6.351	4	1.588	1.254	0.290
Error	216.491	171	1.266		

Table A.38. Analysis of variance of runner production.

Source	SS	DF	MS	F-ratio	P
Replication	7.905	9	0.878	1.467	0.164
Treatment	4.630	4	1.157	1.933	0.107
Inoculation	17.405	1	17.405	29.066	0.000
Soil Pasteurization	0.005	1	0.005	0.008	0.927
Trt*Inoc	4.370	4	1.093	1.824	0.126
Trt*Soil Past.	0.670	4	0.168	0.280	0.891
Inoc*Soil Past.	1.125	1	1.125	1.879	0.172
Trt*Inoc*Soil Past	1.450	4	0.363	0.605	0.659
Error		171			

Table A.39. Analysis of variance of visual root rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	0.655	9	0.073	0.967	0.469
Treatment	0.996	4	0.249	3.308	0.012
Inoculation	0.437	1	0.437	5.803	0.017
Soil Pasteurization	0.151	1	0.151	2.007	0.158
Trt*Inoc	0.547	4	0.137	1.817	0.128
Trt*Soil Past.	0.413	4	0.103	1.371	0.246
Inoc*Soil Past.	0.410	1	0.410	5.448	0.021
Trt*Inoc*Soil Past	0.053	4	0.013	0.177	0.950
Error	12.872	171	0.075		

Experiment II

Table A.40. Analysis of covariance of whole plant fresh weight.

Source	SS	DF	MS	F-ratio	P
Start Fresh Wt.	580.272	1	580.272	70.206	0.000
Replication	20.566	6	3.428	0.415	
Treatment	72.372	4	18.093	2.189	0.079
Inoculation	39.893	2	19.947	2.413	0.092
Soil Pasteurization	1.320	1	1.320	0.160	0.690
Trt*Inoc	124.802	8	15.600	1.887	0.064
Trt*Soil Past.	3.760	4	0.940	0.114	0.978
Inoc*Soil Past.	22.208	2	11.104	1.343	0.101
Trt*Inoc*Soil Past	45.656	8	5.707	0.690	0.700
Error	1479.490	173	8.265		

Table A.41. Analysis of variance of whole plant dry weight.

Source	SS	DF	MS	F-ratio	P
Replication	3.435	6	0.573	1.013	0.418
Treatment	5.321	4	1.330	2.355	0.056
Inoculation	3.240	2	1.620	2.868	0.060
Soil Pasteurization	0.034	1	0.034	0.061	0.805
Trt*Inoc	7.596	8	0.949	1.681	0.106
Trt*Soil Past.	0.934	4	0.234	0.413	0.799
Inoc*Soil Past.	2.662	2	1.331	2.356	0.098
Trt*Inoc*Soil Past	0.983	8	0.123	0.217	0.987
Error	98.293	174	0.565		

Table A.42. Analysis of variance of visual root rating; transformed by $(x+0.5)^{1/2}$.

Source	SS	DF	MS	F-ratio	P
Replication	1.103	6	0.184	1.293	0.263
Treatment	1.500	4	0.375	2.639	0.036
Inoculation	0.091	2	0.046	0.322	0.725
Soil Pasteurization	0.798	1	0.798	5.616	0.019
Trt*Inoc	1.322	8	0.165	1.162	0.325
Trt*Soil Past.	0.239	4	0.060	0.420	0.794
Inoc*Soil Past.	1.458	2	0.729	5.128	0.007
Trt*Inoc*Soil Past	2.145	8	0.269	1.894	0.064
Error	24.734	174	0.142		

APPENDIX B
STANDARD OPERATING PROCEDURES (SOPS)

SOP for a TECHNIQUE

Title: Preparing oats and fungal inoculum medium

SOP No.: T101

OBJECTIVE: to prepare an oats growth medium and to inoculate it with a plant pathogen in order to use the oats plus pathogen medium in experiments with live plants in greenhouse or field experiments.

MATERIALS:
Wide mouth 250 ml erlenmeyer flasks
non-absorbent cotton
aluminum foil
distilled H_2O
whole (feed-type) grain oats
wire-tipped tool
spoonula
autoclave
alcohol lamp
laminar flow hood
inoculant material
75% ETOH spray bottle
90% ETOH in bottle

SAFETY PRECAUTIONS: no hazards to humans or animals

SHELF LIFE AND STORAGE CONDITIONS OF REAGENT/SOLUTION/MEDIUM: once the oats/fungal inoculum has grown at room temperature for approx. 7 days, it should be used within 5 days.

METHODS: **(Note that eight 250 ml flasks of wet inoculum will yield approx. 240 g dry groung inoculum.)**

For each 250 ml flask:
1. put 75 ml of oats in flask;
2. add distilled H_20 up to 100-125 ml mark on flask; (this determines how moist the final product will be);
3. stuff the mouth of the flask with enough non-absorbent cotton to completely block the opening; leave some cotton sticking out to serve as a handle;
4. cover the cotton and entire neck of flask tightly with a double thickness square of aluminum foil (approx. 6" square).

