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ALTERNATIVES TO METHYL BROMIDE FOR CONTROLLING THE BLACK ROOT ROT DISEASE COMPLEX OF STRAWBERRY

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By

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ABSTRACT

ALTERNATIVES TO METHYL BROMIDE FOR CONTROLLING THE BLACK ROOT ROT DISEASE COMPLEX OF STRAWBERRY

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The United States strawberry industry is heavily dependent on methyl bromide to control soil-borne diseases and weeds. The continued phase-out of methyl bromide has left a void in the arsenal growers have traditionally used to manage these pests. Strawberry black root rot is a disease complex of *Rhizoctonia fragariae* Husain & McKeen, *Pythium* species, and the nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven that affects the productivity and longevity of strawberry plantings. In a Michigan study, 13 commercial bio-control or reduced-risk fungicides, applied as drenches or pre-plant dips, were evaluated for their efficacy in the control of black root rot at the time of planting in a naturally infested field. This experiment was also replicated in the greenhouse. A pre-plant root dip of azoxystrobin (Abound) with a potassium salt (ProPhyt) has shown a measure of control. Experiments conducted to evaluate combinations of crop rotations, in conjunction with two commercially available biological control products, in an effort to create suppressive soils prior to the establishment of strawberries, and a study to evaluate the 'best practices' for controlling black root rot at the time of planting consisting of crop rotation, fungicide dip, compost, a biocontrol product, and the use of resistant varieties have shown that while fumigation resulted in the largest, healthiest plants, the use of the rotational crops squash, rye, and *Brassica* spp. tended to cause healthier strawberry plants. Compost and the Abound + ProPhyt pre-plant dip offered some improvement in plant health.

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SYMBOLS AND ABBREVIATIONS

ANOVA: Analysis of variance

BRR: Black root rot

ITS: Internal transcribed spacer

PDA: Potato dextrose agar

PDAamp: Potato dextrose agar amended with 50 μl ampicillin/ml

CHAPTER ONE: LITERATURE REVIEW

Introduction

Strawberry black root rot (BRR) is a disease complex that is widespread around the world wherever strawberries have been planted for multiple years (Watanabe *et al.*, 1977; D'Ercole *et al.*, 1989). Initial symptoms of BRR include brown lesions on the feeder and structural roots of the strawberry plant and blackening of the root cortex while the stele remains white, initially (Maas, 1998). Root lesions range from 0.5 to 5 cm. Feeder roots disintegrate due to infection. Infected roots darken and die, eventually becoming completely black in severe cases. Infected plants produce smaller leaves, fewer main and lateral roots, have slower growth, and reduced runner production (Hancock *et al.*, 2001). Severely infected plants wilt at the onset of dry weather. The disease can be spread via infected nursery stock, movement of infested soil, or infected plant debris (Maas, 1998; Strong and Strong, 1927; Hildebrand, 1934). Also known as strawberry decline, many organisms and abiotic factors have been implicated in the cause of the disease; however, *Rhizoctonia fragariae* Husain & McKeen, *Pythium* spp., and the root lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven are generally considered the primary pathogens (D'Ercole *et al.*, 1989; Wing *et al.*, 1995; Maas, 1998).

In Michigan, the matted-row system is used to grow strawberries, where plants are typically planted at wide spacing in spring so that runners fill in between the plants. Harvest occurs the year after planting and continues for 3 to 4 years. The fruit ripens for 3 to 5 weeks in May to mid-June (Pritts and Handley; 1998). In Michigan 'U-Pick' operations, continual strawberry production or short rotations are common due to the few

suitable locations for these enterprises. The shorter a rotation is between strawberry plantings, the higher the potential economic return to the grower. However, continual cropping allows pathogen establishment and build-up to deleterious levels. In this system, fumigation is often employed to help control soil-borne pathogens and weeds before planting (Perry and Ramsdell, 1994; Rosskopf *et al.*, 2005).

A common and effective fumigant used by strawberry growers is methyl bromide. In 1992, the Montreal Protocol established methyl bromide as an ozone-depleting substance and instituted its ban by January 1, 2005 (Anonymous, 1998; Rosskopf *et al.*, 2005). Methyl bromide was predominantly used as a soil fumigant, but also in the disinfestation of durable and perishable commodities, and structures. Besides being detrimental to the environment, concerns for operator safety, residues in food, effects on soil biodiversity, and pollution of surface and ground water all influenced the decision to ban methyl bromide use (Anonymous, 1998; Rosskopf *et al.*, 2005). Methyl bromide is emitted from many sources, some being natural, but it is estimated that 30% of emissions come from soil fumigation (Rosskopf *et al.*, 2005).

Currently, control of BRR consists of using crop rotation, cover crops, good aeration and drainage, and fumigation (Maas, 1998; Martin and Hancock, 1983; Perry and Ramsdell, 1994). Some chemicals, such as Telone (1,3-dichloropropene) and chloropicrin have shown success as alternative fumigants. Vapam (metam sodium) and Basamid (dazomet) have shown comparable results in some countries to methyl bromide as well (Anonymous, 1998). Chemical control can cost between $700-$2000 depending on chemical and application method (Anonymous, 1998; Rosskopf *et al.*, 2005). With one of the most effective fumigants being banned, and no specific crop rotation that has

shown consistent, effective control, growers are left with vague recommendations. Prevention is key; planting disease-free stock in fertile, well-drained sandy loam is the best way to avoid this disease (Perry and Ramsdell, 1994).

One problem when trying to develop a management strategy for BRR stems from the variability of the disease. *Rhizoctonia fragariae* does not always have to be present to get BRR symptoms (Wing *et al.*, 1994; Wing *et al.*, 1995). While studies have implicated *P. penetrans*, the disease can occur with low nematode populations or even in their absence (Wing *et al.*, 1994; Wing *et al.*, 1995). It seems that a combination of abiotic and biotic factors predispose strawberry plants to invasion by perhaps otherwise saprophytic or weakly parasitic fungi that then cause severe damage to the root system (Wing *et al.*, 1994; Wing *et al.*, 1995).

With the primary control measure due to be eliminated, it becomes necessary to find alternative reduce-risk chemicals, biological, and cultural alternatives. These alternatives should not be expected to work as effectively alone as methyl bromide did. The best control still resides in integrated management practices. Thus the objectives of this research are: 1) Evaluate reduced-risk fungicides and biological products for the control of BRR at the time of planting, 2) Evaluate various crop rotations in combination with biological control products at the time of planting, 3) Assess integration of host plant resistance with chemical and cultural controls to develop a "best practices" approach, in order to make a recommendation to growers and 4) Evaluate reduced-risk fungicides in already established strawberry fields.

Strawberry Production

The cultivated strawberry, *Fragaria* x *ananassa* Duchesne, is thought to be a cross between *Fragaria virginiana* Duch. and *Fragaria chiloensis* Linn. (Galleta, 1990; Hancock, 1999; Pritts and Handley, 1998). The strawberry plant, a member of the rose family, is a perennial that consists of a crown from which leaves, stolons, branch crowns, flower clusters, and adventitious roots grow (Hancock, 1999; Pritts and Handley, 1998). Axillary buds produced at the base of each leaf may become a stolon or branch crown depending on the environment, with long days and warm temperatures encouraging runners, while cooler short days favor branch crowns (Hancock, 1999; Pritts and Handley, 1998). The stolons have two nodes, the first of which may become another runner or remain dormant while the second becomes a daughter plant. A healthy plant can develop 10-15 runners a year (Hancock, 1999; Pritts and Handley, 1998). 'Allstar' is currently a widely grown variety in the Eastern, Mid-Atlantic, and Midwestern United States. It is an early variety with large to medium sized, light colored fruit developed in Maryland by the United States Department of Agriculture; and it also has resistance to Verticillium wilt (Hancock, 1999).

Flower buds are initiated in one season for the following season. Initiation occurs after certain day length and temperature requirements are met, which is often variety specific. The inflorescence has a primary flower, two secondary, four tertiary, and possibly eight quarternary flowers (Pritts and Handley, 1998; Hancock, 1999). In the Northeast, plants are often short-day or June-bearing, meaning that flower bud initiation occurs during September and October when the days are shortening and cooler. Some varieties are not sensitive to day length, and are called day neutral; these initiate flower

buds between 4.4-29.4°C, and still others have a weak day length response (Pritts and Handley, 1998; Hancock, 1999). Flowering in day neutral varieties occurs about six weeks after bud initiation which is continuous from late spring through fall. The plants are self-fertile, but fruit size is greatly improved with pollinators. The fruit develops from the flower receptacle (Pritts and Handley, 1998; Hancock, 1999).

The fruit is composed of numerous ovaries, each with seeds referred to as achenes (Pritts and Handley, 1998; Hancock, 1999). Ripening depends on the pre-harvest environment and the cultivar, with pectinmethylesterases and cellulases thought to be the most important enzymes involved in strawberry softening; anthocyanins cause the characteristic reddening (Hancock, 1999). In Michigan, three to four harvests are possible, but plant yield and berry weight decrease through the season as the tertiary and quarternary flowers develop(Pritts and Handley, 1998; Hancock, 1999).

Strawberries have two types of roots, perennial (primary) and lateral (feeder, secondary) (Pritts and Handley, 1998; Hancock, 1999). Primary roots arise from the crown chiefly in late summer and fall while lateral roots originate in the pericycle, push through the cortex of the primaries and are the main source of absorption. Root growth occurs primarily during non-fruiting and vegetative dormancy periods (Pritts and Handley, 1998; Hancock, 1999). Good aeration is important as low soil oxygen and water-logging favors root damaging fungi and death of rootlets depending on the duration of standing water (Galleta, 1990; Hancock, 1999). The lateral roots live 1 to 2 years while primary roots may live 2 to 3 years. The largest root concentration is in the top 15 cm of the soil with each plant usually maintaining about 20 to 30 primary roots with an average length of 10 to15 cm (Pritts and Handley, 1998; Hancock, 1999). Dormancy is

caused by 4 to 6 weeks of short-day periods and is broken after sufficient chilling at -1 to 10°C (Hancock, 1999).

Commercially, strawberries are primarily propagated by digging runners in late fall and early spring, then storing them at 0°C until spring planting. Runner tips are also used for fall planting in the annual plasticulture system used in California and Florida where most of the strawberries in the United States are produced. In the annual plasticulture system, the plants are set at a high density on raised beds covered with black polyethylene plastic in late summer after the day length decreases. The plants produce large crowns during fall and they fruit in spring (Hancock, 1999). In polyethylene-mulched production systems, the use of pre-plant soil fumigants is essential to control soil pathogens, weeds, and nematodes (Locascio, 2005; and Rosskopf *et al.*, 2005).

The United States produces approximately 20% of the world's supply of strawberries (Hancock, 1999). The strawberry crop is the highest valued berry crop, accounting for about two-thirds of all berry revenues since the 1980's in the United States (Pollack and Perez, 2005). The United States is a net exporter of fresh strawberries, primarily to Canada, but it is a primary importer of frozen strawberries from Mexico (Cook, 2002). In 2006, the United States' strawberry crop was valued at $1.5 billion and harvested from 53,280 acres. California's crop was valued at $1.2 billion and harvested from 35,800 acres. Florida had a strawberry crop valued at $239 million in 2006. Michigan's crop was valued at $6.3 million and harvested from 950 acres (NASS, 2007). California produces strawberries from January through October, and Florida's harvest covers the winter months. (NASS, 2007).

California is able to have such high yields because of the annual, plasticulture system that utilizes soil fumigation to control pests and improved region-specific varieties that can produce for 6 months instead of 4 weeks (Pritts and Handley, 1998; Hancock, 1999; Cook, 2002).

The plasticulture system is not suitable for northern regions because of the risk of spring frosts and the shorter growth period. In these regions, the matted row system is used. This system requires that the flowers are removed the first year. In this system the strawberry is grown as a perennial for 3 to 4 years. Harvest occurs for 3 to 5 weeks in May to mid-June (Pritts and Handley; 1998). In Michigan, the strawberry season starts in early June in the Lower Peninsula and ends in late July in the Upper Peninsula. Berrien, Leelanau and Van Buren are Michigan's largest strawberry-producing counties (Long, 2002). Most of the Michigan crop is produced on "U-Pick" operations. In 2006, Michigan had 200 strawberry farms totaling 850 acres (MDA, 2007).

In both of these systems, fumigation is employed to help control soil-borne pathogens and weeds. Roots in fumigated soils have deeper penetration with more root branching, and have lateral roots that live longer. Lateral roots are continuously replaced as they die so the plant can continue to get nutrients from the same soil area (Galletta, 1990).

Strawberry growers in the United States use chemicals to control a variety of diseases including: gray mold (*Botrytis cinerea)*, anthracnose (*Colletotrichum* spp.), powdery mildew (*Podosphaera aphanis)*, leather rot (*Phytophthora cactorum*), angular leaf spot (*Xanthomonas fragariae*), red stele (*Phytophthora fragariae* var. *fragariae)*, common leaf spot (*Mycosphaerella fragariae)*, phomopsis leaf blight (*Phomopsis*

obscurans), leaf scorch (*Diplocarpon earlianum)* and BRR. In 2002, the ten primary strawberry-producing states purchased and applied a total of 864,000 pounds of fungicides, at a value of $12.09 million. A loss of 1.1 billion pounds of production with a value of $707 million was predicted if fungicides had not been used (Gianessi and Reigner, 2005). In 2001, it was estimated that in Georgia, methyl bromide fumigation accounted for the largest single expenditure for disease control in strawberries. Root rots, especially BRR, were estimated to have reduced crop value by 3%, with total costs, including damage and control expenses, amounting to $255,000 (Williams-Woodward, 2001). It has been estimated that yields can be reduced by 50% if BRR is not controlled, and control with fumigation can cost $1000 per acre (Long, 2002; EPA, 2006). Yuen *et al.* (1991) found that soil fumigation with methyl bromide and chloropicrin (MBC) reduced the severity of BRR of strawberries in California. Root density was increased by 19-61% over the non-fumigated control, while harvests from fumigated plots were 24-29% greater than untreated controls.

Black Root Rot Complex

First reported by Zeller in 1932, BRR is characterized by reddish brown lesions on the feeder and structural roots of the strawberry plant, which darken to black with age and result in the death of the root (Zeller, 1932). Many organisms and abiotic factors have been implicated in the cause of the disease; however, *Rhizoctonia fragariae* Husain & McKeen, *Pythium* spp., and the nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven are generally accepted as the primary pathogens (D'Ercole *et al.*, 1989; Wing *et al.*, 1995; Maas, 1998). The role of *R. solani* Kühn in the disease is

unclear, as much work done prior to Husain and McKeen's discovery of *R. fragariae* does not identify, or may have misidentified, the species of *Rhizoctonia* (Husain and McKeen 1963a; Parmeter *et al.*, 1967). *Rhizoctonia fragariae* has been more frequently isolated than *R. solani* in several studies, but both have been found on strawberry roots (Wilhelm *et al.*, 1972; D'Erocole *et al.*, 1989; Martin, 1988; Watanabe *et al.*, 1977).

Hildebrand (1934) found *Gliocladium*, *Fusarium*, *Pythium*, *Hainesia*, *Cylindrocladium*, *Coniothyrium*, *Rhizoctonia*, *Helminthosporium*, *Asterocystis* spp., and members of the Plasmodiophoraceae associated with BRR, while Nelson (1957) demonstrated the pathogenicity of *Idriella lunata* P.E. Nelson & S. Wilh. on strawberry in California. *Idriella lunata* was also recovered during root isolations from strawberry in Italy (D'Ercole *et al.*, 1989). Katznelson and Richardson (1948) found *Cylindrocarpon* associated with 'Premier' strawberries. Yuen *et al.* (1991) isolated *Cylindrocarpon destructans*, *Pythium ultimum* Trow, and *Pythium irregulare* most frequently from diseased strawberry plants in California. Damage caused by *R. fragariae* and other primary pathogens may allow secondary fungi to invade the strawberry roots (Husain and McKeen, 1963b). Weak parasites and saprophytes are more likely to be found in samples collected in late spring and early summer, while samples taken in the late fall and early winter have a better chance of containing *R. fragariae* (Husain and McKeen, 1963a).

Infection is most severe in moderately wet soils and when environmental conditions are not conducive to plant growth. D'Ercole *et al.* (1989) found that the appearance of decline 10 to 15 days after planting was caused by agronomic problems, while plants declining 50 to 60 days after planting harbored pathogens such as *Rhizoctonia, Verticillium, Pythium, Idriella, Fusarium* and *Cylindrocarpon.*

***Rhizoctonia* spp.**

While *Rhizoctonia* spp. are an immense group of fungi that are grouped largely by their lack of distinctive taxonomic features, with teleomorphic states in both the basidiomycetes and ascomycetes, the three groups associated with plant diseases includes *R. solani*, the binucleate species, and the isolates with a *Waitea* teleomorph (Vilgalys and Cubeta, 1994).

Rhizoctonia spp. have a highly variable growth rate, and some isolates may produce spores. Under certain conditions, some species produce sclerotia-like tufts consisting of short, broad cells that function as chlamydospores. The hyphae display characteristic right-angle branching with dolipore septa and a moniliform resting cell (Maas, 1998). The branches are slightly constricted and cross walls are present near the junction. Infrequently, the perfect stage, *Thanatephorus cucumeris* (A.B. Frank) Donk, is formed in the multinucleate species (*R. solani*), or *Ceratobasidium* spp. in the binucleate species (Ogoshi and Ui, 1983; Burpee *et al.*, 1980).

Rhizoctonia solani is a significant pathogen of many crops. *Rhizoctonia solani* can cause rot in the strawberry crown and affects roots near the crown at 2 to 18°C with crown infection favored at 18 to 32°C (Maas 1998). This and other *Rhizoctonia* species are "collective" species consisting of several more or less unrelated strains. Strains are distinguishable from each other by their relative ability to form anastomoses. Ogoshi and Ui (1983) developed anastomosis groups (AG) for Japanese isolates, while Burpee *et al.* (1980) developed groups for North America. Ogoshi (1985) then compared the two different groups and found that Burpee's seven groups corresponded to several groups in the Ogoshi system. Ogoshi's anastomosis groups AG-A, AG-G, and AG-I have often

been implicated in BRR of strawberries (Martin, 1988). The fungus overwinters usually as mycelium or sclerotia in soil, infected perennial plants, propagation material, or even seed depending on the host. It is present in most soils, and once established, impossible to eliminate.

Rhizoctonia fragariae is represented in groups AG-A, AG-G, and AG-I. Isolation frequency and virulence vary between and within each group and by site (Burpee *et al.*, 1980; Martin, 1988; Mass, 1998; Martin, 2000). Martin (2000) found that isolates within AG-I were particularily virulent and that AG groups differed between locations, and even within a single location depending on the time of year. Each of these AG's have different host ranges that should be considered before incorporating certain crops into a rotation prior to strawberry (Martin, 1988).

Husain and McKeen (1963a) first implicated *R. fragariae* in BRR, and reported that it was primarily isolated from roots during cold periods. Disease seems to be conditional on temperature, with *R. fragariae* germinating most rapidly in exudates on strawberry roots grown at 5-10° C (Husain and McKeen, 1963b).

In some cases, *Rhizoctonia fragariae* has also been found to stimulate plant growth. Scott *et al.* (2003) found that *R. fragariae* may be tolerated by strawberries during cool weather due to more favorable conditions for root growth over fungal growth. However, as temperatures increase the plant begins to show symptoms of infection that had occurred earlier in the season (Scott *et al.*, 2003). In greenhouse trials using the variety 'Redgauntlet', *R. fragariae* was not as aggressive as *R. solani* (Molot and Ferriere, 1989). Ribeiro and Black (1971) found that *R. fragariae* could actually stimulate plant growth depending on environmental and nutritional conditions. This may

explain why some *Rhizoctonia* spp. have been isolated from apparently healthy plants. Another consideration is the stage of the infection process.

Pythium spp.

Pythium species belong to the class Oomycetes and are primarily known for causing damping off in seeds and seedlings. The damage caused by *Pythium* spp. is proportional to the amount of soil moisture present with it being greatest near the saturation point (Hendrix and Campbell, 1973). These pathogens are pandemic.

Pythium produces a white, fast-growing mycelium that gives rise to sporangia, which then form a vesicle, containing 100 or more zoospores. When the zoospores are released, they swarm about and then encyst (Hendrix and Campbell, 1973). Zoospores are attracted to the roots by exudates, with soil moisture influencing the distance over which the exudates can stimulate the fungus (Hendrix and Campbell, 1973). Germination of encysted zoospores occurs with the production of a germ tube, which usually directly penetrates the host through the use of pectinolytic enzymes; the fungus then grows between and through the cells, while proteolytic and sometimes cellulytic enzymes break down protoplasts and cause complete collapse of invaded cells (Hendrix and Campbell, 1973).

The mycelium may give rise to oogonia, which, once fertilized, produce a thick wall and become oospores that serve as the survival and resting stage on dead plant and animal material as a saprophyte, or as a parasite on fibrous roots. Stanghellini and Hancock (1970) found that sporangia are important survival structures for some species such as *P. ultimum.* Germination of oospores is similar to that of sporangia, but is

favored at temperatures above 18°C while temperatures between 10 to 18°C favor zoospore germination (Hendrix and Campbell, 1973). Oospore germination is stimulated by root exudates that contain sugars and amino acids (Hendrix and Campbell, 1973; Nelson, 1990).