For all 250 ml flasks:

5. autoclave at 14 psi (**SLOW**) for 30 minutes;
6. remove from autoclave when temperature reaches approx. 80°C (must be below 100°C);
7. let flasks stand at room temperature for 24 hours.
8. repeat autoclaving procedure for 24 hours.

Inoculation Process:

1. turn-on hood, spray with 75% ETOH, and wipe-down with paper towelling; (if it is already on, spray and wipe again);
2. place flasks and petri plates of inoculum into hood; along one side
3. spray flasks lightly with 75% ETOH and wipe-down with paper towelling;

Steps 4-13 will be performed using 1 flask and 1 petri plate at a time to prevent contamination; care will be taken to minimize the amount of time either the flask or the petri plate remain open;

4. open a petri plate and divide medium into large pieces (approx. 1 X 2 cm.) using the sterile blunt end of a spoonula, a wire-tipped tool or equivalent tool;
5. use 1/2 of the medium in a standard plate (100 X 15 mm) per 250 ml flask, or all the medium in a 60 X 15 mm plate;
6. carefully remove aluminum foil cap from a flask and save, **top side down;**
7. remove cotton and place on aluminum foil cap;
8. while holding flask, flame mouth of flask over alcohol lamp approximately 30 seconds;
9. drop pieces of medium into flask with sterile spoonula or wire-tipped tool; be careful not to touch the flask or insert tool into the mouth of the flask;
10. flame the flask mouth again for 30 seconds;
11. replace the cotton and then the aluminum foil cap; fit cap tightly;
12. shake flask gently to mix oats and medium;
13. seal edges of aluminum foil with parafilm;
14 repeat steps 4-13 with another petri plate and flask;
15. let flasks incubate at room temperature (approx. 30 ° C) for approx. 7 days on the lab bench.

SOP for a TECHNIQUE

Title: CFU Counts

SOP No.: T102

OBJECTIVE: To determine presence and population density of inoculum (colony forming units per volume of media)

MATERIALS:
9 test tubes and covers
100 ml cool sterile distilled H_2O
8 sterile 1 ml pipettes
1 sterile 10 ml pipette
sterile bent glass rod
200 micro liter pipettes (pipette-man)
sterile pipette tips for above
sterile plates of media PCAL or PDAL 3 per each sampling
250 ml beaker 1/2 full of 90% ETOH
spray bottle with 75% ETOH
pipette-aid-electric pump
Vortex
SAFETY PRECAUTIONS:
no hazards

SHELF LIFE AND STORAGE CONDITIONS OF REAGENT/SOLUTION/MEDIUM(if applicable):
Once dilutions are plated, they will be counted within 48 hrs. (before plates are over-grown.)

METHODS:
Setting up the experiment
1. At least one day prior to dilution, autoclave the following on FAST (14psi) for 15 min.: test tubes (t.t), glass rod wrapped in foil, pipette tips in their box, empty beaker topped with foil.
2. If the 1 ml and 10 ml pipettes are not sterile, they can be sterilized as follows (if they are glass; plastic pipettes are never reused):
   a. Stuff non absorbent cotton pieces into the blunt end of the glass 10 ml and 1 ml pipettes.
   b. Wrap pipettes in separate bundles in heavy duty aluminum foil.
   c. Autoclave as described above.
3. Turn-on hood and wipe-down with 75% ETOH.
4. After autoclave has cooled to 80^{o}C remove instruments and place in hood.
5. Separately autoclave H_2O on SLOW setting (14 psi) for 30 min. Water must be at room temperature before dilutions can be started.

Making the dilution series

1. Bring-in to the hood the pipette-aid, vortex pipette-man and sterile H_2O, along with the instruments already stored in hood.
2. Spray all these down very well with 75% ETOH and wipe dry. (Only the 'gun' part of the pipette-aid need be in the hood. The pump can sit on the table.)
3. Using the 10 ml pipette fitted into the pipette-aid 'gun', measure water into test tubes, **10** ml in first tube, **9** ml in the rest.
4. Hold test tube at 45^{o} angle. Be sure not to insert pipette into the tube. Instead, squirt a stream of H_2O into the tube, flame mouth of tube 30 seconds before re-covering.
5. Weigh out your sample to be used in the dilution. Approximately 1 gram for dry material and 1.5 or more for wet materials (e.g. moist soil). Record exact weight.
6. To compensate for moisture, place sample in weighing paper and fold up into sealed bundle.
7. In hood, open bundle and pour contents into 10 ml test tube, flame t.t. and cover Vortex t.t., for 30 seconds.
8. Using 1 ml pipette; extract 1 ml of solution from t.t. containing sample, flame mouth and replace cover on t.t.
9. Transfer this 1 ml of solution to the next test tube (9 ml), flame and recover, then Vortex.
10. Repeat procedure for each test tube.

Plating the dilutions

1. Use **3** plates for each dilution. (You will sample from the first tube away from the initial sample in 10 ml H_2O will be 10^{-1}, next 10^{-2} etc.) Label your plates accordingly prior to plating.
2. Using the pipette-man fitted with a sterile tip, start at the **most** dilute tube (highest #).
3. Set pipette-man at 200 microliters (2/10 ml).