Pythium spp. rarely kill older plant hosts, but the plants develop root and stem lesions and root rots; their growth is retarded, yields decrease, and they may wither and die. Rootlets may be attacked at any point in the plant growth and the fungus can proliferate quickly. *Pythium* diseases are favored by prolonged wetness and unfavorable temperatures for the host, excess nitrogen, and growing the same crop for several years at the same location (Hendrix and Campbell, 1973).

Watanabe *et al.* (1977) found that *Pythium* spp. played an important role in the occurrence of strawberry stunt disease in the cool, wet conditions of Japan. Of the 58 fungal genera isolated from strawberry roots in Japan, *Rhizoctonia* spp. accounted for 25.2%, followed by *Fusarium* spp. at 19.6%, and *Pythium* spp. at 14.5%. Despite the lower frequency of isolation, Watanabe *et al.* (1977) found that *P. ultimum* was a primary pathogen causing BRR symptoms in strawberry at temperatures below 20°C. They suggested that the wetness of the drained rice paddy fields in which strawberries were grown in Japan may be a contributor to the severity of root rot caused by *Pythium* spp.

In Japan, Watanabe *et al.* (1977) isolated *P. sylvaticum* Hendrix & Campbell more frequently than *P. ultimum* from strawberry roots. In the United States, *P. sylvaticum* was the most isolated *Pythium* species from strawberry roots in Southern Illinois, but *P. irregulare*, and *P. perniciosum* were also common (Nemec and Sanders,

1970; Nemec, 1970). Wilhelm (1953) found *P. ultimum* to be one of the most common fungi associated with strawberry roots in California and established its role as a pathogen.

Increasing moisture affects the pathogen, which multiplies and disperses best in wet soils, but higher moisture may also decrease the ability of the host to defend itself because oxygen availability and soil temperature are reduced (Hendrix and Campbell; 1973). *Pythium ultimum* has been found to survive at -18°C for 24 months and in air-dried soil for 12 years (Hendrix and Campbell, 1973). *Pythium* spp. frequently occurs in a complex with other fungi such as in peanut pod rot with *Fusarium* spp. (Hendrix and Campbell, 1973).

Nematodes

Nematodes in the genus *Pratylenchus* are commonly called root lesion nematodes. Although the nematodes typically damage plant roots through the formation of lesions, they are capable of damaging other underground shoot tissues such as those of potatoes or peanuts. Many species have a wide host range, with some hosts able to support multiple species (Mai and Mullin, 1960; Mai *et al.*, 1977). *Pratylenchus penetrans* is a migratory, endoparasitic root lesion nematode with nearly 400 known hosts. It is considered the most important plant parasitic nematode in the northern United States and Canada (Maas, 1998).

Pratylenchus penetrans has been shown to play a role in BRR. Hildebrand (1934) originally suggested that BRR was caused by nematodes, while in the Netherlands Klinkenberg (1955) concluded that nematodes may be a primary cause, but that fungi move in secondarily to the wound sites to create more disease symptoms. Raski (1956)

provided evidence that *P. penetrans* was probably not the most important factor in plantings showing BRR symptoms, while Goheen and Bailey (1955) found that BRR was associated with small as well as large populations of nematodes. Goheen and Smith (1956) then showed that nematode-infested soils contributed to typical BRR symptoms and severe stunting of strawberries, but Chen and Rich (1962) found that fungi more readily infected necrotic tissue, suggesting that the nematodes predisposed the host for infection by surrounding soil fungi. Finally, Townshend (1963) demonstrated the pathogenicity of *Pratylenchus penetrans* on strawberries, showing that the nematode causes root necrosis and polyderm formation beneath the damaged endodermis in the stele.

The root lesion nematode can be identified by its flat, rounded head, overlapping esophagus, a stylet that is 14 to19 μm long with a prominent basal bulb at the base of the stylet (Mai and Mullin, 1960). The overlapping esophagus and head are the primary means to make a positive identification; other characteristics discussed by Mai and Mullin (1960) include a body that is less than 1.0 mm long, phasmids that are 1/3 the length of the tail or more behind the anus, and a blunt rounded tail. On the lateral field, four incisures can be found. When viewed under a microscope root lesion nematodes often move slowly and gracefully (Mai and Mullen, 1960).

The root lesion nematode overwinters as eggs, juveniles, or adults in infected roots (Dunn, 1972). Females lay a cluster of eggs in the cortex cells of the root or in the soil. The first-stage larva stays in the egg and molts into a second-stage larvae, which emerges from the egg 9 to 25 days after egg deposition, depending on temperature, and starts feeding on root parenchyma cells (Mamiya, 1971). All stages are vermiform, and

are morphologically similar except for reproductive structures. The nematode can infect roots or other below-ground plant structure, but the third juvenile and females appear in roots more often than males (Mai *et al.*, 1977; Olthof, 1982). After hatching, the nematode may move to new sites of infection, but all stages are usually found on the same host (Dunn, 1972). The male-to-female ratio and life expectancy are influenced by temperature, with higher temperatures shortening the life cycle. At 30°C, the life cycle can be completed in 30 days but survival is better at 15°C. On average, the life cycle is 45 to 65 days (Mai *et al.*, 1977; Kable and Mai, 1968).

Soil with a moisture tension (pF) around 1.8 to 2.5 maximizes root penetration, while tensions above pF 5.06 result in the death of nematodes (Kable and Mai, 1968). Soil texture plays a critical role in determining water tension, while organic soil amendments can also influence populations of *P. penetrans* by changing soil structure and water holding capacity (Miller *et al.*, 1973).

Lee (2002) reported that the root lesion nematode finds it host most likely by chemo-attraction or by sensing a potential gradient of ions. Root invasion occurs preferentially 3-13 mm behind the root tip. A potential feeding site is created by rubbing the epidermal cells with the lips and stylet, which is then thrust into the cell wall at rates of up to 140 times per minute, starting at the corners of the cell and working along the cell wall till it breaks (Mountain and Patrick, 1959; DiEdwardo, 1960; Freckman and Chapman, 1972; Oyekan *et al.*, 1972; Olthof, 1982; Kurppa and Vrain, 1985; Lee, 2002). Penetration of the root is complete within 6-12 hours, and the mid-cortex is reached by 18-24 hours after inoculation. The endodermis acts as a barrier to invasion, except in some plants after prolonged feeding (Mountain and Patrick, 1959; DiEdwardo, 1960;

Freckman and Chapman, 1972; Oyekan *et al.*, 1972; Olthof, 1982; Kurppa and Vrain, 1985).

Feeding can last for hours after a short salivation period, but feeding and migration are interrupted by periods of rest that can last for hours (Lee, 2002). The nematode moves through the cortical cells (Mai *et al.*, 1977). The lesions appear mainly on the younger feeder roots but may appear anywhere along the roots. Affected cortex cells in the lesions collapse and the lesion area appears constricted. Cell death is often delayed until after the nematode leaves, with nematode numbers often higher in diseased tissue than in dead tissue (Lee, 2002).

Pathogenicity of *P. penetrans* is influenced by host resistance due to phenolics produced in the roots or anatomical differences among plant species. Some hosts can support large numbers of nematodes without showing injury. Other hosts may be penetrated, but only a few cells around the infection site die where as others, such as alfalfa, have numerous cells that die along the pathway of the nematode (Mai *et al.*, 1977; Lee, 2002). Secondary fungi and bacteria usually invade the lesions and contribute to the discoloration and rotting.

Strawberry plants infected with *P. penetrans* appear stunted, have increased drought sensitivity, fewer runners, and shorter, more erect petioles (Maas, 1998). *Pratylenchus penetrans* alone or in combination with *Rhizoctonia fragariae* was found to reduce strawberry yield over time (LaMondia, 1999). Stunted plants with BRR have harbored *R. fragariae* and *P. penetrans* while symptomless plants nearby were infected only with *R. fragariae*. The two pathogens act additively and not synergistically (LaMondia and Martin, 1989). *Pratylenchus penetrans* can directly damage plants and

may lower the host's natural resistance to fungi making the combined damage of the nematode and fungi worse than either alone (Powell, 1971). The nematode was found to interact with *R. fragariae* among several other pathogens, allowing the associated diseases to develop more quickly (Szczygiel and Profic-Alwansiak, 1989; Powell, 1971). The interaction was more pronounced in sterilized than in unsterilized soil, probably due to decreased antagonism by any other organisms naturally present. Szczygiel and Profic-Alwansiak (1989) found that *P. penetrans*, *Meloidogyne hapla* Chitwood 1949, and *Longidorus elongatus* (de Man, 1876) Thorne & Swanger 1936 could all interact with *R. fragariae* to the detriment of the strawberry plant.

Other nematodes associated with strawberry roots include *Longidorus elongatus*, *Xiphinema americanum*, and *Meloidogyne* spp. (Brown *et al.*, 1993). Chapman (1956) found *Xiphinema*, *Pratylenchus*, and *Tylenchorhynchus* spp., all of which he felt should be considered potential pathogens in Kentucky. Any nematode which damages the root offers a potential entry point for fungi.

Other Factors Influencing Black Root Rot

Drought, winter injury, excessive fertilizer application, and excessive soil moisture are all detrimental to the strawberry plant and can cause symptoms similar to BRR (Perry and Ramsdell, 1994; Miller, 1948). Wing *et al.* (1995) discussed that no single factor accounted for the majority of the observed variation in root health, and that BRR may be caused by different factors in different fields. They also suggested that several interacting factors are necessary. Poor root health was associated with soil compaction and high soil clay and silt content. Wing *et al.* (1995) also found that raised

beds (10-20 cm) were associated with better root health, while those under 10 cm had poor root health. The age of the current planting, strawberry production on the site within the previous 5 years, and cumulative period of strawberry production were all significantly associated with poor root health (Wing *et al.*, 1995). Higher rates of the herbicide terbacil were associated with poor health, possibly due to the stress imposed on plants, predisposing them to infection by pathogens. Recently, however, Mervosh and LaMondia (2004) found that rates of terbacil four times the maximum recommended dosage did not result in an increased occurrence of BRR or reduced yield. Use of the fungicide metalaxyl was associated with good root health (Wing *et al.*, 1995). Fumigation had a negative correlation with root health, possibly due to the 'boomerang' effect where fumigated areas are more quickly re-colonized by pathogens than by beneficial antagonists, or it could be that areas with a history of disease were more likely to have been fumigated (Wing *et al.*, 1995).

Black Root Rot in Michigan

Over three years, seven samples suspected of BRR from various locations across the state of Michigan, were processed. Over all these locations, *Fusarium* spp. were commonly isolated (Glass, unpublished). While the cause of the decline at two of these sites could not be adequately determined, *Rhizoctonia* spp., and at a separate site *Pythium* spp., were each suspected to be the cause of decline. *P. penetrans* was determined to be the cause at another location, while *L. elongatus* was causing the decline on a different farm (Glass, unpublished).

Through personal communications with extension staff and cooperators, the use of fumigation, particularily methyl bromide seems to be limited. While a nursery in Michigan fumigates for production of their nursery stock, each grower has a preferred method that works at their location and in their management scheme (Bardenhagen and DeLange, personal communication).

Current Control

Currently, recommendations for control of BRR consist of using crop rotation, cover crops, good aeration and drainage, and fumigation (Maas, 1998; Martin and Hancock, 1983; Perry and Ramsdell, 1994). If an existing planting develops BRR, a new site should be chosen, or the old plants plowed under and the soil cultivated for several months followed by fumigation and planting of healthy strawberries in the spring (Perry and Ramsdell, 1994). Prevention is key; planting disease-free stock in fertile, well-drained sandy loam is the best way to avoid this disease (Perry and Ramsdell, 1994).

Incorporation of organic matter can encourage beneficial organisms that are antagonistic to pathogens (Perry and Ramsdell, 1994, Hoitink *et al.*, 1997). Also, good cultural practices to prevent drought stress and winter injury, and a 3-to 5-year rotation between strawberry plantings helps prevent disease (where fumigation is not possible) (Perry and Ramsdell, 1994). LaMondia (2004) evaluated 21 commercial strawberry varieties in naturally infested BRR soil for up to 3 years after planting, and found that loss in plant vigor and increased plant mortality occurred during harvest, especially under conditions of environmental stress. The best performing cultivars were 'Earliglow' (early season), 'Cavendish' and 'Lester' (early-midseason), 'Primetime' (mid-season), and

'Idea' and 'Latestar' (late-season). LaMondia (2004) found that evaluations should be done over many years due to the complexity, and often specificity, of the disease within a field. In addition, out of 20 strawberry genotypes evaluated over 2 years, 'Cavendish,' 'Bounty,' and 'Cabot' were all found to perform well on soil naturally infested with *Rhizoctonia fragariae*, *Pythium* spp., and *P. penetrans* in Michigan (Particka and Hancock, 2005).

Methyl Bromide Fumigation

Fumigation with methyl bromide and chloropicrin was reported by Wilhelm *et al.* (1963) to control Verticillium wilt of strawberry. Since that time it has been used as a pre-plant eradicant of weeds, nematodes, and soil-borne pathogens. They showed that soil productivity was limited not just by nutrients, but could be enhanced by fumigation that eliminates pathogens (Wilhelm, 1965, 1984). The effectiveness of methyl bromide in root rot disease control minimized the need for developing strawberry varieties with resistance to root disease.

In 1992, the Montreal Protocol established methyl bromide as an ozone-depleting substance and banned its use by January 1, 2005 in developed countries, or January 1, 2015 in developing countries (Anonymous, 1998; Rosskopf *et al.*, 2005). When methyl bromide is applied, it reacts in the soil to leave bromide ions, various methylated products and carbon dioxide. The remainder of the gas escapes into the atmosphere (WMO, 2003). Reactions involving bromide are thought to contribute to 50% of the loss of the ozone over Antarctica annually (Rosskopf *et al.*, 2005).

In 1996, global usage of methyl bromide for fumigation was 66,750 tonnes with 76% of that being used to fumigate soil within the United States (Anonymous, 1998). Methyl bromide was widely used due to its penetrative nature and effectiveness over a broad range of temperatures. Furthermore it did not disrupt farming practices due to the quick efficacy and ability to air rapidly after application (Rosskopf *et al.*, 2005). Methyl bromide was of particular use as a pre-plant fumigant where a broad range of soil pests limited economic production and where land was limited, forcing continuous same-crop production. It was of particular value to the vegetable, fruit, ornamental, tobacco, and nursery industries (Rosskopf *et al.*, 2005). Until recently, methyl bromide was widely used in the strawberry industry as a soil fumigant in production fields, but also in nurseries to guarantee disease-free transplants. Florida alone accounted for 36% of pre-plant methyl bromide use in 1997, with strawberry accounting for 9% of the total use in that state (Rosskopf *et al.*, 2005).

In 1998, the Methyl Bromide Technical Options Committee (MBTOC), met to develop feasible alternatives to methyl bromide. Although there are critical use exemptions for areas and industries that could not operate without methyl bromide, there has been a re-examination of existing fumigants. These materials are limited in their applicability as fumigants, as they can be highly variable in efficacy both by year and location. In Florida, the best available alternative for strawberry consists of Telone + 35% chloropicrin, applied in-bed at 331 liters per treated hectare, 3-5 weeks before transplanting. Fumigant application is supplemented by an herbicide tank mix of Goal (oxyfluorfen) 0.56 kg/ha plus napropamide 4.5 kg/ha. A minimum 30-day interval is required for Goal between applications and before transplanting (Rosskopf *et al.*, 2005).

There are many chemicals still under regulatory scrutiny including compounds from plants or processed plant by-products (Rosskopf *et al.*, 2005).

Each region will have to find alternatives that best fit the production schemes and pests for that area. Soil type, climate, social, economic, regulatory, and political situations all play into the success of a particular alternative. Factors limiting the acceptance of alternatives include local availability, registration status, costs, labor, and efficacy of control (Anonymous, 1998). Methyl bromide will continue to be used in limited land areas where replant is essential and for pest-free propagation material, but the methyl bromide use for these is small (Rosskopf *et al.*, 2005). Although there is no single replacement for methyl bromide, further research promises to develop an integrated approach that will be safer for the environment and society. The future of chemicals as a replacement for methyl bromide is unknown, and a movement towards a more sustainable system is essential. One of the alternatives is biological control.

Biological Control

The rhizosphere is a dynamic environment. The mucilage excreted by the roots supports both beneficial and potentially harmful organisms seeking to colonize the growing root. Mycorrhizal fungi colonize the root cortex, forming arbuscules, vesicles, and extramatrical hyphae or ensheath short roots helping the plant to acquire nutrients, especially iron (Buyer and Sikora, 1991). Other microbial interactions involve antibiosis against other microbes and induction of plant resistance mechanisms. The beneficial microbes are antagonistic to plant pathogens by competition for food, essential elements, and space (Whipps, 2001; Parke, 1991). Beneficial microbes include bacteria in the

genera *Streptomyces*, *Pseudomonas*, and *Bacillus*, and fungi in several genera, with potentially the most important being *Trichoderma*. These microbes enhance plant growth by suppressing major pathogens, increasing nutrient availability, decreasing chemical toxicity levels around the plant, or a combination of these (Whipps, 2001; Parke, 1991). Some of the microbes parasitize pathogenic fungi by producing chitinases or antibiotics. For instance, *Streptomyces hygroscopicus* var. *geldonus* produces a toxin called geldanomycin which can inhibit *R. solani* (Chet *et al.*, 1991; Fravel and Keinath, 1991). Temperature, soil moisture, soil texture, pH, varietal differences and overall health of the host, and growth rate of the microbe all influence the potential success of a biocontrol organism (Parke 1991).

One biocontrol organism that has been used in the past is *T. harzianum* Rifai. Chet and Henis (1983) reported 70% control of *R. solani* with 150 g (dry wt) of their *T. harzianum* preparation per square meter in a broadcast application in carnation. Control was enhanced further by establishing carnations in peat moss with 15% by volume of the *T. harzianum* preparation prior to planting in the infested field. Chet and Henis (1983) also report that under field conditions, seed treatment with *T. hamatum* (Bonord.) Bainier reduced cotton damping-off caused by *R. solani* by 60%, and bare patches 23 days later by 39%. It also increased density of plants by 14%. An integrated approach with soil solarization or PCNB (pentachloronitrobenzene) improved control further. The use of fumigation in conjunction with *Trichoderma* helped prevent the re-establishment of *R. solani* and *S. rolfsii* in a peanut field. The *Trichoderma* prolonged control over methyl bromide alone (Chet and Henis, 1983). Chet and Henis (1983) detail the findings that *Trichoderma* is antagonistic through parasitism, and competition for nutrients for

germinating sclerotia of *R. solani* and *S. rolfsii* . *Trichoderma harzianum* can use the *R. solani* cell wall as a sole carbon source. Isolates of *T. harzianum* differed in the levels of hydrolytic enzymes produced when mycelia of *S. rolfsii*, *R. solani*, and *Pythium aphanidermatum* (Edson) Fitzp. were attacked in the soil. This correlated with the level of control of each pathogen, suggesting that a specific *Trichoderma* species may be required to control a specific pathogen (Chet and Henis, 1983).

Different groups within *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx produce different antibiotics that differ in their control of *Pythium ultimum* versus *Rhizoctonia solani* (Whipps, 2001). D'Ercole *et al.* (1989) found a reduction of post-transplant blight incidence from 24.5% in the control to 11.5% in the treatment with *T. harzianum* as a liquid dip on 'Gorella' strawberries. Studies found that T-22 (*Trichoderma harzianum*) could increase nitrogen fertilizer efficiency in corn (Harman, 2000). It causes plants to be more robust and have more extensive root systems by suppressing disease as well as stimulating plant metabolism (Harman, 2000). A combination of a binucleate *Rhizoctonia* spp. and *Gliocladium virens* (J.H. Mill., Giddens & A.A. Foster) Arx provided control of Rhizoctonia blight on tall fescue in laboratory assays decreasing percent blighted plants to 14 to 26%, compared to 30-36% in the control (Yuen *et al.*, 1994).

Walker and Morey (1999) found that in pot and field experiments, commercial microbial nematicidal products were not as effective as conventional control measures in controlling the nematodes *Tylenchulus semipenetrans* Cobb, *Paratrichodorus lobatus* Colbran, and the root rot fungi *Pythium ultimum* and *Phytophthora nicotianae* var. *parasitica* (Dastur.) Waterhouse in citrus. They did find that Actizyme [*Bacillus subtilis*

(Ehrenberg) Cohn.] stimulated citrus growth, as did the herbicide oryzalin, but effective control was not obtained by biological measures, and some of the products harmed the plant.

Cultural Control

Besides biological controls, other non-chemical measures have been tested. Katznelson and Richardson (1948) found that treatment of soil infested with strawberry BRR with dried blood, acetic acid, or steam sterilization all resulted in the reduction of the disease, while oat straw appeared to increase the severity of the disease. In California, chloride salts decreased *Pythium's* ability to colonize the soil resulting in a chance for antagonists to become established (Martin and Hancock, 1983).

The build up of BRR pathogens over time has led to the recommendation of crop rotation (Maas, 1998; Martin and Hancock, 1983; Perry and Ramsdell, 1994; LaMondia *et al.*, 2002). In Michigan 'U-Pick' operations, continual strawberry production is common due to the few suitable locations for these enterprises and other economic pressures. Continual cropping allows pathogen establishment and inoculum build-up. In potato, wheat, barley, and corn, the more frequently a crop is in a rotation, the higher the decrease in yield of that crop (Schippers *et al.*, 1987). Hildebrand and West (1941) found that strawberries grown after a soybean series in greenhouse experiments approached the same level of disease as sterilized soil. 'Saia' oats (*Avena strigosa* Ard.) has had some success in controlling *P. penetrans* and *R. fragariae* in a pot trial in the greenhouse (Townshend, 1989). Elmer and LaMondia (1999) found that an application of ammonium sulfate with 'Saia' oats or sorgho-sudangrass reduced *P. penetrans*

populations in strawberry roots, and that a rotation with 'Garry' oats and ammonium sulfate reduced root colonization by *R. fragariae* in a microplot study using 'Honeoye' strawberries. *Brassica* species such as oilseed radish, mustard, and canola have also received attention due to the formation of isothiocyanates that have fungicidal and nematicidal properties (Ettlinger and Kjaer, 1968; Snapp and Mutch, 2003).

Compost offers another means of control. Hoitink and Fahy (1986) discuss the use of organic material for disease control, such as the incorporation of ammoniated Douglas fir bark into soil which provided control for red stele in strawberry during the first two years of the planting, and the use of composted hardwood bark to suppress several species of nematodes including *P. penetrans*. Plant pathogens are removed from composted materials by three mechanisms discussed by Hoitink and Fahy (1986): (1) the high temperature achieved during the composting procedure, (2) release of lethal chemicals during the process, and (3) microbial antagonism. Once the pathogens have been eliminated, successful disease suppression is affected by the final particle size, nitrogen, cellulose, lignin, and soluble salts content, as well as pH, any inhibitory compounds released by the compost, and the population of beneficial microbial populations (Hoitink *et al.*, 1997). The microorganisms provide control through the same concepts as biocontrol products such as competition, antibiosis, hyperparasitism, and the induction of systemic acquired resistance (Hoitink *et al.*, 1997). The success of the compost depends on the raw product from which it is derived and how it is handled. The variability in compost stability is one obstacle that must be overcome. For instance, control of *R. solani* by *Trichoderma* is better in mature compost as compared to fresher organic matter because *R. solani* is a more fit saprophyte under those conditions (Hoitink

et al., 1997). At the same time, excessively mature compost does not support as diverse a microbial population, allowing pathogens to cause disease (Hoitink *et al.*, 1997).

The recommendations for controlling BRR have not changed much over the past seventy years. The central difficulty is that BRR is a disease complex and the pathogens vary from site to site. Studies have implicated *P. penetrans*, but the disease can occur with low populations of this nematode, or without them at all. *Rhizoctonia fragariae* does not always have to be present to get BRR symptoms either. It seems that a combination of abiotic and biotic factors predispose strawberry plants to invasion by perhaps otherwise saprophytic or weakly parasitic fungi that then cause severe economic loss. With the primary control measure being eliminated, it becomes necessary to find alternative environmentally safe chemicals, biological alternatives, or cultural controls.

LITERATURE CITED

Anonymous. 1998. United Nations Environmental Programme (UNEP) Methyl Bromide Technical Options Committee (MBTOC). 1998 Assessment of the Alternatives to Methyl Bromide. United Nations Environmental Programme, Nairobi: 374 pp.

Brown, D. J. F, Dalmasso, A., and Trudgill, D. L. 1993. Plant Parasitic Nematodes in Temperate Agriculture. Nematode Pests of Soft Fruits and Vines. Evans, K., D. L. Trudgill, and J. M. Webster, Eds. CAB, International.

Burpee, L. L., Sanders, P. L., and Cole Jr., H. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia 72: 689-701.

Buyer, J. S. and Sikora, L. J. 1991. Rhizosphere interactions and siderophores. Pages 263-269 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Chapman, R. A. 1956. Plant parasitic nematodes associated with strawberries in Kentucky. Plant Dis. Reporter 40:179-183.

Chen, T., and Rich, A. E. 1962. The role of *Pratylenchus penetrans* in the development of strawberry black root rot. Plant Disease Reporter. 46:839-843.

Chet, I., and Henis, Y. 1983. *Trichoderma* as a biocontrol agent against soilborne root pathogens. *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul. 110-112.

Chet, I., Ordentlich, A., Shapira, R., and Oppenheim, A. 1991. Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria. Pages 229-336 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cook, R. 2002. Strawberry Production in the United States 1990-2000. Department of Agriculture Resources and Resource Economics.

D'Ercole, N., Nipoti, P., and Manzali, D. 1989. Research on the root rot complex of strawberry plants. Acta Horticulturae 265:497-501.

DiEdwardo. 1960. Time-lapse studies of movement, feeding and hatching of *Pratylenchus penetrans*. Phytopathology 50:570-571.

Dunn, R. A. 1972. Importance of depth in soil, presence of host roots, and role of eggs as compared to vermiform stages in overwintering of *Pratylenchus penetrans* at Ithaca, New York. Journal of Nematology 4:221-222.

Elmer, W. H., and LaMondia, J. A. 1999. Influence of ammonium sulfate and rotation crops on strawberry black root rot. Plant Dis. 83:119-123.

EPA (Environmental Protection Agency). 2006. Metam sodium as an alternative to methyl bromide for fruit and vegetable production. U.S. Environmental Protection Agency: Ozone Depletion Rules and Regulations.

Ettlinger, M. G., and Kjaer, A. 1968. Sulfur compounds in plants. Pages 59-144 *In* Recent advances in phytochemistry. T. J. Mabry, ed. New York: Appleton-Century-Crofts.

Fravel, D. R., and Keinath, A. P. 1991. Biocontrol of soilborne plant pathogens with fungi. Pages 237-243 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Freckman, D. W., and Chapman, R. A. 1972. Infection of red clover seedlings by *Heterodera trifolii* (Goffart) and *Pratylenchus penetrans* (Cobb.) Journal of Nematology 4:23-28.

Galletta, G. J., and Himelrick, D. G. 1990. Pages 83-156 *In* Small Fruit Crop Management. Prentice Hall, New Jersey.

Gianessi, L., and Reigner, N. 2005. The Value of Fungicides in U.S. Strawberry Production: Preliminary Findings. CropLife Foundation: Crop Protection Research Institute.

Goheen, A. C., and Bailey, J. S. 1955. Meadow nematodes in strawberry plantings in Massachusetts. Plant Disease Reporter 39:879-880.

Goheen, A. C., and Smith, J. B. 1956. Effects of inoculation of strawberry roots with meadow nematodes, *Pratylenchus penetrans*. Plant Disease Reporter 40:146-149.

Hancock, J. F., Callow, P. W., Serçe, S., and Schilder, A. C. 2001. Relative performance of strawberry cultivars and native hybrids on fumigated and nonfumigated soil in Michigan. HortScience 36:136-138.

Hancock, J. F. 1999. Strawberries. Crop Production Science in Horticulture. CABI Publishing, New York.

Harman, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease 84:377-392.

Hendrix Jr., F. F., and Campbell, W. A. 1973. *Pythiums* as plant pathogens. Ann. Rev. Phytopathol. 11: 77-98.

Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, 11:18-31.

Hildebrand, A. A., and West, P. M. 1941. Strawberry root rot in relation to microbiologiclal changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research Sec. C 19:183-198.

Hoitink, H. A. J., and Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts. Ann. Rev. Phytopathol. 24:93-114.

Hoitink, H. A. J., Stone, A. G., and Han, D. Y. 1997. Suppression of plant diseases by composts. HortScience 32:184-187.

Husain, S. S., and McKeen, W.E. 1963a. *Rhizoctonia fragariae* sp. nov. in relation to strawberry degeneration in southwestern Ontario. Phytopathology 53:532-540.

Husain, S. S., and McKeen, W.E. 1963b. Interactions between strawberry roots and *Rhizoctonia fragariae*. Phytopathology 53:541-545.

Kable, P. F., and Mai, W. F. 1968. Influence of soil moisture on *Pratylenchus penetrans*. Nematologica 14: 101-122.

Katznelson, H., and Richardson, L.T. 1948. Rhizosphere studies and associated microbiological phenomena in relation to strawberry root rot. Scientific Agriculture 28:293-307.

Klinkenberg, C. H. 1955. Nematode diseases of strawberries in the Netherlands. Plant Dis. Reporter 9:603-606.

Kurppa, S., and Vrain, T. C. 1985. Penetration and feeding behavior of *Pratylenchus penetrans* in strawberry roots. Revue Nematology 8:273-276.

LaMondia, J. A. 2004. Field performance of twenty-one strawberry cultivars in a black root rot infested site. Journal of the American Pomological Soc. 58:226-232.

LaMondia, J. A., Elmer, W. H., Mervosh, T. L., and Cowles, R. S. 2002. Integrated management of strawberry pests by rotation and intercropping. Crop Protection 21:837-846.

LaMondia, J. A. 1999. Effects of *Pratylenchus penetrans* and *Rhizoctonia fragariae* on vigor and yield of strawberry. Journal of Nematology 31:418-423.

LaMondia, J. A., and Martin, S. B. 1989. The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. Plant Disease 73:107-110.

Lee, D. L. 2002. The Biology of Nematodes. University of Leeds, United Kingdom. Taylor and Francis, London.

Locascio, S. J. 2005. Alternatives to methyl bromide fumigation for polyethylene-mulched strawberries. University of Florida.

Long, S. 2002. MSU Researchers seek multicropping strawberry variety. Project Greeen. Michigan State University.

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Mai, W. F., and Mullin, P. G.. 1960. Plant-Parasitic Nematodes: A Pictorial Key to Genera. 5th Ed. Cornell University Press. Ithaca, New York.

Mai, W. F., Bloom, J. R., and Chen, T. A. 1977. Biology and Ecology of the Plant-Parasitic Nematode *Pratylenchus penetrans*. Pennsylvania State University. Bulletin 815.

Mamiya, Y. 1971. Effect of temperature on the life cycle of *Pratylenchus penetrans* on Cryptomeria seedlings and observations on its reproduction. Nematologica 17:82-89.

Martin, F. N. 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. Phytopathology 90:345-353.

Martin, F. N., and Hancock, J. G. 1983. Chemical factors in soils suppressive to *Pythium ultimum*. Pages 113-116 *In* Ecology and Management of Soilborne Plant Pathogens. C.A. Parker, A.D. Rovira, K.J. Moore, and P.T.W. Wong, Eds. American Phytopathological Society. St. Paul.

Martin, S. B. 1988. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78:379-384.

MDA (Michigan Department of Agriculture). 2007. Michigan Fruit Inventory 2006-2007. United States Department of Agriculture.

Mervosh, T. L., and LaMondia, J. A. 2004. Strawberry black root rot and berry yield are not affected by terbacil herbicide. HortScience 39:1339-1342.

Miller, P. M., Sands, D. C., and Rich, S. 1973. Effect of industrial mycelial residues, wood fiber wastes, and chitin on plant-parasitic nematodes and some soilborne diseases. Plant Disease Reporter 57:438-442.

Miller, P. W. 1948. Relation of desiccation to the development of black root rot of strawberries. Plant Dis. Reporter 32:315-316.

Molot, P. M., and Ferriere, H. 1989. Susceptibility of strawberry cultivars to *Rhizoctonia solani* and *R. fragariae* as influenced by inoculation technique, seasonal variations and physiological condition of the plants. Acta Horticulturae 265:535-538.

Mountain, W. B., Sands, D. C, and Rich, S. 1959. The peach replant problem in Ontario. VII. The pathogenicity of *Pratylenchus penetrans* (Cobb, 1917) Filip. and Stek., 1941. Canadian Journal of Botany 37:459-470.

NASS (National Agricultural Statistics Service). 2007. 2006 Strawberry Data. United States Department of Agriculture.

Nelson, E. B. 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant and Soil 129:61-73.

Nelson, P. E. 1957. Pathogenicity of *Idriella lunata* on strawberry. Phytopathology 47:438-443.

Nemec, S. 1970. *Pythium sylvaticum* pathogenic on strawberry roots. Plant Dis. Rep. 54:416-418.

Nemec, S., and Sanders, H. 1970. *Pythium* species associated with strawberry root necrosis in southern Illinois. Plant Disease Reporter 54:49-51.

Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Fitopatol. Brasileira 10: 371-390.

Ogoshi, A., and Ui, T. 1983. Anastomosis groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Pages 57-58 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Olthof, Th. H. A. 1982. Effect of age of alfalfa root on penetration of *Pratylenchus penetrans*. Journal of Nematology 14:100-105.

Oyekan, P. O., Blake, C. D., and Mitchell, J. E. 1972. Histopathology of pea roots axenically infected by *Pratylenchus penetrans*. Journal of Nematology 4:32-35.

Parke, J. L. 1991. Root colonization by indigenous and introduced microorganisms. Pages 33-42 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Parmeter Jr., J. R., Whitney, H. S., and Platt, W. D. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. Phytopathology 57: 218-223.

Particka, C. A., and Hancock, J. F. 2005. Field evaluation of strawberry genotypes for tolerance to black root rot on fumigated and nonfumigated soil. J. Amer. Hort. Sci. 130:688-693.

Perry, S., and Ramsdell, D. 1994. Strawberry diseases in Michigan. Ag Facts, Michigan State University Extension Bulletin E-1728: 1-4.

Pollack, S., and Perez, A. 2005. Fruit and Tree Nuts Outlook. Economic Research Service. USDA. FTS-315.

Powell, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annual Review of Phytopathology 9:253-274.

Pritts, M., and Handley, D. Eds. 1998. Strawberry Production Guide: For the Northeast, Midwest, and Eastern Canada. Northeast Regional Agricultural Engineering Service Cooperative Extension. Ithaca, New York.

Raski, D. J. 1956. Meadow *Pratylenchus penetrans* tested on strawberries grown in black root rot soil. Plant Disease Reporter 40:690-693.

Ribeiro, O. K., and Black, L. L. 1971. *Rhizoctonia fragariae*: a mycorrhizal and pathogenic fungus of strawberry plants. 55:599-603.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Management Network 27 Oct.

Schippers, B., Bakker, A. W., and Bakker, P. A. H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Rev. Phytopahtology 25:339-358.

Scott, R., Pritts, M., and Kelly, M. J. 2003. Effects of *Rhizoctonia fragariae* infection on growth and productivity of strawberry plants grown under different temperature regimes. Advances in Strawberry Research 22: 26-33.

Snapp, S. S., and Mutch, D. R. 2003. Cover crop choices for Michigan vegetables. Michigan State University Extension Bulletin E 2896: 1-6.

Stanghellini, M. E., and Hancock, J. G. 1970. The sporangium of *Pythium ultimum* as a survival structure in soil. Phytopathology 61:157-164.

Strong, F. C., and Strong, M. C. 1927. Investigations on the black root of strawberries. Phytopathology 21:1041-1059.

Szczygiel, A., and Profic-Alwasiak, H. 1989. Studies on the interaction between nematodes and fungi in infecting strawberry plants. Acta Horticulturae 265:561-565.

Townshend, J. L. 1963. The pathogenicity of *Pratylenchus penetrans* to strawberry. Can. J. of Plant Science 43:75-78.

Townshend, J. L. 1989. Population densities of four species of root-lesion nematodes (*Pratylenchus*) in the oat cultivars, Saia and OAC Woodstock. Can. J. Plant Science 69:903-905.

Vilgalys, R., and Cubeta, M. A. 1994. Molecular systematics and population biology of *Rhizoctonia*. Annual Rev. Phytopathol. 32:135-155.

Walker, G. E., and Morey, B. G. 1999. Effects of chemicals and microbial antagonists on nematodes and fungal pathogens of citrus roots. Australian Journal of Exper. Ag. 39: 629-637.

Watanabe, T., Hashimoto, K., and Sato, M. 1977. *Pythium* species associated with strawberry roots in Japan, and their role in the strawberry stunt disease. Phytopathology 67:1324-1332.

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany 52:487-511.

Wilhelm, S. 1953. *Pythium ultimum*, an injurious component of the rhizosphere in certain soils. Phytopathology 43:590.

Wilhelm, S. 1963. The control of Verticillium wilt of strawberry and of weeds by preplant fumigation with chloropicrin and chloropicrin-methyl bromide mixtures. 16th Inter. Hort. Congr. 3 (1962): 263-264.

Wilhelm, S. 1965. *Pythium ultimum* and the soil fumigation growth response. Phytopathology 55:1016-1020.

Wilhelm, S., Nelson, P. E., Thomans H. E., and Johnson, H. 1972. Pathology of strawberry root rot caused by *Ceratobasidium* species. Phytopathology 62:700-705.

Williams-Woodward, J. L. 2001. Georgia Plant Disease Loss Estimate: Strawberry. The University of Georgia College of Agriculture and Environmental Sciences Cooperative Extension Service.

Wing, K. B., Pritts, M. P., and Wilcox, W. F. 1994. Strawberry black root rot: A Review. Advances in Strawberry Research. 13:11-19.

Wing, K. B, Pritts, M. P., and Wilcox, W. F. 1995. Biotic, edaphic, and cultural factors associated with strawberry black root rot in New York. HortScience 30:86-90.

WMO (World Meteorological Organization). 2003. Scientific Assessment of Ozone Depletion: 2002, Global Ozone Research and Monitoring Project -Report No. 47, 498 pp. Geneva.

Yuen, G. Y., Craig, M. L., and Giesler, L. J. 1994. Biological control of *Rhizoctonia solani* on tall fescue using fungal antagonists. Plant Disease 78:118-123.

Yuen, G. Y., Schroth, M. N, Weinhold, A. R., and Hancock, J. G. 1991. Effects of soil fumigation with methyl bromide and chloropicrin on root health and yield of strawberry. Plant Disease 75:416-420.

Zeller, S. M. 1932. A strawberry disease caused by *Rhizoctonia*. Oregon Agricultural Experiment Station Bulletin 295:1-22.

CHAPTER TWO: BIOCONTROL AND REDUCED-RISK FUNGICIDE EFFICACY TRIAL

Introduction

Strawberry black root rot (BRR) is a disease complex that is widespread around the world wherever strawberries have been planted for multiple years (Watanabe *et al.*, 1977; D'Ercole *et al.*, 1989). Infected plants produce smaller leaves, fewer main and lateral roots, have slower growth, and reduced runner production (Maas, 1998; Hancock *et al.*, 2001). Severely infected plants wilt at the onset of dry weather. The disease can be spread via infected nursery stock, movement of infested soil, or infected plant debris (Maas, 1998; Strong and Strong, 1927; Hildebrand, 1934). Also known as strawberry decline, many organisms and abiotic factors have been implicated in the cause of the disease; however, *Rhizoctonia fragariae* Husain & McKeen, *Pythium* spp., and the root lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven are generally considered the primary pathogens (D'Ercole *et al.*, 1989; Wing *et al.*, 1994; Maas, 1998).

In Michigan, the matted-row system is used to grow strawberries, where plants are typically planted at wide spacing in spring so that runners fill in between the plants. In Michigan 'U-Pick' operations, continual strawberry production or short rotations are common due to the few suitable locations for these enterprises. The shorter a rotation is between strawberry plantings, the higher the potential economic return to the grower. However, continual cropping allows pathogen establishment and build-up to deleterious levels. In this system, fumigation is often employed to help control soil-borne pathogens

and weeds before planting. A common and effective fumigant used by strawberry growers is methyl bromide. In 1992, the Montreal Protocol established methyl bromide as an ozone-depleting substance and instituted its ban by January 1, 2005 (Anonymous, 1998; Rosskopf *et al.*, 2005).

The primary problem when trying to develop a management strategy for BRR is that the causative organisms of the disease are variable. *Rhizoctonia fragariae* does not always have to be present to get BRR symptoms (Wing *et al.*, 1994; Wing *et al.*, 1995). While studies have implicated *P. penetrans*, the disease can occur with low nematode populations or even in their absence (Wing *et al.*, 1994; Wing *et al.*, 1995). A combination of abiotic and biotic factors may predispose strawberry plants to invasion by perhaps otherwise saprophytic or weakly parasitic fungi that then cause severe damage to the root system (Wing *et al.*, 1994; Wing *et al.*, 1995). With the primary control measure due to be eliminated, it becomes necessary to find alternative reduce-risk chemicals, biological, and cultural alternatives.

An alternative to methyl bromide are reduced-risk fungicides and biocontrol products which could be applied at planting. This would require the least change in a production scheme that is heavily reliant on pre-plant fumigation.

The mucilage excreted by the roots supports both beneficial and potentially harmful organisms seeking to colonize the growing root. The beneficial microbes are antagonistic to pathogens by competition for food, essential elements, and space (Whipps, 2001; Parke, 1991). These microbes enhance plant growth by suppressing major pathogens, increasing nutrient availability, decreasing toxicity levels around the plant, or a combination of these (Whipps, 2001; Parke, 1991). Some of the microbes

parasitize pathogenic fungi by producing chitinases or antibiotics. For instance, *Streptomyces hygroscopicus* var. *geldonus* produces a toxin called geldanomycin which can inhibit *R. solani* (Chet *et al.*, 1991; Fravel and Keinath, 1991). *Trichoderma* is antagonistic through parasitism, and competition for nutrients for germinating sclerotia of *R. solani* and *S. rolfsii* . *Trichoderma harzianum* can use the *R. solani* cell wall as a sole carbon source (Chet and Henis, 1983).