4. Vortex test tube 30 seconds. Draw up sample. Eject sample onto plate held open just a crack **away** from you (facing towards rear of hood, make sure no equipment is blocking air flow.)
5. Repeat procedure with other two plates.
6. Take bent glass rod and immerse it into beaker of 90% ETOH and remove, allowing excess to drip off, and flame.
7. Repeat and let cool for 30 seconds.
8. Using bent end gently spread liquid over and around plate surface on each of the three plates. (Put glass rod back in ETOH.)
9. Discard pipette tip and put on a fresh one.
10. Repeat these procedures on remaining tubes you want to sample from, changing tips after each dilution. (You don't have to save from all test tubes.)

*You might only want 10^{-2} ---> 10^{-5} or 10^{-1} ---> 10^{-3} but you still need 3 plates from each, and at least 3 dilutions in series to get workable data.

SOP for a TECHNIQUE

Title: Drying Oats/Inoculum Medium

SOP No.: T106

OBJECTIVE: To effectievely dry fresh oats inoculum prior to grinding, while maintaining inoculum purity.

MATERIALS:
Drying oven
90% ETOH in 600 ml beaker
Long-handled spatula
Alcohol lamp
Sterile paper towels
Sterile tray(s)
70% ETOH spray bottle
Piece of cheese cloth approx. 8" square folded once

SAFETY PRECAUTIONS: Wear latex gloves to protect hands from ETOH, and maintain sterile conditions.

SHELF LIFE AND STORAGE CONDITIONS OF RAGENT/SOLUTION/MEDIUM (if applicable): once oats are dried and ground, they can be stored in a zip-loc bag in refridgerator at 4°C for a month without losing viability.

METHODS:
Setting up the experiment
1. Prepare drying oven by removing rack and spraying down interior and inside door with 70% ETOH and wiping dry with paper towel.
2. Spray rack and wipe dry. Return rack to oven.
3. Spray oven interior, inside door, and rack again. Close door and turn on oven to approx. 35°C. Place clean cheesecloth over oven vent.
4. Use metal trays that have been coveren in aluminum foil and autoclaved on **FAST** (14psi) for 15 minutes to sterilize.
5. Place tray(s) in oven and close door.
6. Assemble beaker of 90% ETOH, alcohol lamp, and spatula on side of oven.
7. Bring flasks or jars of inoculum and sterile paper towels to side of oven.
8. Open oven.
9. Line trays with paper towelling.
10. Light alcohol lamp and place spatula in beaker of 90% ETOH.
11. Grasp flask of jar of inoculum; remove foil top and cotton, or unscrew ring and remove vacuum seal.
12. While holding jar of flask at 45° angle, flame mouth of jar for 30 secs.
13. Dip spatula in 90% ETOH and flame; repeat.
14. Scoop out inoculated oats on to paper towel-lined tray. Spread oats to evenly cover tray .
15. Cover tray with another layer of sterile paper towels.
16. Repeat as needed with different jar/flast of inoculum making sure to thoroughly sterilize spatula in between jar/flask.

17. Close oven door.
18. Check the cheese cloth to make sure it is covering the vent hole on top of the oven.
19. Let dry for 3 days or so before grinding. If trays are filled deeply it might take longer. It must be completely dry before grinding.
20. Verify that the oven remained at approx 35°C at least once during the 3 days and at the end.

SOP for a TECHNIQUE

Title: Grinding Dried Oats/ Inoculum Medium

SOP No.: T109

OBJECTIVE: To prepare a finely textured dry product of dried oats and fungal inoculum medium that will be uniform in particle size, and will be easy to measure and deliver as an inoculum.

MATERIALS:
Spray bottle of 75% ETOH
Blender
Paper towels
Zip-loc bags
Latex gloves
Lab marker

SAFETY PRECAUTIONS:
No hazards

SHELF LIFE AND STORAGE CONDITIONS OF REAGENT/SOLUTION/MEDIUM(if applicable):
Ground material will be kept in refrigerator (4°C) indefinitely.

METHODS :
1. Grinding **MUST NOT** be done in lab to avoid contamination of lab. Take blender out to hall-way by double sink (potting area).
2. Use the extension cord that powers over-head lights. (There is no other outlet with table nearby.)
3. Spray interior and lid of blender until **soaked** with 75% ETOH, wipe dry with clean paper towelling.
4. Run blender in order to evaporate any excess ETOH, keep lid on.
5. Wearing latex gloves, place several chunks of unground dry oats/inoculum in blender. **No more than about one cup at a time**.
6. Run blender on "grind" or "chop" setting. (You might have to tilt blender while it's running to get all of it ground up.)
7. Blend until oats reach consistency of fine sawdust.
8. Pour into Zip-loc bag, seal.
9. Repeat until all oats are ground.
10. Wash blender out with hot soapy water, rinse.
11. Repeat alcohol procedure and return blender to lab.
12. Store ground oats in "dirty refrigerator," i.e. not where sterile plates are kept.

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