One biocontrol organism that has been used in the past is *T. harzianum* Rifai. Chet and Henis (1983) reported 70% control of *R. solani* with 150 g (dry wt) per square meter in a broadcast application in carnation. Chet and Henis (1983) also report that under field conditions, seed treatment with *T. hamatum* (Bonord.) Bainier reduced cotton damping-off caused by *R. solani* by 60%, and bare patches 23 days later by 39%. It also increased density of plants by 14%. *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx produce different antibiotics that differ in their control of *Pythium ultimum* versus *Rhizoctonia solani* (Whipps, 2001). D'Ercole *et al.* (1989) found a reduction of post-transplant blight incidence from 24.5% in the control to 11.5% in the treatment with *T. harzianum* as a liquid dip on 'Gorella' strawberries.

This experiment was conducted to evaluate biocontrol agents and reduced-risk fungicides applied at planting, or through the establishment season, as possible alternatives to methyl bromide for strawberry production, and to enable the selection of promising products for further assessment in an integrated approach to managing BRR (Chapter 4).

All the products tested were fungicides, except for Ditera, a biological nematicide. Some products, such as Abound, claim to control root rot caused by *Rhizoctonia* spp., but

have not been tested in Michigan. Some of the chemicals, like Ridomil Gold EC, are used in strawberry production, but not necessarily in this application method or timing. Other products are not labeled for strawberries, but claim to control similar diseases on other hosts such as brown patch and large patch caused by *Rhizoctonia* spp. on turf (Endorse). There are also many biological products that make claims of disease control or enhancement of plant growth based on *Streptomyces* or *Trichoderma* spp.

Materials and Methods

Field Establishment and Measurements

On 16 June 2005, 15 treatments, replicated four times, were established in 3.05 x 3.05 m plots in a randomized complete block design to establish efficacy against BRR (table 1). The field was a sandy-loam located at the Michigan State University Horticulture Farm (East Lansing, MI) with a 4-year history of continuous strawberry production and BRR. Each plot consisted of four rows of strawberries planted 61 cm apart with six plants set 45.7 cm apart. There were 45.7 cm of cultivated buffers around each plot. Plots used as positive controls received fumigation with 448 kg/ha of methyl bromide and chloropicrin (methyl bromide 66%, Chlor-o-pic 33%) 14 d before planting. Plots receiving no treatment served as negative controls. Napropamide (Devrinol) was applied over the whole planting 10 d before planting for weed control. Susceptible cultivar 'Allstar' transplants were obtained from Krohne Plant Farms (Hartford, MI). These were pulled out of storage three days before planting due to severe etiolation and placed in lugs with Baccto High Porosity Professional Planting Mix (Michigan Peat Company, Houston, TX) to ensure the plants were healthy for transplanting.

Table 1. Products, active ingredient, application method, and label rate used of strawberry biocontrol and fungicide products applied at planting in an efficacy trial in East Lansing, MI from 2005-2007.

Product	Active Ingredient	Application Method	Label Rate
Abound**	Azoxystrobin	5 Minute Dip	8 fl oz/100 gal
Actinovate	*Streptomyces lydicus*	Drench	1 tsp/ gal
C/G	Fatty acids	Drench	1% v/v
Ditera	*Myrothecium verrucaria*	Drench	2.5 lbs/acre
Endorse	Polyoxin D zinc salt	Drench	11 lbs/acre
Methyl bromide + Chloropicrin	Methyl bromide 66%, chloropicrin 33%	Fumigant	400 lbs/acre
Mycostop	*Streptomyces griseoviridis*	Drench	2 g/ 100 ft^2
Plantshield**	*Trichoderma harzianum*	5 Minute Dip	2.5 lbs/5 gal
Polyversum	*Pythium oligandrum*	Drench	0.1 g/m^2
T-10*	*Trichoderma rossicum*	5 Minute Dip	$5.25x10^7$
ProPhyt**	Potassium phosphite	15 Minute Dip	2 pt/100 gal
ProPhyt + T-10*	Potassium phosphite + *T. rossicum*	30 Minute Dip	As above
ProPhyt + Abound	Potassium phosphite + azoxystrobin	30 Minute Dip	As above
Ridomil Gold EC**	Mefenoxam	Drench	1 pt/acre

* As prepared in the laboratory.

** Products currently labeled for use in strawberry production.

C/G was applied two days before planting to avoid phytotoxicity that had been observed in previous experiments and to allow any precipitation to further distribute it in the soil. Rainfall totaled 68 mm over the two days after planting. All strawberry transplants were planted the same day with the rest of the drenches applied as 473 ml per planting hole and the plant placed in the hole immediately. T-10 was grown on potato dextrose agar (PDA) for 7-10 d until sufficient spore production was achieved for making a spore suspension the day of planting. The drench application procedure was done to decrease the chance of contamination between treatments, increase consistency of volume applied, and get each product around the roots. Since many of the products are biocontrol agents, getting them in the root zone was essential to assist in potential colonization, and consequently, disease control.

Roots receiving the dip treatments were treated with product mixed in 3.79 liters of water in buckets. Planting was followed by 25 mm of irrigation. Standard cultural practices were implemented including a spring and fall fertilization of 19-19-19 at the rate of 32 kg/ha and weekly hand weeding. In August, 57.7 kg/ha of nitrogen was applied as urea. Flowers were removed the first year and runners were racked back into the rows to establish a perennial bed 50 cm wide. Irrigation was applied as needed.

A count was taken of the mother plants, within the two center rows, two weeks after planting to determine loss due to planting and establish a base number of plants used for data collection. The mother plant count difference was calculated using the starting count from 2005 through the fall count in 2005. Ditera, although originally applied at 2.8 kg/ha,was re-applied each month for the first season at a rate of 5.6 kg/ha as a 473-ml drench around each plant.

Each application of Ditera was followed by at least 6.4 mm of irrigation except for the last treatment in September which was not irrigated because the irrigation system was being repaired. On 13 September 2005, 100 g of soil surrounding the plant dug for root samples were taken from each replication of the control, fumigated, and Ditera plots to assess nematode populations. All samples were taken from the outside rows of the treatments so that interior plants were kept for future data collection. Plant vigor and bed fill ratings were obtained in 13 September 2005 and were based on a 1 to 5 scale (table 2). The total number of crowns and number of remaining mother plants were counted on 18 November 2005 from the middle two rows. Total crown counts include daughter and mother crowns. Crown counts and erect petiole biomass were calculated for an area of 38.1 cm x 61 cm for a single row. Approximately 7 cm of straw was placed over the plants in November.

Table 2. Plant vigor and bed fill ratings used in the strawberry biocontrol and fungicide products applied at planting efficacy trial in East Lansing, MI during 2005-2007.

PlantVigor	Characteristics
5	Plants in excellent health, vigorous growth, superior runnering
4	33% plants diseased or stunted
3	50% plants diseased or stunted
2	Majority of plants diseased or stunted
1	Plants very stunted, diseased
Bed Fill	**Characteristics**
5	Runners healthy and establishing well, full bed
4	Fewer runner plants than in 5
3	Thinning evident, runner size and establishment good
2	Few runners, mother plants obvious
1	Few to no runners establishing, mother plants dead

The straw was removed on 9 April 2006. Three harvests were taken a week apart starting on 8 June 2006. The middle meter of each of the interior two rows was harvested. Ripe berries were picked regardless of condition or disease. Yield was standardized to one meter of a single row by dividing collected data by two. All plots were subsequently clean picked to ensure plant health for the following year. Bed fill and plant vigor ratings were taken on 4 August 2006. In September, lime and 19-19-19 fertilizer were applied at the rates of 271.5 kg/ha and 254.5 kg/ha, respectively. Plants were dug for fungal assays on 9 October 2006, and then the rows were roto-tilled by treatment to prevent cross contamination. All samples for fungal isolations were taken from the outside rows of the treatments so that interior plants were kept for data collection. Plants were covered with approximately 7 cm of straw in November 2006 .

On 21 April 2007 the straw was removed. Berries were counted as they were harvested in June. There were two harvests 8 d apart. The same interior meters and procedures were used as in 2006. Bed fill, plant vigor, and fresh erect petiole and leaf biomass were collected on 19 July 2007. A wooden frame measuring 38.1 cm x 61 cm was centered over each of the center rows and all leaves were collected within this area. The collected material was subsequently dried for 48 h at 80°C and weighed.

Root and Soil Analysis

The plants and soil collected in 2005 were submitted to the Michigan State University Diagnostic Lab for nematode analysis. Roots were taken from the untreated and fumigated treatments and put on water agar for fungal analysis. A total of ten root pieces (five root pieces per plate) were analyzed in addition to five root pieces from

plants showing root tip discoloration after obtaining whole plant fresh weights. Root pieces with lesions were selected. Isolated fungi were sub-cultured one week later after growing at room temperature. They were subsequently identified to genus using classic taxonomic procedures and growth characteristics on media (Barnett and Hunter, 1998; Domsch *et al.*, 1980; Barron, 1968). Those fungi that could not be identified in this manner were subsequently subjected to DNA sequencing of the internal transcribed spacer (ITS) regions by Dr. Mursel Catal (Sambrook *et al.* 1989; Altschul *et al.*, 1990).

On 9 and 10 October 2006 mother plants were again taken from the outside rows of each treatment for a total of 120 mother plants. These were selected as representatives of the entire plot. These plants were dug for fresh and dry biomass, percent root necrosis, and a visual root quality rating (on a scale of 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots). Dead material and daughter plants were removed, the roots washed under running cold water, and then the plants were air dried before the fresh weight was taken. Approximately 200 g of soil was collected from the Ditera, untreated, and fumigated plots for nematode analysis done by the Michigan State University diagnostic lab. Nematode presence was determined using the modified Jenkins technique and a modified root extraction process (Jenkins, 1964; Bird, 1971).

The foliage and roots were separated by cutting the crown in half above the uppermost adventitious roots. After rating and taking the fresh weights, the foliage and roots were dried at 80°C for 48 h and weighed again.

Additional 1 year old plants were also dug for fungal isolations from roots to evaluate general plant health. After washing as above, root pieces were surface sterilized

in 20% bleach for 2 min followed by two rinses in sterile deionized water for 1 min. Root pieces were dried on sterile paper towels. Ten 0.64-cm pieces were placed onto water agar (5 pieces per plate) with 80 pieces/treatment. Isolated fungi were sub-cultured over the next 7-10 d, being incubated on the laboratory bench at room temperature, and identified to genus as described previously. In 2007, despite the treatments Plantshield, Endorse, and the untreated control having biomass measurements and crown count not being taken from the two center rows, but one center and one outside row, these data were not removed and no significant differences were observed. The Endorse and Mycostop plots in block 2 were improperly harvested by not harvesting the same interior meter as the previous year on the first harvest date, no significant differences are evident. The frequency of fungi recovered was calculated from total fungi that grew. Total roots showing no growth was calculated from total root number.

On 11 September 2007, two mother plants were dug from the middle rows of each treatment plot to obtain a root quality rating, percent root necrosis, and fresh and dry weights as previously described. An additional mother plant was dug for fungal analysis from the same treatments assayed in 2006. The same procedure was used as previously described except that there were only 10 root pieces per treatment. A mother plant and 200 g of soil were also removed from the untreated, fumigated, and Ditera plots for nematode analysis.

Data Analysis

All statistical analyses were performed using the ANOVA and mean separation functions (Fisher's protected least significant difference test p=0.05) of the StatGraphics

Plus 4.1 (StatPoint Inc., VA) statistical computer program. Initially a variance check was performed; all data that did not pass the variance check were subsequently transformed. When analyzing the data over multiple years, it was necessary to utilize SAS 9.1 (SAS Institute Inc., Cary, N.C.) using repeated measurement ANOVA in Proc GLM.

Results and Discussion

Year effect was significant in the repeated measurements ANOVA of the field data ($p<0.0001$). There was not a block x treatment interaction ($p=0.3033$).

During establishment in 2005, the fumigated and control treatments did not differ from one another significantly for any parameter, except total crown number where fumigated plants had more crowns than the untreated (table 3). Ditera, Abound, ProPhyt and ProPhyt + Abound treated plants tended to have the highest vigor, best bed fill, and most crowns, but were not significantly superior to those untreated. C/G was the only treatment causing a significant reduction in mother crowns from the untreated plants in 2005. The reason for this difference is that C/G can be phytotoxic to strawberries if not properly applied. Plants receiving Ridomil Gold EC also appeared stunted, but were not significantly different than the untreated controls. Wing *et al.* (1995) discusses that Ridomil is typically associated with healthier plants, but it is only effective in BRR sites with oomycetes. Ridomil Gold EC is typically applied to older strawberry plantings, so the age of the plants and the method used to apply it may not have been optimal for strawberry growth. This toxicity was also observed by Louws *et al.* (2004).

Table 3. Effects of different biocontrol and fungicide products applied at planting on plant vigor and bed fill of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2005 and 2006.[z]

[x] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[y] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size andestablishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

[z] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p =0.05. Pairwise comparisons performed with n=4.

Treatment	Bed fill rating (1-5)[y]	Plant vigor rating (1-5)[x]	Total crown number/m[2]	Difference in mother crown number	Fruit yield/m (kg)
			2005		
Fumigated	4.50 d	4.75 NS	12.31 c	0.00 c	-
Abound	4.00 cd	4.25	11.67 bc	-0.25 bc	-
Actinovate	3.75 cd	3.75	10.38 bc	-0.50 bc	-
C/G	1.25 a	3.25	6.46 a	-5.75 a	-
Ditera	4.00 cd	4.50	10.36 bc	-0.25 bc	-
Endorse	3.50 cd	3.75	9.70 bc	-1.50 b	-
Mycostop	4.00 cd	3.75	9.02 ab	-0.25 bc	-
Plantshield	2.75 bc	3.50	8.84 ab	-0.25 bc	-
Polyversum	3.25 bcd	3.50	10.04 bc	-1.00 bc	-
ProPhyt	4.00 cd	4.50	11.59 bc	0.00 c	-
ProPhyt + Abound	3.50 cd	4.50	11.06 bc	0.00 c	-
ProPhyt + T-10	3.50 cd	4.25	9.77 bc	-0.50 bc	-
Ridomil Gold EC	2.00 ab	2.75	8.84 ab	-1.00 bc	-
T-10	3.00 bc	3.50	9.70 bc	-1.25 bc	-
Untreated	3.25 bcd	3.75	8.92 ab	-1.00 bc	-
ANOVA					
Effect	Df		Significance (p)		
Treatment	14	**0.0035**	0.0546	**0.0305**	**0.0000**	-
Block	3	0.7981	0.5958	**0.0019**	0.7605	-
Residual	42				
Total (Corr.)	59				
			2006		
Fumigated		5.00 a	5.00 a	13.79 NS	-	1.31 abc
Abound		4.25 ab	4.50 ab	12.41	-	1.67 a
Actinovate		3.75 bc	3.75 bcde	12.39	-	1.11 abc
C/G		2.75 c	3.25 def	11.36	-	0.44 d
Ditera		4.00 ab	4.00 bcd	12.77	-	1.31 abc
Endorse		2.75 c	3.50 cde	13.50	-	1.19 abc
Mycostop		3.50 bc	3.50 cde	12.80	-	1.16 abc
Plantshield		3.50 bc	3.50 cde	10.89	-	0.96 bcd
Polyversum		3.25 bc	3.00 ef	10.95	-	1.10 abc
ProPhyt		4.25 ab	4.25 abc	11.91	-	1.31 abc
ProPhyt + Abound		3.75 bc	3.50 cde	11.91	-	1.29 abc
ProPhyt + T-10		4.00 ab	4.00 bcd	10.93	-	1.25 abc
Ridomil Gold EC		2.75 c	2.50 f	10.36	-	0.76 cd
T-10		3.75 bc	3.50 cde	9.85	-	1.09 bc
Untreated		3.50 bc	3.75 bcde	11.12	-	1.36 ab
ANOVA						
Effect	Df			Significance (p)		
Treatment	14	**0.0074**	**0.0003**	0.0652	-	**0.0505**
Block	3	0.6471	0.1363	0.5244	-	0.2621
Residual	42					
Total (Corr.)	59					

In 2006, the negative impact of C/G and Ridomil Gold EC on bed fill and plant vigor was still apparent. Abound, Ditera, ProPhyt, and ProPhyt + T-10 all tended to increase bed fill and plant vigor ratings over the untreated plants, but the differences were not significant over the untreated control (table 3). Although total crown number was not significantly different among treatments, Ditera, Endorse, and Abound tended to have numerically more crowns (table 3). Abound, ProPhyt, and ProPhyt + Abound did not have the highest averages for total crown number in 2006, which may be a result of already having well established beds from 2005, meaning that the beds did not have as many new additional crowns. Although the fumigated and untreated control treatments significantly differed for plant vigor and bed fill, the lack of yield differences may be a result of the fumigated strawberries putting on more vegetative growth, making them bigger and more vigorous, but not necessarily produce more fruit (table 3).

Although there was a lack of significant differences amongst the majority of the treatments, and even between the treatments and the untreated control, there were three treatments that consistently tended to have averages closer to the fumigated control for each parameter in 2005 and 2006. These treatments were ProPhyt, Abound, and Ditera. When examining just the biocontrol products over 2005 and 2006, Actinovate also tended to have higher plant vigor and bed fill than other biocontrol treatments.

Treatments subjected to fungal assays were selected because they showed initial promising results and were being considered for evaluation in the Integrated Management Experiment (Chapter 4). These included: untreated control, fumigated control, ProPhyt + Abound, Abound, ProPhyt, Actinovate, and Plantshield.

The treatments were not significantly different for a single parameter in 2007. However, Abound, Ditera, ProPhyt, and ProPhyt + Abound-treated plants continued to show averages approaching those of the plants in the fumigated control (table 4). Plants receiving the Actinovate and T-10 dip also had averages approaching those of plants in the fumigated plots for fill, vigor, total berries, fresh biomass, and yield. The untreated plants had the lowest average bed fill and plant vigor rating, and dry biomass weights, while the fumigated control had the highest averages. The significance of the blocks in the plant vigor rating data may be due to some of the Ridomil Gold EC treated plants recovering more fully than others due to possible differences in local soil fertility and microbiology. In the third season (2007), treatment effects, especially from those treatments that appeared detrimental initially, had disappeared.

Despite the set backs the strawberry plants suffered from the C/G and Ridomil Gold EC treatments, the ability of strawberry plants to compensate for the death of plants is apparent from the 2007 yield data (table 4). The plants had also been able to outgrow any of the initial toxicity. Due to the lack of a general decline being observed and the increasing risk of not being able to confidently identify all surviving mother crowns in each treatment, a total surviving mother crown count was not attempted in 2007.

Table 4. Effects of different biocontrol and fungicide products applied at planting on plant vigor and bed fill of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2007.[z]

Treatment	Bed fill rating (1-5)[y]	Plant vigor Rating (1-5)[x]	Total crown number/m^2	Single berry wt. (g)	Total berry number	Fruit yield/m (kg)	Fresh biomass /m^{2x} (g)	Dry biomass /m^{2x} (g)
Fumigated	5.00 NS	5.00 NS	22.00 NS	4.56 NS	129.50 NS	0.61 NS	295.10 NS	89.61 NS
Abound	4.75	4.75	24.00	5.48	95.75	0.53	219.49	69.78
Actinovate	5.00	4.75	22.25	5.10	123.63	0.63	220.49	71.18
C/G	4.00	4.50	15.75	4.83	91.25	0.45	145.51	49.54
Ditera	5.00	4.75	21.50	5.28	117.00	0.62	217.40	71.31
Endorse	4.25	4.50	19.25	4.12	108.00	0.44	187.06	60.98
Mycostop	5.00	4.75	21.50	4.93	115.88	0.57	201.73	67.90
Plantshield	5.00	4.75	21.50	5.43	108.88	0.60	201.73	66.55
Polyversum	4.00	4.25	19.75	4.44	101.75	0.45	186.59	59.95
ProPhyt	4.50	4.75	23.25	5.09	111.00	0.57	217.73	68.31
ProPhyt + Abound	5.00	4.75	22.88	4.86	117.00	0.55	221.01	72.26
ProPhyt + T-10	5.00	4.75	23.25	4.95	111.63	0.57	233.38	75.97
Ridomil Gold EC	4.50	4.75	18.63	5.20	97.50	0.52	179.04	59.48
T-10	5.00	4.75	23.00	5.43	131.25	0.71	239.90	77.42
Untreated	4.00	4.25	19.88	4.99	132.50	0.67	189.39	60.28

ANOVA

Effects	Df	Significance (p)							
Treatment	14	0.0668	0.4535	0.2364	0.3601	0.7003	0.6700	0.0546	0.1277
Block	3	**0.0467**	**0.0000**	0.0887	0.9186	0.3237	0.4233	**0.0000**	**0.0000**
Residual	42								
Total (Corr.)	59								

[x] Plant vigor rating 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants

[y] Bed fill rating 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased

[z] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p =0.05. Pairwise comparisons performed with n=4.

For the two years that yield data were collected, Abound treated plants had the highest average, but plants in the two control treatments did not differ significantly from one another. This lack of difference between years is maybe due to a late spring frost in 2007 that reduced fruit set. This was also a hotter, drier year resulting in lower yields.

Over the three years that the fill rating was obtained, plants treated with Ditera or Abound were the closest to the plants in the fumigated control, but none of the treatments resulted in significantly fuller beds than the untreated control (table 5). Ditera, Abound, ProPhyt, and ProPhyt + T-10 treated plants also had the same plant vigor as the fumigated control, but treatment effects were not significant. ProPhyt and Abound treated beds tended to have the same number of crowns as beds in the fumigated control, but there were no significant differences among treatments. Average total crowns are expected to increase each year in healthy strawberry beds, this is particularly true in beds that may have started out thin due to phytotoxic treatments. This compensation would also be reflected in bed fill.

Table 5. Effects of different biocontrol and fungicide products applied at planting on plant vigor, bed fill, and yield of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2005-2007.[x]

	All three years			*2006-2007*
Treatment	**Bed fill rating (1-5)[z]**	**Plant vigor rating (1-5)[y]**	**Total crown number/m^2**	**Total yield/m (kg)**
Fumigated	4.83 a	4.92 a	16.03 NS	0.96 NS
Abound	4.33 ab	4.50 ab	16.02	1.10
Actinovate	4.17 abc	4.08 bcd	15.01	0.87
C/G	2.67 e	3.67 cde	11.19	0.44
Ditera	4.33 ab	4.42 ab	14.88	0.96
Endorse	3.50 cd	3.92 bcde	14.15	0.82
Mycostop	4.17 abc	4.00 bcd	14.44	0.86
Plantshield	3.75 bcd	3.92 bcde	13.74	0.78
Polyversum	3.50 cd	3.58 de	13.58	0.77
ProPhyt	4.25 abc	4.50 ab	15.58	0.94
ProPhyt + Abound	4.08 abc	4.25 bc	15.28	0.92
ProPhyt + T-10	4.17 abc	4.33 ab	14.65	0.91
Ridomil Gold EC	3.08 de	3.33 e	12.61	0.64
T-10	3.92 bc	3.92 bcde	14.18	0.90
Untreated	3.58 bcd	3.92 bcde	13.30	1.01

ANOVA

Effects	Df	Significance (p)			
Treatment	14	**<0.0001**	**<0.0001**	0.8729	0.4867
Block	3	0.9227	**0.0052**	0.9763	0.6857
Residual	162				
Total (Corr.)	179				

[x] Results of pairwise comparison of averages performed on n=15, for block, n=4. Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05.

[y] Plant vigor rating 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

[z] Bed fill rating 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

When considering individual plant parameters in 2006, average fresh foliage weight and fresh root weight there were not significantly different among treatments, although the plants from the fumigated treatments were significantly larger than all other treatments (table 6). A similar pattern was found for fresh and dry total weights. There was no difference between the plants from the untreated control for any weight parameter except for Plantshield which had lower whole plant weights. Plants receiving the ProPhyt + Abound, ProPhyt + T-10, Ditera, Mycostop, and Polyversum treatments tended to have root quality ratings similar to those of the plants in the fumigated treatment. Plants treated with Polyversum, Mycostop, and ProPhyt + T-10 had the lowest percent root necrosis next to plants from the fumigated plots, although they were not significantly different than the untreated control.

Table 6. Effects of different biocontrol and fungicide products applied at planting on individual plant parameters of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006.[y]

Treatment	Fresh foliage wt. (g)	Fresh root wt. (g)	Dry foliage wt. (g)	Dry root wt. (g)[z]	Total fresh plant wt. (g)	Total dry plant wt. (g)	Root necrosis (%)	Root quality rating (1-5)[x]
Fumigated	132.24 a	74.68 a	38.23 a	21.13 a	206.91 a	59.35 a	13.75 NS	4.63 a
Abound	46.79 b	31.31 bc	13.59 bc	8.41 bc	78.10 bc	22.00 bcd	45.00	3.50 abcd
Actinovate	40.54 b	30.68 bc	11.70 bc	7.34 bc	71.21 bc	19.04 cd	65.00	2.75 cd
C/G	61.63 b	42.53 bc	19.63 bc	10.76 bc	104.15 bc	30.39 bcd	65.63	3.38 abcd
Ditera	56.24 b	28.28 bc	16.05 bc	7.41 bc	84.51 bc	23.46 bcd	49.38	3.75 abc
Endorse	46.53 b	37.81 bc	13.21 bc	9.34 bc	84.34 bc	22.55 bcd	58.13	3.13 bcd
Mycostop	61.74 b	41.33 bc	17.98 bc	10.68 bc	103.06 bc	28.65 bcd	31.00	3.63 abcd
Plantshield	34.88 b	20.75 c	10.49 c	5.28 c	55.63 c	15.76 d	72.50	2.38 d
Polyversum	57.06 b	39.14 bc	14.39 bc	9.90 bc	96.20 bc	24.29 bcd	30.00	3.88 abc
ProPhyt	52.23 b	29.08 bc	16.09 bc	7.36 bc	81.30 bc	23.45 bcd	78.13	2.63 cd
ProPhyt + Abound	64.99 b	44.43 b	17.53 bc	11.31 ab	109.41 bc	28.84 bcd	46.25	4.25 ab
ProPhyt + T-10	53.48 b	45.09 b	16.45 bc	12.73 ab	98.56 bc	29.18 bcd	38.13	4.13 ab
Ridomil Gold EC	69.63 b	41.54 bc	20.41 bc	16.76 ab	111.16 bc	37.18 b	51.88	3.38 abcd
T-10	51.20 b	34.14 bc	14.70 bc	9.23 bc	85.34 bc	23.93 bcd	42.50	3.38 abcd
Untreated	74.56 b	43.93 b	22.73 b	11.45 ab	118.49 b	34.18 bc	38.75	4.13 ab

ANOVA

Effects	Df	Significance (p)							
Treatment	14	**0.0154**	**0.0118**	**0.0138**	**0.0444**	**0.0122**	**0.0070**	0.0507	**0.0446**
Block	3	0.8246	0.7448	0.9080	0.4707	0.7944	0.7192	0.4825	0.9339
Residual	42								
Total (Corr.)	59								

[x] Scale of 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots.

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p =0.05. Pairwise comparisons performed with n=4. Dry root and total dry weights did not pass variance.

[z] Statistical analysis was performed after log(x) transformation.

In 2007 the effect of treatment was not significant for fresh foliage, dry foliage, or total dry weights, according to the ANOVA. Although no treatment caused plants to significantly differ from the untreated control plant weights, Ditera, ProPhyt + Abound, ProPhyt + T-10, and T-10 had average weights that approached the same fresh and dry foliar weights as the fumigated treatment (table 7). The plants within the two controls did differ from one another in fresh root, dry root, and total fresh weights, and root necrosis. Although plants within the treatments Actinovate, Mycostop, ProPhyt + T-10, and T-10 alone approached the same average necrosis as plants within the fumigated control, they did not differ from the untreated. Actinovate and T-10 having plants with better root quality than the untreated plants.

Table 7. Effects of different biocontrol and fungicide products applied at planting on individual plant parameters of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2007.[y]

Treatment	Fresh foliage wt. (g)[z]	Fresh root wt. (g)	Dry foliage wt. (g)[z]	Dry root wt. (g)	Total fresh plant wt. (g)	Total dry wt. (g)[z]	Root necrosis (%)	Root quality rating (1-5)[x]
Fumigated	52.55 NS	74.18 a	15.73 NS	26.20 a	126.73 a	41.93 NS	18.75 a	4.75 a
Abound	33.64	42.83 bc	9.86	15.54 bc	76.46 bcd	25.40	42.50 bcde	3.25 bcde
Actinovate	26.35	45.03 bc	7.98	15.85 bc	71.38 bcd	23.83	29.38 ab	4.25 ab
C/G	31.13	34.33 c	8.96	12.84 c	65.46 bcd	21.81	53.13 e	2.50 e
Ditera	33.01	42.12 bc	10.06	15.09 bc	75.13 bcd	25.15	51.88 e	3.88 abcd
Endorse	19.42	36.46 bc	5.66	13.07 c	55.88 d	18.73	48.75 cde	3.00 cde
Mycostop	42.89	49.53 bc	12.65	17.53 bc	92.42 abc	30.18	32.50 abc	3.88 abcd
Plantshield	34.12	42.10 bc	9.58	14.68 bc	76.22 bcd	24.25	37.50 bcde	4.00 abc
Polyversum	24.21	35.78 bc	7.51	13.38 bc	59.99 cd	20.89	50.00 cde	3.13 cde
ProPhyt	27.83	39.76 bc	8.13	13.65 bc	67.59 bcd	21.77	36.88 bcde	2.88 de
ProPhyt + Abound	36.97	46.52 bc	10.71	16.49 bc	83.49 bcd	27.19	40.63 bcde	3.25 bcde
ProPhyt + T-10	34.24	45.81 bc	10.29	16.67 bc	80.05 bcd	26.96	33.75 abcd	3.50 bcde
Ridomil Gold EC	27.87	39.67 bc	8.27	13.87 bc	67.54 bcd	22.14	51.25 de	2.75 e
T-10	46.29	51.55 b	14.88	19.66 b	97.84 ab	34.53	30.00 ab	4.25 ab
Untreated	35.28	46.77 bc	10.91	17.82 bc	82.04 bcd	28.74	36.25 abcde	3.13 cde

ANOVA

Effect	Df	Significance (p)							
Treatment	14	0.2676	**0.0048**	0.2746	**0.0193**	**0.0416**	0.1262	**0.0071**	**0.0021**
Block	3	0.2716	0.2782	0.3114	0.3091	0.4877	0.6194	0.0899	0.2649
Residual	42								
Residual (Corr.)	59								

[x] Scale of 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, and 5= >80% secondary roots intact.

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p =0.05. Pairwise comparisons performed with n=4.

[z] Statistical analysis was performed after log(x) transformation.

In 2006 and 2007, the fumigated treatment had significantly smaller *P. penetrans* populations than the untreated or Ditera-treated plants (table 8). This difference was also evident for total parasitic nematodes present. In 2007 a higher average number of *Criconemella* spp. were observed in the Ditera treatment when compared to the controls. Using the risk rating table for parasitic nematodes on strawberries in Michigan (Appendix C), the field did not exceed a risk rating of two after 2005.

For the root isolations, ProPhyt + Abound had the lowest recovery of *Rhizoctonia* in 2006. With 2007 being a hotter, drier year, recovery overall was lower compared to 2006. *Pythium* was recovered at low rates indicating the primary fungus present in the BRR complex was *Rhizoctonia*. The exact strain of Rhizoctonia was not determined, so the influences of temperature and other strain specific characteristics observed by Martin (2000, 1988) can not be discussed. *Cylindrocarpon* spp. was also recovered (table 9). It is not surprising that *Rhizoctonia* was recovered from plants in the fumigated treatment as the plots were surrounded by non-fumigated buffers, allowing a fast-growing fungus, like *Rhizoctonia*, to quickly re-establish, as has been noted by other authors (Wing *et al.*, 1994). There may have also been pathogens present on the planting stock.

Table 8. Root (1 g) and soil (100 cm^3) nematode samples taken from efficacy trial of different biocontrol and fungicide products applied at planting on individual plant parameters of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2005, 2006, and 2007.[x]

		Pratylenchus penetrans	
Treatment	**2005**	**2006[z]**	**2007**
Fumigated	-	1.8 a	0.0
Untreated	11.5	19.5 b	19.5
Ditera	27.5	12.8 b	12.8

ANOVA

Effects		Significance(p)	
Treatment	0.3805	**0.0227**	0.1171
Block	0.6129	0.5519	0.8872

		Criconemella spp.	
Treatment	**2005**	**2006**	**2007[z]**
Fumigated	-	0.0	0.0 a
Untreated	5.0	18.0	0.5 a
Ditera	8.5	13.8	8.0 b

ANOVA

Effects		Significance (p)	
Treatment	0.5919	0.2708	**0.0003**
Block	0.4594	0.5279	0.1318

		Total Plant Parasitic Nematodes Present[y]	
Treatment	**2005**	**2006[z]**	**2007**
Fumigated	-	1.8 a	0.0
Untreated	19.5	39.0 b	22.0
Ditera	43.0	31.5 b	39.5

ANOVA

Effects	Df	p value	Df	p value	Df	p value
Treatment	1	0.2167	2	**0.0100**	2	0.0615
Block	3	0.4573	3	0.7808	3	0.2672
Residual	3		6		6	
Total (Corr.)	7		11		11	

[w] Appendix C has risk ratings for parasitic nematodes on strawberries in Michigan.

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p =0.05. Pairwise comparisons performed with n=4.

[y] Total nematode averages includes: *Pratylenchus penetrans*, *Trichodorus* spp., *Criconemella* spp., *Meloidogyne* spp., *Longidorus elongatus*, and *Paratylenchus* spp.

[z] Statistical separations were performed on log(x+1) transformation.

Table 9. Frequency of fungal genera isolated from roots in different biocontrol and fungicide products applied at planting to strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2005-2007.

Genera Isolated	**Fumigated**			**Untreated**			**ProPhyt + Abound**		**Abound**		**ProPhyt**		**Actinovate**		**Plantshield**	
	2005	*2006*	*2007*	*2005*	*2006*	*2007*	*2006**	*2007*	*2006*	*2007*	*2006*	*2007*	*2006*	*2007*	*2006**	*2007*
Acremoium spp.	-	-	5.0	-	-	-	-	5.0	-	5.0	1.4	2.5	-	7.5	-	12.5
Alternaria spp.	7.7	-	2.5	23.3	-	2.5	-	2.5	-	-	-	-	-	-	-	-
Botrytis spp.	-	-	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceratosporella spp.	-	-	-	-	-	-	-	-	-	-	-	-	2.0	-	-	-
Cercosporella spp.	-	1.5	-	-	-	-	-	-	1.7	-	-	-	-	-	-	-
Chaetomella spp.	15.4	-	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium spp.	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-
Chalara spp.	-	-	-	-	-	-	-	12.5	-	2.5	-	10.0	-	-	-	2.5
Codinaea spp.	-	1.5	-	-	-	-	3.2	-	8.6	-	5.4	2.5	4.0	-	8.2	-
Cylindrocarpon spp.	-	-	10.0	-	16.7	5.0	25.8	7.5	8.6	2.5	9.5	2.5	6.0	5.0	20.4	-
Cylindrocladium spp.	-	-	-	-	3.3	-	-	-	3.4	-	1.4	-	-	-	-	-
Epicoccum spp.	7.7	-	-	13.3	-	-	-	-	1.7	-	-	-	-	-	-	-
Fusarium spp.	-	4.5	-	10.0	3.3	-	9.7	2.5	5.2	-	14.9	-	22.0	2.5	12.2	-
Gilmaniella spp.	-	-	-	3.3	-	-	-	-	-	-	-	-	-	-	-	-
Hainesia spp.	-	3.0	-	-	-	-	-	-	-	-	-	-	-	-	2.0	-
Penicillium spp.	15.4	1.5	2.5	-	-	-	-	-	-	2.5	-	-	-	-	2.0	-
Pestalotia spp.	-	-	-	-	3.3	-	-	-	-	-	-	-	-	-	-	-
Phoma spp.	-	38.8	12.5	26.7	26.7	30.0	38.7	27.5	37.9	30.0	31.1	20.0	24.0	42.5	18.4	37.5
Pythium spp.	-	3.0	-	3.3	3.3	-	6.5	-	-	-		5.0	-	-	2.0	2.5
Rhizoctonia spp.	-	37.3	20.0	3.3	36.7	27.5	3.2	25.0	17.2	12.5	28.4	2.5	22.0	2.5	24.5	10.0
Stigmella spp.	-	-	-	10.0	-	-	-	-	-	-	-	-	-	-	-	-
Tetracladium spp.	-	-	-	-	-	-	-	-	3.4	-	-	-	-	-	2.0	-
Thielavia spp.	7.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma spp.	30.8	1.5	2.5	-	3.3	-	3.2	-	-	-	-	-	4.0	-	2.0	-
Verticillium spp.	-	-	-	6.7	-	-	-	-	1.7	-	-	-	-	-	-	-
Zalerion spp.	-	-	-	-	-	-	-	-	1.7	-	-	-	-	-	2.0	-
Unidentified	-	-	2.5	-	5.8	-	3.2	5.0	-	5.0	2.8	2.5	4.0	2.5	2.0	5.0
No growth	74.0	16.3	32.5	40.0	62.5	32.5	62.0	20.0	27.5	45.0	7.5	40.0	37.5	30.0	39.2	35.0

* Isolations were done from 79 root pieces instead of 80.

** Total root pieces per treatment for each year were: 2005 (n=50), 2006 (n=80), and 2007 (n=40). Percentage of fungal genera isolated calculated from total fungi isolated.

With initial differences due to chemical toxicity, either due to unsuitable application or the products being detrimental to strawberry plants, it became evident that strawberry plants can compensate for initial damage, if properly grown. Although this evaluation only covered three years, BRR is a progressive disease, with disease symptoms taking varying amount of times to become evident depending on location.

Over the duration of the study, it did not seem that BRR was a significant problem at this location; however, the pathogens implicated in this disease complex were present. While others have had success in inhibiting *Rhizoctonia* blight on tall fescue using biocontrols, the chemicals ProPhyt and Abound showed the most promising trends when compared to the untreated control (Yuen, 1994). The trends observed for ProPhyt and Abound may be the result of these dips controlling any pathogens still on the crown from the nursery. In addition, the ProPhyt may have provided some initial nutritional benefits that assisted in initial establishment. Since this location had low levels of nematodes, and the strawberries were optimally maintained, BRR was not a severe problem. Thus it is evident that carefully cultivated strawberries, even in the presence of pathogens, will be productive, especially if they are established well initially. Developing a threshold for *Rhizoctonia fragariae*, like that which exists for *P. penetrans* (Appendix C), would be useful when developing a recommendation for a specific BRR site.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Anonymous. 1998. United Nations Environmental Programme (UNEP) Methyl Bromide Technical Options Committee (MBTOC). 1998 Assessment of the Alternatives to Methyl Bromide. United Nations Environmental Programme, Nairobi: 374 pp.

Barnett, H. L., and Hunter, B. B. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press. St. Paul, Minnesota.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins Company, Baltimore.

Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. J. of Nematology 3:378-385.

Chet, I., and Henis, Y. 1983. *Trichoderma* as a biocontrol agent against soilborne root pathogens. Pages 110-112 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul. 110-112.

Chet, I., Ordentlich, A., Shapira, R., and Oppenheim, A. 1991. Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria. Pages 229-336 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

D'Ercole, N., Nipoti, P., and Manzali, D. 1989. Research on the root rot complex of strawberry plants. Acta Horticulturae 265:497-501.

Domsch, K. H., Gams, W., and Anderson, T-H. 1980. Compendium of Soil Fungi. Academic Press Inc., New York.

Fravel, D. R. and Keinath, A. P. 1991. Biocontrol of soilborne plant pathogens with fungi. Pages 237-243 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B, Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hancock, J. F., Callow, P. W., Serçe, S., and Schilder, A. C. 2001. Relative performance of strawberry cultivars and native hybrids on fumigated and nonfumigated soil in Michigan. HortScience 36:136-138.

Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, 11:18-31.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Reporter 48:692

Louws, F. J., Driver, J. G., and Leandro, L. 2004. Evaluation of fungicide pre-plant dip and spray applications to manage Phytophthora crown rot on strawberry plugs. American Phytopathological Society. Fungicide and Nematicide Tests, 60:SMF041

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society. St. Paul, MN.

Martin, S. B. 1988. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78:379-384.

Martin, F. N. 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. Phytopathology 90:345-353.

Parke, J. L. 1991. Root colonization by indigenous and introduced microorganisms. Pages 33-42 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Management Network 27 Oct.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Strong, F. C., and Strong, M. C. 1927. Investigations on the black root rot of strawberries. Phytopathology 21:1041-1059.

Watanabe, T., Hashimoto, K., Sato, M. 1977. *Pythium* species associated with strawberry roots in Japan, and their role in the strawberry stunt disease. Phytopathology 67:1324-1332.

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany 52:487-511.

Wing, K. B., Pritts, M. P., and Wilcox, W. F. 1994. Strawberry black root rot: A Review. Advances in Strawberry Research. 13:11-19.

Wing, K. B, Pritts, M. P., and Wilcox, W. F. 1995. Biotic, edaphic, and cultural factors associated with strawberry black root rot in New York. HortScience 30:86-90.

Yuen, G. Y., Craig, M. L., and Giesler, L. J. 1994. Biological control of Rhizoctonia solani on tall fescue using fungal antagonists. Plant Disease 78:118-123.

CHAPTER THREE: GREENHOUSE BIOCONTROL AND REDUCED RISK FUNGICIDE EFFICACY TRIAL

Introduction

Strawberry black root rot (BRR) is a disease complex that is widespread around the world wherever strawberries have been planted for multiple years (Watanabe *et al.*, 1977; D'Ercole *et al.*, 1989). Infected plants produce smaller leaves, fewer main and lateral roots, have slower growth, and reduced runner production (Maas, 1998; Hancock *et al.*, 2001). The disease can be spread via infected nursery stock, movement of infested soil, or infected plant debris (Maas, 1998; Strong and Strong, 1927; Hildebrand, 1934). Also known as strawberry decline, many organisms and abiotic factors have been implicated in the cause of the disease; however, *Rhizoctonia fragariae* Husain & McKeen, *Pythium* spp., and the root lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven are generally considered the primary pathogens (D'Ercole *et al.*, 1989; Wing *et al.*, 1994; Wing *et al.*, 1995; Maas, 1998).

In Michigan 'U-Pick' operations, continual strawberry production or short rotations are common due to the few suitable locations for these enterprises. Continual cropping allows pathogen establishment and build-up to deleterious levels. In this system, fumigation is often employed to help control soil-borne pathogens and weeds before planting. In 1992, the Montreal Protocol established methyl bromide as an ozone-depleting substance and instituted its ban by January 1, 2005 (Anonymous, 1998; Rosskopf *et al.*, 2005).

The primary problem when trying to develop a management strategy for BRR is that the disease expression is variable. *Rhizoctonia fragariae* does not always have to be

present to get BRR symptoms (Wing *et al.*, 1994; Wing *et al.*, 1995). A combination of abiotic and biotic factors may predispose strawberry plants to invasion by perhaps otherwise saprophytic or weakly parasitic fungi that then cause severe damage to the root system (Wing *et al.*, 1994; Wing *et al.*, 1995). With the primary control measure due to be eliminated, it becomes necessary to find alternative reduce-risk chemicals, biological, and cultural alternatives. Applying reduced-risk fungicides and biocontrol products at planting would require the least change in a production scheme that is heavily reliant on pre-plant fumigation.

The mucilage excreted by the roots supports both beneficial and potentially harmful organisms seeking to colonize the growing root. The beneficial microbes are antagonistic to pathogens by competition for food, essential elements, and space (Parke, 1991). These microbes enhance plant growth by suppressing major pathogens, increasing nutrient availability, decreasing toxicity levels around the plant, or a combination of these (Parke, 1991). Some of the microbes parasitize pathogenic fungi by producing chitinases or antibiotics. For instance, *Streptomyces hygroscopicus* var. *geldonus* produces a toxin called geldanomycin which can inhibit *R. solani* (Chet *et al.*, 1991; Fravel and Keinath, 1991). *Trichoderma* is antagonistic through parasitism, and competition for nutrients for germinating sclerotia of *R. solani* and *S. rolfsii* . *Trichoderma harzianum* can use the *R. solani* cell wall as a sole carbon source (Chet and Henis, 1983). D'Ercole *et al.* (1989) found a reduction of post-transplant blight incidence from 24.5% in the control to 11.5% in the treatment with *T. harzianum* as a liquid dip on 'Gorella' strawberries.

These experiments were performed to support field trials (Chapter 2). The greenhouse provided a more controlled environment to evaluate the effects on the two pathogens separately and in a co-inoculation experiment. In addition, experiments were conducted to establish an inoculation protocol that will consistently cause disease (Appendix A and B) in evaluating biological and reduced risk fungicide efficacy against *Rhizoctonia* spp. and *Pythium* spp. as other work on strawberry resulted in disease development, but the amount of inoculum was not as quantitative (Olatinwo and Schilder, 2002).

A product evaluation trial was conducted in 2005 to test fungicides targeting *R. fragariae*. Products were initially chosen for their reduced-risk characteristics and potential efficacy against *R. fragariae* and *Pythium* spp. Some of the products (Ridomil Gold EC, ProPhyt, Abound, Plantshield) were already labeled for use on strawberry. Several of the products are biocontrol products that offer a unique control mechanism, but need further evaluation for efficacy (Actinovate, Mycostop, Plantshield, *Trichoderma rossicum*, Polyversum, Ditera). From the 2005 trial and field experiments (Chapter 2), the five most promising products were chosen for further assessment. These were evaluated in 2006 against *R. fragariae* and *P. ultimum* var. *ultimum*, independently, and co-inoculated. Efficacy was tested against *R. fragariae* again in 2007.

All the products tested in this study were fungicides, except for BioNem and Ditera, which are biological nematicides. Some products, such as Abound, claim to control root rot caused by *Rhizoctonia* spp., but have not been tested in Michigan. Some of the chemicals, like Ridomil Gold EC, are used in strawberry production, but not necessarily in this application method or timing. Other products are not labeled for

strawberries, but claim to control similar diseases on other hosts such as brown patch and large patch caused by *Rhizoctonia* spp. on turf (Endorse). There are also many biological products that make claims of disease control or enhancement of plant growth using *Streptomyces* or *Trichoderma* spp.

Materials and Methods

Plant Material

'Allstar' strawberry plants were obtained from Krohne Plant Farms, Inc. (Hartford, MI). These plants were potted in Baccto High Porosity Professional Planting Mix (Michigan Peat Company, Houston, TX) soil in 15 x 15 cm pots. Daughter plants were propagated in August and September 2005 into 10 x 10 cm black plastic pots filled with 2 NS-grade sand which was autoclaved in a metal bin at 121°C for at least 16 h prior to planting. The plants were kept outside on a bench between greenhouses at Michigan State University. Once established in the sand, they were stored in a coldroom at 4°C with a light bank set for 11-h days until January 24, when they were brought to the greenhouse to acclimate. Plants were selected for uniformity and root health when the experiment was setup.

Due to the possibility of contamination when establishing the daughter plants outside and with overhead hand watering, a modification was made for subsequent experiments. During July through September 2006, the same mother plants from 2005 were kept under a polyethylene tunnel outside and used to propagate daughter plants into 52 x 64 x 6.4-cm aluminum pans filled with 2 NS-grade sand. The sand was autoclaved in the pans for 4 h prior to plant establishment. Drip nozzles were used to prevent splash

contamination from the mother plants. Once the daughter plants were established in the sand, the pans were moved into the greenhouse where they were placed under grow lights set at 12-h day length.

In July of 2007, daughter plants were established from the same mother plants as had been previously used into autoclaved, 2 NS-grade sand that had been placed in pans in the greenhouse. These plants were carefully hand watered to prevent contamination.

In 2006 and 2007, the planting stock and soil were examined for any fungal presence prior to planting.

Pathogen Evaluation in Planting Stock

In 2006, prior to the single pathogen inoculation experiment, ten 6-mm long, root pieces were collected from each of four randomly selected daughter plants. The root pieces were surface disinfested for 2 min in 20% bleach solution, followed by two 1-min rinses in sterile distilled water. The root pieces were dried on sterile paper towels, and placed aseptically on water agar. No fungal growth was observed. This procedure was repeated prior to the co-inoculation experiment, using three randomly selected daughter plants. When fungal growth was observed, fungi were sub-cultured, and identified based on morphology. In September 2007, five daughter plants were assessed using the procedure previously described.

Inoculum Preparation

All cultures used in the experiments were obtained from Dr. Annemiek Schilder's laboratory at Michigan State University, East Lansing, MI. Cultures were

maintained on potato dextrose agar amended with ampicillin (50 μg/ml) (PDAamp) throughout the duration of the experiments. Isolates were from strawberry roots from samples taken in Michigan.

For the experiment in 2005, *R. fragariae* (Rhfr03038) was grown on PDAamp. Three to four plugs were then placed on 240 g of oat bran in a 500-ml beaker with 143 ml of sterile deionized water. Beakers with oat bran were autoclaved for 30 min. After 1 wk of growth at 25°C the bran was stirred with a sterile rod; after the second week the colonized bran was air dried in a laminar flow hood for 2 d. The bran was broken into small pieces (<2.5 cm) prior to air drying to aid in the drying process and to ensure even inoculation. The bran was then stored covered on a laboratory bench overnight.

The same procedure was utilized in October 2006 except that flasks were used instead of beakers. The bran was inoculated using two to three 6-mm mycelial plugs from cultures of either *P. ultimum* var. *ultimum* (Pyth03008) or *R. fragariae* (Rhfr03038) after the bran had cooled. The bran was dried in a laminar flow hood for a total of 26.5 h over a period of 3 d. When the hood was not in operation, the bran was stored, covered, on a laboratory bench. The bran was stirred and broken up the morning of each day the bran was in the hood to help it dry thoroughly. Inoculum for the co-inoculation experiment was started on 8 November 2006. The same isolates and procedure were used as previously described.

In September 2007, only *R. fragariae* (Rhfr03038) was grown on bran as previously described. After one week of growth at 25°C, the bran was stirred with a sterile rod; after the second week the bran was air dried in a laminar flow hood for 36 hr over 4 d. The bran was stored covered on a laboratory bench overnight. The bran was

mixed the morning of each day to aid in drying. The bran was ground with a mini prep plus food processor (Cuisinart, Stamford, CT) before drying. A sieve was used to ensure that the bran was less than 0.6 cm in size. The procedure was as described by Martin (2000) except that the bran was not passed through nested sieves.

Pathogen Evaluation in Soil Prior to Experiment

For the 2006 efficacy trial, the sandy-sandy loam greenhouse soil mix was evaluated and found to contain *Fusarium* spp., P*enicillium* spp., *Periconia* spp., making autoclaving the soil necessary. After autoclaving for 4 hr, 5 g of soil was taken from a single pot in each of the four autoclaved batches of soil, and was prepared by mixing the soil with 125 ml of sterile distilled water in sterile 125-ml flasks for a 1:25 dilution. One milliliter of this dilution was taken to make a 1:500 dilution. Each dilution was plated twice onto water agar amended with ampicillin (50 µg/ml), streptomycin (20 µg/ml), and gentomycin (1 µg/ml) for a total of four plates per batch of autoclaved soil. A sterile bent glass rod was used to spread the soil dilution over the plate evenly, and the plates were evaluated 5 d later. No pathogens were recovered. On 30 November 2006, soil dilution plating was done as before from batches of soil autoclaved for the co-inoculation experiment. One milliliter of a 1:25 dilution was plated onto a water agar plate for each of the three autoclaved soil batches. In 2007, using a 1:25 dilution on PDA and water agar, the autoclaved soil was assessed for fungi as previously described.

Inoculation Procedures

On 2 February 2005, 13 x 13-cm diameter standard clay pots were filled with the greenhouse mixed sandy loam to mimic field conditions such as drainage and conditions conducive to disease development. The soil was autoclaved within the pots for 4 h. The following day the contents of each pot were placed into sterile aluminum pans and incorporated with 0.75% wt/wt inoculum. This mixture was then returned to the pots. No bran and autoclaved bran without *Rhizoctonia* served as controls. Each pot contained approximately 1 kg of oven-dry soil. For treatments to be applied as a drench, it was determined that 250 ml of each treatment would wet the soil, but not cause excess run off out of the bottom of the pot.

On 8 November 2006, clay pots were prepared as previously described for the 2005 trial. Pots were prepared for the co-inoculation experiment 21 d later. The day following autoclaving, the pots were individually inoculated with 1.5% wt/wt for the single pathogen trials (*Rhizoctonia* and *Pythium*). Each organism was inoculated at 0.75% wt/wt per pot for the co-inoculation (total=1.5% wt/wt). The single fungus control treatments within the co-inoculation experiment received 0.75% wt/wt of either *Rhizoctonia* or *Pythium* inoculum. The pots were prepared with their respective pathogens as previously described. Autoclaved bran served as a control. In September 2007, pots were inoculated with 3% wt/wt of non-inoculated bran or bran with *R. fragariae* using the same procedure as previously described (table 10).

Table10. Potted plant experiments conducted, inoculum level used, pathogens tested against, and number of blocks used in greenhouse trials evaluating efficacy of strawberry biocontrol and reduced-risk fungicide applied at planting in East Lansing, MI.

Year of Experiment	Inoculum Level (v/v)	Pathogen Used in Experiment*	Number of Replicates
2005	0.75%	*R. fragariae*	4
2006	1.5%	*R. fragariae*	8
2006	1.5%	*P. ultimum* var. *ultimum*	6
2006	0.75% of each organism	*R. fragariae* and *P. ultimum* var. *ultimum*	7
2007	3.0%	*R. fragariae*	9

*Isolates used were Pyth03008 and Rhfr03038.

Treatments

In 2005, the day after autoclaving, all pots were inoculated and lightly watered to rehydrate the soil as it was not watered prior to autoclaving and became too dry to plant and apply treatments. The treatments were applied at the label rate using the method as described in table 11. There were four replications per treatment.

The plants in the dip treatments and untreated controls were watered after planting with 50 ml of water per pot. Pots were arranged in a randomized complete block design. Each pot was placed in an individual 8-cm deep plastic tray to prevent cross contamination and to prevent contact with the greenhouse bench. All plants were potted and treatments applied the same day, with the exception of the C/G treatments, in which the plants were potted one day after the treatments were applied. The C/G was allowed to maintain contact with the soil and inoculum for 24 h. The C/G treated pots were then drenched with 300 ml of water to wash out the C/G, since previous studies had found it to be phytotoxic (Sabaratnam and Schilder, unpublished data; Chapter 2). Strawberry plants were then planted in the pots when the water had disappeared from the soil surface. The T-10 (*Trichoderma rossicum*) spore suspension was prepared the morning of the application in sterile deionized water, using sporulating cultures that had been growing on PDAamp for 23 d. This culture (Trsp02024), originally isolated from strawberries, is available from Dr. Annemiek Schilder's laboratory, Michigan State University, East Lansing, MI. The spore concentration was selected due to information available on commercial products utilizing *Trichoderma* spp. Plants were hand watered using a plastic beaker as needed. Any flowers or runners that formed were removed.

Table 11. Treatments, manufacturer rates, active ingredient and application method used in a greenhouse trial evaluating the efficacy of biocontrol and reduced-risk fungicide applied at planting to strawberry cv. Allstar for control of black root rot pathogens in East Lansing, MI from 2005.

Product	Label Rate	Active Ingredient	Application Method
Abound	8 fl oz/100 gal	Azoxystrobin	5 min dip
Actinovate	1 tsp/ gal	*Streptomyces lydicus*	250 ml drench
BioNem	135 lbs/acre	*Bacillus firmus*	250 ml drench
C/G	0.2% v/v	Fatty acid	250 ml drench
C/G	0.5% v/v	Fatty acid	250 ml drench
Ditera	2.5 lbs/acre	*Myrothecium verrucaria*	250 ml drench
Endorse	11 lbs/acre	Polyoxin D zinc salt	250 ml drench
Mycostop	2 g/ 100 ft^2	*Streptomyces griseoviridis*	250 ml drench
Plantshield	2.5 lbs/5 gal	*Trichoderma harzianum*	250 ml drench
Polyversum	0.1 g/m^2	*Pythium oligandrum*	250 ml drench
ProPhyt	2 pt/100 gal	Potassium phosphite	30 min dip
ProPhyt + Abound	Rates as above	Potassium phosphite + azoxystrobin	15 min dip
ProPhyt + T-10*	Rates as above	Potassium phosphite + *Trichoderma rossicum*	5 min dip
Ridomil Gold EC	1 pt/acre	Mefenoxam	250 ml drench
T-10*	$1.38x10^7$ spores/ml	*Trichoderma rossicum*	5 min dip

*Dosage determined in laboratory.

Table 12. Treatments, manufacturer rates, and application method, used in a greenhouse trial evaluating biocontrol and reduced-risk fungicide applied at planting in strawberry cv. Allstar inoculated with black root rot pathogens in East Lansing, MI in 2006 and 2007.

Treatment	Label Rate	Application Method
T-10*	1.75×10^7 spores/ml	5 min dip
ProPhyt + Abound	2 pt/100 gal + 8 fl oz/100 gal	15 min dip
Actinovate	1 tsp/ gal	Drench
Plantshield	2.5 lbs/5 gal	Drench
Ditera	2.5 lbs/acre	Drench

*Dosage determined in the lab.

In the 2006 experiments strawberries were planted and treatments applied the day following inoculation with *Rhizoctonia* and *Pythium* on 1 December. Treatments were applied, according to the label, either as a 250-ml drench per pot or as pre-plant root dip (table 12). Plants receiving the fungicide dips were placed into plastic buckets containing 3.79 L of water for the appropriate amount of time. Plants receiving the T-10 dip were placed in a 500-ml beaker containing the spore suspension.

Untreated control plants and those plants receiving dip treatments received an additional 50 ml of water to ensure good establishment. Drenches were applied post-planting. Plants were grown under 400 W grow lights (P.L. Light Systems, Hortilux Shreder Group, The Netherlands) set at 12-h day length and were hand watered using a plastic beaker as needed to prevent splash between treatments. The greenhouse temperature was kept between 21-27°C. Pots were arranged in a randomized complete block design replicated eight times for the *Rhizoctonia* only experiment, six times for the *Pythium* only experiment, and seven times for the combined inoculation experiment (table 11). The co-inoculation experiment pots were arranged in a randomized complete block design with seven replications per treatment. The T-10 suspension of 1.26×10^7 conidia/ml was prepared the morning of application in sterile deionized water when all treatments, except ProPhyt +Abound, were applied. ProPhyt + Abound was not applied until 4 wk after other treatments in the co-inoculation experiment, using fresh inoculum and following the procedures previously described. This delay was due to a mistake during the initial set-up. Each pot was individually placed on a plastic tray to prevent contact with the greenhouse bench. Imidacloprid (Marathon) was applied 11 December

2007 and 8 February 2007 to control fungal gnats. Flowers and runners were removed if they developed.

On 26 September 2007 the trial was repeated. The T-10 suspension $1.77x10^7$ conidia/ml was prepared the morning of the application. Approximately 14 d after planting, replicate 3 of the T-10 treatment was replanted and treated due to initial planting error. Imidacloprid (Marathon) was applied on 17 October. Flower and runners were removed as they developed. All treatments were applied as previously described (table 12). Pots were placed in a randomized complete block design upon inverted 8-cm deep plastic trays. Each treatment was replicated 9 times.

Evaluation Procedure

In 2005, all plants were evaluated 6 weeks after planting. Percent root necrosis was visually estimated and root quality rated using scale of 1-5 (1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots.) (Appendix A, Figure 1). Fresh weight of the foliage and roots were obtained separately by splitting the crown horizontally just above the uppermost adventitious roots. Plant parts were then dried at 80°C for 48 h and weighed. Total fresh and dry weights were obtained by adding the foliage and root weights together. For fungal isolations, fresh roots were then taken from all plants and kept in a separate Petri plate containing a piece of moist Watman filter paper for each treatment/inoculation combination. The root pieces were stored at 4°C until processing the next day. Soil was reserved from each pot

in each treatment and kept at 4°C for evaluation of fungal contamination using Czapek's media.

The 2006 experiments were evaluated 68 d after planting. Plants were washed under running water and then placed in trays with water to keep roots moist to evaluate root quality as previously described. Percent root necrosis was also visually estimated. The biomass measurements were obtained as previously described. One plant of each treatment was taken for root plating on water agar to assess pathogen recovery rate. Soil was taken randomly from different pots to enumerate colony forming units (CFUs) of *Rhizoctonia*. Roots and soil were stored at 4°C until processing.

In December 2007, 72 d after planting, plants were processed as previously described. For each treatment, the roots of the eighth replicate were set aside for root isolations after taking the fresh weight. These were plated the next day. CFUs per unit soil were not determined in 2007.

Pathogen Recovery

The day following fresh biomass assessment, in 2005, 10 root pieces with lesions per treatment were selected and plated on water agar (five 6-mm pieces per plate). Each piece was taken from a different lesion margin and surface sterilized in a 20% bleach solution for 2 min, rinsed in sterile water twice for 1 min, and dried on sterile paper towels. Hyphae from the root pieces were subcultured onto PDA and identified based on morphological characteristics after plate colonization. The frequency of recovery was calculated by dividing the number of root pieces that yielded *Rhizoctonia* sp. by the total number of fungi that grew from all root pieces.

The same day, 0.5 g of soil was taken from the soil from each treatment. The soil was dried for 72 h in a fume hood, diluted in 100 ml sterile water in sterile flasks, and then spread on Czapek's media plates with a sterile bent glass rod. This procedure served to evaluate the extent of any possible fungal contamination.

In 2006, at the time of fresh weight measurements, roots from the last replication of each treatment in the single pathogen trials were set aside. Roots from the first replication of each treatment in the co-inoculation experiment were set aside, after taking the fresh weight. Two or three days later, five 6-mm root pieces with lesions were placed on each of two water agar plates, for a total of 10 root pieces per plant, using the technique described previously. The roots in the ProPhyt +Abound treatment were evaluated in the same manner after the same number of days as the other treatments. Fungi growing from the root pieces were subcultured onto PDA identified based on morphological characteristics (Barnett and Hunter, 1998; Domsch *et al.*, 1980; Barron, 1968). Frequency of recovery of *Rhizoctonia* was calculated as previously defined. The same isolation procedure as previously described was used in 2007 using roots from the plants in the eighth replicate of each treatment.

Recovery of Rhizoctonia from Soil

For the 2006 greenhouse experiments, 30-g soil samples were taken from random pots within the inoculated and non-inoculated control treatments within the *Rhizoctonia*-only evaluation trial. The non-inoculated control, *Rhizoctonia*-only control, and the *Rhizoctonia* + *Pythium* control were evaluated from the co-inoculation experiment. The soil was plated 2 d after experiment evaluation using a pellet soil sampler described by

Henis *et al.* (1978) after spreading the soil in a sterile Petri dish bottom. The soil was plated onto semi-selective media for *Rhizoctonia* spp., with three plates per treatment for a total of 45 pellets (Gutierrez *et al.*, 1997). Four sets of pellets were taken, with one set randomly put aside to obtain the weight for calculation of CFUs per gram of soil. Plates were evaluated once mycelium became evident to the unaided eye. CFUs were calculated by taking the average weight of pellets set aside for all treatments evaluated, and then multiplying by the counts for each treatment.

Data Analysis

For each year that entire root systems were sacrificed for fungal isolations, the replicate utilized was removed from the analysis for both dry root weight and total dry weight data.

All data were analyzed, after performing a variance check, using ANOVA and means separated by Fisher's protected least significant difference test (p=0.05) in StatGraphics Plus 4.1 (StatPoint Inc., VA). In the interaction analysis, the following effects were analyzed: treatment (T), inoculation (I), Block (B), I x T, and error.

Results and Discussion

2005

Based on the inoculation procedure evaluation (Appendix A) the 0.75% bran inoculum rate was chosen. The soil was autoclaved within the clay pots to ensure that both the soil and the pots were pathogen free and to allow more control over the process than was obtained in an earlier trial (Appendix A).

In 2006, the fungi found (*Epicoccum* sp. and *Periconia* sp.) were isolated from the roots of the strawberries sampled prior to the setup of the experiment. In 2007 the fungi found (*Alternaria* sp., *Cladosporium* sp., *Phoma* sp. and yeast), all are considered saprophytic. When assessing the soil prior to experiment setup in 2006, no growth was observed on any of the plates 5 d later. In 2007 a *Penicillium* sp. and a Zygomycete were found, but these were thought to be contaminants on the plates while they were on the bench in the laboratory.

Inoculated control weight averages for each variable tended to be lower than the non-inoculated control averages. Plants treated with ProPhyt + Abound had significantly higher average weights and better root quality than the control plants (table 13). C/G 0.5% and Polyversum may have damaged the plants and allowed *Rhizoctonia* sp. to cause more necrosis in inoculated pots, since these two treatments consistently produced the lowest means in all variables. A combination of ProPhyt and Abound resulted in plants with greater fresh total weight than the untreated control.

Although Ridomil Gold EC is labeled for control of oomycetes in strawberries, this product is usually applied to older plants, so the young transplants may have been adversely affected. In addition, the method used may have resulted in a higher rate than is recommended on strawberry. Also, *Rhizoctonia* is a basidiomycete, so control was not expected. Control was also not expected from the two biocontrol nematicides, BioNem and Ditera. Plants treated with Ditera showed promising growth, however, it did not differ from the control for any parameter (table 13).

Table13. Effects of different biocontrol and fungicide products on plant biomass and root health of strawberry cv. Allstar in a greenhouse study in soil with and without inoculation with *Rhizoctonia fragariae* in East Lansing, MI, in 2005.[v]

[v] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons of treatments performed with n=8. For inoculum presence, n=64.
[w] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).
[x] Statistical analysis performed after log(x+1) transformation.
[y] Statistical analysis performed after sqrt(x) transformation.
[z] Statistical analysis performed after sqrt(x+1) transformation.

Treatment	Fresh foliage weight (g)[z]	Fresh root weight (g)[y]	Dry foliage weight (g)	Dry root weight (g)[x]	Fresh whole plant weight (g)[z]	Dry whole plant weight (g)[z]	Root quality rating (1-5)[w]	Root necrosis (%)[y]
Control	3.71 bcd	2.18 de	1.16 bcd	0.46 cdef	5.89 cde	1.63 bcde	2.25 efgh	21.50 abc
Abound	3.69 bcd	3.26 bcde	1.10 bcd	0.76 bcd	6.95 bcde	1.86 bcd	2.38 defg	36.13 bcde
Actinovate	3.03 cde	3.31 bcde	0.90 cd	0.54 cde	6.34 cde	1.44 cde	3.63 bc	7.25 a
BioNem	3.55 cde	3.98 bcde	1.01 cd	0.63 cde	7.53 bcde	1.64 cde	3.00 bcde	24.38 abc
C/G 0.2%	3.03 cde	1.85 ef	0.94 cd	0.49 cdef	4.88 def	1.43 cde	2.00 fghi	58.75 ef
C/G 0.5%	2.18 ef	1.23 fg	0.70 cde	0.29 ef	3.40 fg	0.99 ef	1.75 ghi	68.75 f
Ditera	3.03 cde	3.21 bcde	1.01 cd	0.63 bcd	6.24 cde	1.64 bcde	2.75 def	31.50 abcd
Endorse	5.59 ab	5.61 b	1.60 ab	1.04 b	11.20 b	2.64 b	3.75 ab	20.38 abc
Mycostop	4.00 bcd	4.46 bcd	1.16 bcd	0.79 bcd	8.46 bcd	1.95 bcd	2.63 def	16.63 abc
Plantshield	3.86 bcd	4.34 bc	1.19 bcd	0.78 bcd	8.20 bcd	1.96 bcd	3.13 bcd	26.25 abc
Polyversum	1.09 f	1.00 g	0.35 e	0.20 f	2.09 g	0.55 f	1.38 i	75.88 f
ProPhyt	4.24 bcd	3.66 cde	1.23 bcd	0.75 bcd	7.90 bcde	1.98 bcd	2.88 cde	53.75 def
ProPhyt + Abound	7.59 a	8.84 a	2.09 a	1.78 a	16.43 a	3.86 a	4.50 a	4.75 ab
Prophyt + T-10	3.03 cde	2.60 cde	1.04 cd	0.66 bcd	5.63 cdef	1.70 bcde	1.50 hi	50.63 cdef
Ridomil Gold EC	2.35 def	2.05 ef	0.69 de	0.44 def	4.40 efg	1.13 def	2.38 defg	27.63 abc
T-10	4.64 bc	3.76 bcde	1.24 bc	0.93 bc	8.40 bc	2.16 bc	3.75 ab	7.50 a
Inoculum Presence								
Inoculated	3.00 a	3.02 a	0.89 a	0.61 a	6.02 a	1.50 a	2.31 a	45.88 a
Non-inoculated	4.32 b	3.90 b	1.29 b	0.78 b	8.22 b	2.07 b	3.14 b	20.57 b

ANOVA

Effects	Df	Significance (p)							
Treatment (T)	15	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Inoculation (I)	1	**0.0001**	**0.0017**	**0.0001**	**0.0188**	**0.0003**	**0.0006**	**0.0000**	**0.0001**
Block	3	0.6822	0.3368	0.8293	0.6136	0.5761	0.7623	0.1540	0.1342
TxI	15	0.0709	0.0673	0.1636	0.5338	0.0799	0.2982	**0.0087**	0.1912
Residual	93								
Total (Corr.)	127								

Pathogen Recovery

Rhizoctonia was recovered from all treatments applied to inoculated soil, but not from the non-inoculated control. The lowest frequency was found in C/G 0.2% and Abound, at 55.6% and 80%, respectively. In addition to the inoculated pots, *Rhizoctonia* was also isolated from the non-inoculated Abound, C/G 0.2%, C/G 0.5%, Ditera, ProPhyt+T-10, and Plantshield treatments, indicating there was some of contamination. A zygomycete, *Penicillium* spp., and *Fusarium* spp. were found in all treatments, using Czapek's media, except C/G 0.2%, C/G 0.2% inoculated with *Rhizoctonia* sp., and the control treatment without bran.

Fungal gnats may explain some contamination that became evident after plating the root lesions. There was also some doubt as to the success that *Rhizoctonia* sp. was kept out of the transplants in 2005. This problem was remedied the following year. The contamination by a zygomycete, *Penicillium* spp., and *Fusarium* spp. may be a result of contaminated bran used in the controls since there was some problem with contamination in the laboratory. Although the contaminated bran was disposed of, the contamination may only have become evident after planting due to the moisture from watering. However, they may have come from outside sources once the plants were in the greenhouse.

The fungicide combination of ProPhyt + Abound seems to offer a measure of control. The lower rate of recovery of *Rhizoctonia* sp. from root lesions in the Abound-treated inoculated pots indicates that Abound may prevent root infection by *Rhizoctonia.* ProPhyt may be providing extra potassium to the plant boosting the plants ability to cope with disease and possibly by inducing plant defenses. This is made more likely since

ProPhyt is labeled for control of *Phytophthora*, an oomycete. ProPhyt + Abound, T-10, Actinovate, Ditera, and Plantshield were chosen for further evaluation due to the tendency of producing higher average weights and higher root quality and performance in the field (Chapter 2). Actinovate was also chosen due to its selection for evaluation in the Integrated Management Trial (Chapter 4) and low root necrosis.

It was decided that the experiment should be able to produce reproducible results in approximately two months time. To achieve a definitive assessment of these products, it was also necessary to increase the number of replications.

2006

The check for pathogens in the planting stock and the assessment of the autoclaved soil yielded no pathogenic fungi. The condition of plants (disease free) and methods (sterile soil) was important for the accurate assessment of pathogen and treatment effects. The incorporation of inoculum simulated infested field conditions with *Rhizoctonia*, *Pythium*, and a co-inoculation of the two, something a grower may face after continuous strawberries in the perennial systems used in Michigan.

Rhizoctonia

For the *Rhizoctonia* inoculation, results of the ANOVA showed that dry root weight differences were not significant amongst treatments. As seen in table 14, ProPhyt + Abound and T-10 treated plants had the the highest average weights for all response variables.

All treatments applied to inoculated pots yielded 100% recovery of *Rhizoctonia*. *Rhizoctonia* was also isolated from the Ditera, Actinovate, and Plantshield untreated control treatments, although at lower frequencies (table 15). *Pythium* spp. were isolated from the Ditera control, and *Cylindrocarpon* spp. were isolated from the Actinovate control. Other fungi include *Alternaria* spp., *Myrothecium* spp., and *Phoma* spp. The soil evaluation demonstrated that *Rhizoctonia* was establishing. No fungi were evident from the non-inoculated controls. The *Rhizoctonia*-inoculated control had 17.55 CFU/g of *Rhizoctonia*. For the co-inoculation experiment the combined inoculation control had 16.62 CFU/g *Rhizoctonia*, the *Rhizoctonia* only control had 16.62 CFU/g *Rhizoctonia*, and the untreated bran control had 0.37 CFU/g *Rhizoctonia*.

There were problems with fungal gnats which may supply a reason for the recovery of *Rhizoctonia* sp. from non-inoculated pots.

Table 14. Effects of different biocontrol and fungicide products on plant biomass and root health of strawberry cv. Allstar in a greenhouse study in soil inoculated with and without *Rhizoctonia fragariae* in East Lansing, MI, in 2006.[x]

Treatment	Fresh foliage weight (g)[y]	Fresh root weight (g)[z]	Dry foliage weight (g)	Dry root weight (g)[z]	Total fresh weight (g)[z]	Total dry weight (g)	Root quality rating (1-5)[w]	Root necrosis (%)
Control	1.90 bc	1.05 ab	1.19 c	0.73 NS	2.95 bc	1.97 bc	2.69 ab	45.94 bc
	1.03			0.56		1.45 c	1.38	
Actinovate	d	0.64 c	0.86 c		1.66 d		d	84.38 a
Ditera	1.87 bc	0.99 ab	1.32 bc	0.75	2.86 bc	2.06 abc	2.31 bc	65.31 abc
Plantshield	1.61 c	0.86 bc	1.25 bc	0.67	2.46 c	1.91 bc	1.75 cd	63.38 abc
ProPhyt+ Abound	2.75 a	1.35 a	1.94 a	0.98	4.10 a	2.80 a	3.25 a	39.19 c
T-10	2.71 ab	1.48 a	1.68 a	0.96	4.19 ab	2.65 ab	2.06 bcd	71.88 ab
Inoculum Presence								
Inoculated	2.05 NS	0.93 a	1.38 NS	0.83 NS	3.24 NS	2.15 NS	2.69 a	51.98 a
Uninoculated	1.90	1.19 b	1.37	0.72	2.83	2.12	1.79 b	71.38 b

ANOVA

Effects	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value
Treatment (T)	5	**0.0001**	5	**0.0006**	5	**0.0004**	5	0.1329	5	**0.0001**	5	**0.0113**	5	**0.0009**	5	**0.0122**
Inoculation (I)	1	0.6425	1	**0.0159**	1	0.9515	1	0.1932	1	0.3354	1	0.8760	1	**0.0007**	1	**0.0135**
Block	7	0.1549	7	0.0967	7	0.3109	6	0.1745	7	0.1844	6	0.1705	7	0.3564	7	0.2763
TxI	5	0.6520	5	0.8515	5	0.2067	5	0.9409	5	0.8667	5	0.6676	5	**0.0299**	5	0.2588
Residual	77		77		77		66		77		66		77		77	
Total (Corr.)	95		95		95		83		95		83		95		95	

[w] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Treatment pairwise comparisons performed with n=16. For inoculum presence, n=48. Dry root weight and total dry plant weight, n=15 and inoculum presence, n=42.

[y] Statistical analysis was performed after sqrt(x) transformation.

[z] Statistical analysis was performed after log(x) transformation.

Table 15. Frequency of fungi isolated from roots of strawberry cv. Allstar treated with biocontrol and fungicide products applied to the in a greenhouse study in soil inoculated with and without *Rhizoctonia fragariae* in East Lansing, MI, in 2006.

			Frequency of Isolation					
Treatment	**Inoculation**	**Total Isolations**	***Rhizoctonia* (%)**	***Pythium* (%)**	***Fusarium* (%)**	***Trichoderma* (%)**	***Cylindrocarpon* (%)**	**Non-pathogens (%)***
Uninoculated control	-	9	0	0	22.2	0	0	77.8
Inoculated control	*Rhizoctonia*	9	100	0	0	0	0	0
Actinovate	-	10	30	0	40	0	20	10
Actinovate	*Rhizoctonia*	8	100	0	0	0	0	0
Ditera	-	6	33.3	50	0	0	0	16.7
Ditera	*Rhizoctonia*	8	100	0	0	0	0	0
Plantshield	-	15	26.7	0	66.7	6.7	0	0
Plantshield	*Rhizoctonia*	10	100	0	0	0	0	0
ProPhyt + Abound	-	6	0	0	83.3	16.7	0	0
ProPhyt + Abound	*Rhizoctonia*	6	100	0	0	0	0	0
T-10	-	9	0	0	22.2	55.6	0	22.2
T-10	*Rhizoctonia*	10	100	0	0	0	0	0

* Non-pathogenic fungi recovered included *Alternaria* sp., *Myrothecium* sp., *and Phoma* sp.

** Percentage fungi calculated from total fungi isolated.

Pythium

For the *Pythium* inoculation, ANOVA results showed that no treatment had a significant effect on any of the variables, but plants in pots inoculated with *Pythium* had significantly lower foliage weights and more necrosis (table 16). For fresh foliage weight, the plants treated with T-10 had the highest average fresh foliar weight, followed by ProPhyt +Abound. Plantshield treated plants tended to have higher average dry root and total dry plant weight than those treated with ProPhyt + Abound.

Pythium was only recovered from the Ditera control plants and Actinovate applied to *Pythium*-inoculated soil. *Rhizoctonia* sp. was isolated from Ditera, Actinovate, Plantshield control plants, and Actinovate-treated plants in *Pythium* inoculated pots. *Trichoderma* spp. were recovered from ProPhyt + Abound, Plantshield, and T-10 controls, but only from the T-10 applied to inoculated soil were *Trichoderma* spp. recovered (table 17).

Table 16. Effects of different biocontrol and fungicide products on plant biomass and root health of strawberry cv. Allstar in a greenhouse study in soil inoculated with and without *Pythium ultimum* var. *ultimum* in East Lansing, MI, in 2006.[y]

Treatment	Fresh foliage weight[z] (g)	Fresh root weight (g)[z]	Dry foliage weight (g)[z]	Dry root weight (g)[z]	Total fresh weight (g)[z]	Total dry weight (g)[z]	Root quality Rating (1-5)[x]	Root necrosis (%)
Control	1.48 NS	0.89 NS	1.05 NS	0.66 NS	2.38 NS	1.71 NS	2.08 NS	71.25 NS
Actinovate	1.01	0.67	0.85	0.55	1.68	1.37	1.33	82.92
Ditera	1.28	0.81	0.93	0.59	2.08	1.53	1.33	83.33
Plantshield	1.58	0.95	1.18	0.71	2.53	1.89	1.50	69.50
Prophyt + Abound	1.81	1.14	1.28	0.69	2.95	1.86	1.67	78.33
T-10	2.17	1.06	1.43	0.74	3.23	2.23	1.67	81.25
Inoculum Presence								
Inoculated	1.19 a	0.88 NS	0.89 a	0.64 NS	2.06 NS	1.57 NS	1.33 a	94.33 a
Uninoculated	1.92 b	0.96	1.35 b	0.67	2.88	1.96	1.86 b	61.19 b

ANOVA

Effects	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value
Treatment (T)	5	0.5622	5	0.6140	5	0.6611	5	0.9762	5	0.5120	5	0.8709	5	0.4679	5	0.8521
Inoculation (I)	1	**0.0380**	1	0.3792	1	**0.0312**	1	0.7448	1	0.0523	1	0.2240	1	**0.0305**	1	**0.0001**
Block	5	0.6537	5	0.5716	5	0.8268	4	0.7138	5	0.5701	4	0.8058	5	0.4093	5	0.2069
TxI	5	0.2838	5	0.1102	5	0.3983	5	0.4021	5	0.2422	5	0.4373	5	0.7951	5	0.7714
Residual	55		55		55		44		55		44		55		55	
Total (Corr.)	71		71		71		59		71		59		71		71	

[x] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Treatment pairwise comparisons performed with n=12 and inoculum presence, n=36, except for dry root and total dry weight, n=10, inoculum presence, n=30.

[z] Statistical analysis was performed after log(x) transformation.

Table 17. Frequency of fungi isolated from roots of strawberry cv. Allstar treated with biocontrol and fungicide products applied to the in a greenhouse study in soil inoculated with and without *Pythium ultimum* var. *ultimum* in East Lansing, MI, in 2006.

			Frequency of Isolation				
Treatment	**Inoculation**	**Total Isolations**	***Rhizoctonia* (%)**	***Pythium* (%)**	***Fusarium* (%)**	***Trichoderma* (%)**	**Non-pathogens (%)***
Non-inoculated control	-	9	0.0	0.0	22.2	0.0	77.8
Inoculated control	*Pythium*	3	0.0	0.0	33.3	0.0	66.7
ProPhyt + Abound	-	6	0.0	0.0	83.3	16.7	0.0
ProPhyt + Abound	*Pythium*	4	0.0	0.0	75.0	0.0	25.0
Ditera	-	6	33.3	50.0	0.0	0.0	16.7
Ditera	*Pythium*	7	0.0	0.0	100.0	0.0	0.0
Actinovate	-	10	30.0	0.0	40.0	0.0	30.0
Actinovate	*Pythium*	17	29.4	11.8	29.4	0.0	29.4
Plantshield	-	15	26.7	0.0	66.7	6.7	0.0
Plantshield	*Pythium*	9	0.0	0.0	55.6	0.0	44.4
T-10	-	9	0.0	0.0	22.2	55.6	22.2
T-10	*Pythium*	9	0.0	0.0	0.0	22.2	77.8

* Non-pathogenic fungi recovered included *Alternaria* sp., *Myrothecium* sp., *and Phoma* sp.

** Percentage fungi calculated from total fungi isolated.

Co-inoculation

In the co-inoculation study, the results of ANOVA showed that treatment did not significantly explain differences for any parameter except fresh root weights and percent root necrosis. ProPhyt + Abound treated plants had the highest fresh root weight, but did not differ significantly from the control (table 18). Inoculation with the pathogens did significantly reduce fresh and dry foliage, total plant fresh and dry weights, and root quality.

Fusarium spp. were a common contaminant when isolations were conducted after the experiment. *Pythium* sp. was not recovered from any treatment. *Rhizoctonia* sp. was recovered from every treatment except the ProPhyt + Abound, Ditera, and T-10 treatments applied to non-inoculated soil (table 19). *Trichoderma* sp. was recovered from ProPhyt + Abound and T-10 treatment controls. *Rhizoctonia* colony forming units were obtained only from inoculated and non-inoculated pots that served as controls. Both the co-inoculation and *Rhizoctonia* only control had 13.53 CFUs of *Rhizoctonia*/g of soil while the un-inoculated control had 0.9 CFUs/g of soil.

Overall, plants in inoculated pots were smaller with poorer root quality than those in the non-inoculated pots. The finding that the non-inoculated control had 0.9 CFUs of *Rhizoctonia*/g of soil indicates cross contamination by *Rhizoctonia* sp. among the treatments, especially in the co-inoculation experiment, but since all three of the experiments in 2006 overlapped for several weeks, this potential source of error may have been present for the other evaluations as well.

Table 18. Effects of different biocontrol and fungicide products on plant biomass and root health of strawberry cv. Allstar in a greenhouse study in soil inoculated with and without *Pythium ultimum* var. *ultimum* + *Rhizoctonia fragariae* in East Lansing, MI, in 2006.[y]

Treatment	Fresh foliage weight (g)	Fresh root weight (g)	Dry foliage weight (g)[z]	Dry root weight (g)	Total fresh weight (g)	Total dry weight (g)	Root quality Rating (1-5)[x]	Root necrosis (%)
Control	3.44 NS	2.93 ab	1.28 NS	1.06 NS	6.37 NS	2.42 NS	3.36 NS	29.29 c
Actinovate	2.85	2.41 bc	1.29	1.05	5.26	2.38	2.64	56.43 ab
Ditera	2.98	2.01 c	1.29	0.71	4.99	1.88	2.57	47.50 abc
Plantshield	3.44	2.16 bc	1.48	0.92	5.60	2.43	3.64	35.36 bc
Prophyt + Abound	2.66	3.43 a	0.89	0.87	6.09	1.80	3.21	26.43 c
T-10	2.66	2.74 abc	1.21	1.10	5.41	2.25	2.93	63.57 a
Inoculum Presence								
Inoculated	2.47 a	2.52 NS	0.99 a	0.93 NS	4.99 a	1.95 a	2.74 a	42.38 NS
Uninoculated	3.55 b	2.70	1.49 b	0.97	6.25 b	2.44 b	3.38 b	43.81

ANOVA

Effects	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value
Treatment (T)	5	0.6076	5	**0.0148**	5	0.1145	5	0.0937	5	0.7288	5	0.2094	5	0.1417	5	**0.0115**
Inoculation (I)	1	**0.0025**	1	0.4696	1	**0.0001**	1	0.6998	1	**0.0295**	1	**0.0124**	1	**0.0165**	1	0.8361
Block	6	0.9991	6	0.9506	6	0.9638	5	0.9488	6	0.9992	5	0.9886	6	0.6568	6	0.5298
TxI	5	0.0571	5	0.1261	5	**0.0351**	5	0.0959	5	0.0688	5	0.0672	5	0.8237	5	0.1278
Residual	66		66		66		55		66		55		66		66	
Total (Corr.)	83		83		83		71		83		71		83		83	

[x] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Treatment pairwise comparisons performed with n=14 and inoculum presence, n=42, except for dry root and total dry weight, n=12 and inoculum presence, n=36.

[z] Statistical separations were performed on log(x+1) transformation.

Table 19. Frequency of fungi isolated from roots of strawberry cv. Allstar treated with biocontrol and fungicide products applied to the in a greenhouse study in soil inoculated with and without *Pythium ultimum* var. *ultimum* + *Rhizoctonia fragariae* in East Lansing, MI, in 2006.

			Frequency of Isolation				
Treatment	**Inoculum**	**Total Isolations**	***Rhizoctonia* (%)**	***Pythium* (%)**	***Fusarium* (%)**	***Trichoderma* (%)**	**Non-pathogens (%)***
Un-inoculated	-	11	36.4	0.0	27.3	0.0	36.4
Inoculated	*Pythum+Rhizoctonia*	9	88.9	0.0	0.0	0.0	11.1
Inoculated	*Pythium*	6	66.7	0.0	0.0	0.0	33.3
Inoculated	*Rhizoctonia*	8	100.0	0.0	0.0	0.0	0.0
ProPhyt+Abound	-	8	0.0	0.0	62.5	37.5	0.0
ProPhyt+Abound	*Pythum+Rhizoctonia*	8	87.5	0.0	12.5	0.0	0.0
Ditera	-	7	0.0	0.0	57.1	0.0	42.9
Ditera	*Pythum+Rhizoctonia*	4	100.0	0.0	0.0	0.0	0.0
Actinovate	-	7	12.0	50.0	0.0	0.0	0.0
Actinovate	*Pythum+Rhizoctonia*	10	100.0	0.0	0.0	0.0	0.0
Plantshield	-	9	14.0	50.0	0.0	0.0	0.0
Plantshield	*Pythum+Rhizoctonia*	6	100.0	0.0	0.0	0.0	0.0
T-10	-	9	0.0	0.0	0.0	88.9	11.1
T-10	*Pythum+Rhizoctonia*	7	100.0	0.0	0.0	0.0	0.0

* Other fungi isolated included *Phoma* sp., *Alternaria* sp., *Phialophora* sp., *Myrothecium* sp., *Epicoccum* sp., and a zygomycete.

** Percentage fungi calculated from total fungi isolated.

All three experiments were conducted in the same greenhouse at overlapping times, so fungal gnats could have contributed to cross contamination amongst all treatments. Although fungal gnat control was implemented, the gnats were not preemptively controlled. The possibility of the planting stock harboring pathogens before planting seems unlikely as the plants were evaluated prior to the start of the experiments and did not show evidence of infection. A significant problem in 2006 was lack of proper care for the plants, particularly in the co-inoculation experiment. The plants probably went through a severe wet and dry cycle due to improper watering. However, this source of error was consistent amongst all treatments, therefore the data were considered valid.

Due to the condition of the plants, the experiments were evaluated earlier than had been; if the experiment were conducted longer, significant differences may have become evident. The conditions seem to have inhibited *Pythium* sp., which prefers cool, moist soil conditions instead of warm, dry soil. The presence of *Rhizoctonia* in the co-inoculation and the establishment of contaminant fungi, such as *Fusarium* spp. in all the experiments, could have outcompeted *Pythium* since it is a weak pathogen (Hendrix and Campbell, 1973). This would explain the low recovery rates, particularly in the co-inoculation evaluation. The isolation of *Fusarium* spp. and *Cylindrocarpon* spp., from the control pots may explain the poor health of treated plants, since both have been implicated in BRR (Hildebrand, 1934; Hildebrand and West, 1941). The possibility of infected planting stock still exists, as evidenced by *Rhizoctonia* sp. found in root lesions of non-*Rhizoctonia* inoculated pots, but not in the CFUs of the soil in the treatments in

the *Rhizoctonia* evaluation. However, contamination may have been at such a low level it could not be detected with the soil isolation procedure.

Since the bran was not ground as recommended by Martin (2000) in the 2005 and 2006 experiments, there may have been a problem with the speed with which the bran broke down and the release of inhibitory compounds. The inoculum was mixed wt/wt, so again the treatments were exposed to the same conditions; however, even distribution of the inoculum may also explain the variability observed. The bran is important to have as a carrier and initial nutrient source until the pathogen can establish, but perhaps other carriers, and the quantity used should be evaluated. Despite these concerns, ProPhyt + Abound showed potential as a reduced risk fungicide alternative to minimize damage caused by black root rot. T-10 and Plantshield also have potential as more environmentally benign alternatives to control black root rot as other *Trichoderma* spp. have shown (Chet and Henis, 1983).

2007

After the inoculum optimization experiment (Appendix B), the inoculum level was increased for this experiment. As with all the greenhouse experiments, artificially severe infestations were created. In this experiment a distinct separation was observed between plants in the *Rhizoctonia*-inoculated pots and those receiving the same quantity of non-inoculated bran, which served as control checks for each treatment. Pots with *Rhizoctonia* actually had higher average weights and better root quality. This split may be a result of *Rhizoctonia* actively breaking down the bran, while the bran in the un-inoculated pots hindered plant growth. The lack of mechanical or nematode damage

made this greenhouse experiment, like the others, possibly less severe than if damage had been present.

Although not significantly different from the inoculated control plants, Plantshield-treated plants had the highest average weights in the presence of *Rhizoctonia* for all parameters except fresh and dry root weights (table 20). ProPhyt + Abound-treated plants had the lowest percent root necrosis.

Alternaria, *Fusarium*, and a zygomycete were found to be colonizing the surface of the soil within a few days after planting. These were probably establishing from surrounding greenhouses. *Rhizoctonia* was isolated from every inoculated treatment, but only from the Plantshield control, indicating much of the cross contamination issues had been resolved. *Trichoderma* was isolated from the T-10 and Plantshield controls only (table 21).

Although this inoculation technique worked for Martin (2000), there were several differences from their original protocol. Instead of using 'Selva', the variety 'Allstar' was used. Also, Martin conducted his experiments in a growth chamber, which is a much more controlled environment than the greenhouse. Their nested sieving of the bran is also a consideration. Their soil was also sieved field soil that was pasteurized at 82°C for 2 h. Although the soil used in these experiments was autoclaved for considerably longer, table 22 provides evidence that the autoclaved soil does not hinder the plants. It was also apparent, during 2005, that non-inoculated bran (0.75% wt/wt) did not significantly reduce plant weights compared to inoculated bran. In 2007, the non-inoculated bran reduced plant weights and root quality compared to the inoculated bran, probably because *Rhizoctonia* was breaking down the bran in inoculated pots.

Table 20. Effects of different biocontrol and fungicide products on plant biomass and root health of strawberry cv. Allstar in a greenhouse study in soil inoculated with and without *Rhizoctonia fragariae* in East Lansing, MI, in 2007.[y]

Treatment	Fresh foliage weight (g)	Fresh root weight[z] (g)	Dry foliage weight (g)	Dry root weight[z] (g)	Total fresh weight (g)	Total dry weight (g)	Root quality rating (1-5)[x]	Root necrosis (%)
Control	6.06 NS	3.91 NS	1.97 NS	0.99 NS	9.97 NS	2.85 NS	2.61 NS	60.28 NS
Actinovate	5.22	4.26	1.74	1.10	9.48	2.71	2.33	66.11
Ditera	6.41	3.91	2.13	1.02	10.32	3.04	2.94	58.89
Plantshield	7.06	4.17	2.17	1.06	11.24	3.11	2.89	50.56
Prophyt + Abound	6.11	3.89	2.11	0.99	10.00	3.00	2.72	46.33
T-10	5.20	3.12	1.73	0.84	8.33	2.48	2.11	68.33
Inoculum Presence								
Inoculated	8.58 a	4.66 a	2.75 a	1.19 a	13.23 a	3.81 a	3.56 a	35.74 a
Non-inoculated	3.45 b	3.10 b	1.20 b	0.81 b	6.55 b	1.92 b	1.65 b	81.09 b

ANOVA

Effects	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value	Df	*p* value
Treatment (T)	5	0.7049	5	0.7565	5	0.7353	5	0.8885	5	0.7852	5	0.8326	5	0.2987	5	0.1553
Inoculation (I)	1	**0.0000**	1	**0.0000**	1	**0.0000**	1	**0.0000**	1	**0.0000**	1	**0.0000**	1	**0.0000**	1	**0.0000**
Block	8	**0.0039**	8	**0.0011**	8	**0.0038**	7	**0.0044**	8	**0.0015**	7	**0.0001**	8	**0.0184**	8	0.3401
TxI	5	0.7899	5	0.9574	5	0.6406	5	0.9488	5	0.8526	5	0.7722	5	0.2282	5	0.8972
Residual	88		88		88		77		88		77		88		88	
Total (Corr.)	107		107		107		95		107		95		107		107	

[x] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Treatment pairwise comparisons were performed with n=18, except for dry root and total dry weight, n=16. Inoculum presence pairwise comparisons performed with n=54, except dry root and total dry weight, n=48.

[z] Statistical analysis was performed after sqrt(x) transformation.

Table 21. Frequency of fungi isolated from roots of strawberry cv. Allstar treated with biocontrol and fungicide products applied to the in a greenhouse study in soil inoculated with and without *Rhizoctonia fragariae* in East Lansing, MI, in 2007.

Treatment	Inoculum	Total Isolations	*Rhizoctonia* (%)	*Fusarium* (%)	*Trichoderma* (%)	Non-Pathogens[z] (%)
No Bran	-	2	0.0	50.0	0.0	50.0
Un-Inoculated	-	10	0.0	50.0	0.0	50.0
Inoculated	*Rhizoctonia*	3	100.0	0.0	0.0	0.0
Plantshield	-	13	15.4	7.7	38.5	38.5
Plantshield	*Rhizoctonia*	6	100.0	0.0	0.0	0.0
ProPhyt + Abound	-	8	0.0	100.0	0.0	0.0
ProPhyt + Abound	*Rhizoctonia*	9	100.0	0.0	0.0	0.0
Actinovate	-	7	0.0	71.4	0.0	28.6
Actinovate	*Rhizoctonia*	9	100.0	0.0	0.0	0.0
Ditera	-	10	0.0	20.0	0.0	80.0
Ditera	*Rhizoctonia*	5	80.0	20.0	0.0	0.0
T-10	-	10	0.0	0.0	90.0	10.0
T-10	*Rhizoctonia*	10	100.0	0.0	0.0	0.0

* Other Other fungi isolated included *Alternaria* sp., *Cephalosporium* sp., *Chaetomium* sp., *Phoma* sp., *Ulocladium* sp., *Acremonium* sp., and a yeast.

** Percentage fungi calculated from total fungi isolated.

Table 22. Effects of oat bran as an inoculum carrier on plant weights and root quality in a greenhouse study using cv. Allstar in a greenhouse study using autoclaved soil inoculated with and without *Rhizoctonia fragariae* in East Lansing, MI in 2005 and 2007.[y]

Bran		Fresh foliage weight (g)	Fresh root weight (g)[z]	Dry foliage weight (g)	Dry root weight (g)	Total fresh plant weight (g)[z]	Total dry plant weight (g)[z]	Root quality rating (1-5)[x]
2005 (0.75% wt/wt)								
Inoculated		3.25 NS	1.48 a	0.93 NS	0.40 a	4.73 a	1.33 NS	2.50 a
Non-inoculated		4.18	2.88 b	1.40	0.53 a	7.05 a	1.93	2.00 a
None		7.13	9.70 c	2.18	1.68 b	16.83 b	3.85	4.50 b
ANOVA								
Effects	Df	Significance (p)						
Inoculation	2	0.0813	**0.0016**	0.1103	**0.0165**	**0.0187**	0.0798	**0.0020**
Block	3	0.3556	**0.0320**	0.6019	0.2514	0.1423	0.5131	0.0701
Residual	6							
Total (Corr.)	11							
2007 (3.0% wt/wt)								
Inoculated		8.36 b	4.53 a	2.68 b	1.14 a	12.89 b	3.70 b	3.44 b
Non-inoculated		3.75 a	3.29 a	1.25 a	0.84 a	7.05 a	2.00 a	1.78 a
None		12.94 c	9.57 b	4.06 c	2.43 b	22.51 c	6.22 c	5.00 c
ANOVA								
Effects	Df	Significance (p)						
Inoculation	2	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Block	8	0.0811	**0.0129**	0.1973	0.1956	**0.0154**	0.0594	0.0689
Residual	16							
Total (Corr.)	26							

[x] Scale of 1-5 (1= <20% secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots).

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons performed on n=4 for 2006. Pairwise comparisons performed on n=9 for 2007.

[z] Statistical analysis was performed after log(x) transformation.

Despite the continual modification of the greenhouse trials, it is clear that ProPhyt + Abound offers a likely alternative to fumigation in an integrated management scheme for BRR. The success of this product combination in the field is probably due to the removal of any pathogens that may be on the roots from the nursery, despite their best efforts to prevent this from occurring. In the greenhouse it is harder to determine why this specific treatment results in larger, healthier plants since the planting stock was checked before each trial. However, the Abound may provide a layer of protection that allows these plants to get a 'jump start' over their counterparts receiving other treatments, especially with a little extra nutrition from the ProPhyt. Plantshield, T-10, and Ditera have shown continued promise as well. As Harman (2000) discusses, *T. harzianum* T-22 does not always cause visual improvement of plants, but it can improve root development of ornamentals and protect tomato against Fusarium crown and root rot in the greenhouse. He further discusses that control may be possible with one application at the beginning of the growing season; however, T-22 can be overwhelmed by high disease pressure and must be used as a preventative. In the BRR study conducted, disease pressure was artificially high, this may explain why Plantshield and T-10 did not perform consistently better than control plants.

All the products need further evaluation, and a quantitative, consistent system to evaluate these products in the greenhouse remains to be determined. It would be interesting to examine the efficacy of integrating biologicals with fungicdes as Elmer and McGovern (2004) studied in cyclamen and Harman (2000) suggests for *T. harzianum* T-22, particularly in conjunction with fumigation.

LITERATURE CITED

Anonymous. 1998. United Nations Environmental Programme (UNEP) Methyl Bromide Technical Options Committee (MBTOC). 1998 Assessment of the Alternatives to Methyl Bromide. United Nations Environmental Programme, Nairobi: 374 pp.

Barnett, H. L., and Hunter, B. B. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press. St. Paul, Minnesota.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins Company, Baltimore.

Chet, I., and Henis, Y. 1983. *Trichoderma* as a biocontrol agent against soilborne root pathogens. Pages 110-112 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Chet, I., Ordentlich, A., Shapira, R., and Oppenheim, A. 1991. Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria. Pages 229-336 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

D'Ercole, N., Nipoti, P. and Manzali, D. 1989. Research on the root rot complex of strawberry plants. Acta Horticulturae 265:497-501.

Domsch, K. H., Gams W., and Anderson, T-H. 1980. Compendium of Soil Fungi. Academic Press Inc., New York.

Elmer, W. H., and McGovern R. J. 2004. Efficacy of integrating biologicals with fungicides for the suppression of Fusarium wilt of cyclamen. Crop Protection 23:909-914.

Fravel, D. R., and Keinath, A. P. 1991. Biocontrol of soilborne plant pathogens with fungi. Pages 237-243 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Gutierrez, W. A., Shew, H. D., and Melton, T. A. 1997. Sources of inoculum and management for *Rhizoctonia solani* damping-off on tobacco transplants under greenhouse conditions. Plant Dis. 81:604-606.

Hancock, J. F., Callow, P. W., Serçe, S., and Schilder, A. C. 2001. Relative performance of strawberry cultivars and native hybrids on fumigated and nonfumigated soil in Michigan. HortScience 36:136-138.

Harman, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease 84:377-392.

Hendrix Jr., F. F., and Campbell, W. A. 1973. *Pythiums* as plant pathogens. Ann. Rev. Phytopathol. 11: 77-98.

Henis, Y., Ghaffar, A., Baker, R., Gillespie, S. L. 1978. A new pellet soil-sampler and its use for the study of population dynamics of *Rhizoctonia solani* in soil. Phytopathology 68: 371-376.

Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, Sec. C. 11:18-31.

Hildebrand, A. A., and West, P. M. 1941. Strawberry root rot in relation to microbiologiclal changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research Sec. C 19:183-198.

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Martin, F. N. 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. Phytopathology 90:345-353.

Olatinwo, R. O., and Schilder, A. M. C. 2002. Transplant root dips with biocontrol agents reduce strawberry black rot. *Phytopathology* 92:S61.

Parke, J. L. 1991. Root colonization by indigenous and introduced microorganisms. Pages 33-42 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to methyl bromide: A Florida perspective. Plant Management Network. 27 Oct.

Strong, F. C., and Strong, M. C. 1927. Investigations on the black root of strawberries. Phytopathology 21:1041-1059.

Watanabe, T., Hashimoto, K., Sato, M. 1977. *Pythium* species associated with strawberry roots in Japan, and their role in the strawberry stunt disease. Phytopathology 67:1324-1332.

Wing, K. B., Pritts, M. P., and Wilcox, W. F. 1994. Strawberry BRR: A Review. Advances in Strawberry Research. 13:11-19.

Wing, K. B, Pritts, M. P., and Wilcox, W. F. 1995. Biotic, edaphic, and cultural factors associated with strawberry BRR in New York. HortScience 30:86-90.

CHAPTER FOUR: EVALUATION OF AN INTEGRATED APPROACH FOR THE MANAGEMENT OF BLACK ROOT ROT

Introduction

Strawberry BRR is a disease complex that is widespread in the United States and around the globe from Japan to Europe, wherever strawberries have been planted for multiple years (Watanabe *et al.*, 1977; D'Ercole *et al.*, 1989). Characterized by 0.5-5 cm reddish brown lesions on the feeder and structural roots of the strawberry plant, eventually all root tissue becomes involved, as the stele, vascular cylinder, and cortical tissue become infected (Maas, 1998). As feeder roots disintegrate at the point of infection, shoots become stunted and wilt at the onset of dry weather. Roots darken with age resulting in the death of the root. The roots may become completely black in severe cases, with a corky texture. Infected younger plants will produce smaller leaves, fewer main and lateral roots, have slower growth, and reduced runner production (Maas, 1998).

The disease can be spread through infected nursery stock, movement of infested soil, or infected plant debris (Maas, 1998; Strong and Strong, 1927; Hildebrand, 1934). Also known as strawberry decline, many organisms and abiotic factors have been implicated in the cause of the disease. Wing *et al.* (1995) discussed that no single factor explained a substantial part of the observed variation in root health, and that BRR may be caused by different factors in different fields or that several interacting factors are necessary. Poor root health was associated with soil compaction and high soil clay and silt content. The implication is that specific pathogens are not known to consistently cause the disease; however, *Rhizoctonia fragariae* Husain & McKeen, *Pythium* spp., and the nematode *Pratylenchus penetrans* (Cobb) Filipjev and Shuurmans Stekhoven are

generally accepted as the primary pathogens (D'Ercole *et al.*, 1989; Wing *et al.*, 1995; Maas, 1998).

Currently, control of black root rot consists of using crop rotation, cover crops, good aeration and drainage, and fumigation (Maas, 1998; Martin and Hancock, 1983; Perry and Ramsdell, 1994). Recommendations for alternative control measures include planting resistant varieties, crop rotations, and incorporating organic matter. Prevention is key; planting disease-free stock in fertile, well-drained sandy loam is the best way to avoid this disease (Perry and Ramsdell, 1994). Also, good cultural practices to prevent drought stress and winter injury, and a 3 to 5-year rotation between strawberry plantings helps prevent disease (Perry and Ramsdell, 1994).

The strawberry cultivar 'Cavendish' has been found to perform well on naturally BRR infested soil (LaMondia, 2004; Particka and Hancock, 2005). In Michigan 'U-Pick' operations, continual strawberry production is common due to the few suitable locations for these enterprises and other economic pressures. Continual cropping allows pathogen establishment and inoculum build-up to deleterious levels. Hildebrand and West (1941) found that strawberries grown after a soybean series in greenhouse experiments approached the same level of disease as sterilized soil. 'Saia' oats (*Avena strigosa*) has had some success in controlling *P. penetrans* and *R. fragariae* in greenhouse pots (Townshend, 1989). Elmer and LaMondia (1999) found that an application of ammonium sulfate with 'Saia' oats or sorgho-sudangrass reduced *P. penetrans* populations in strawberry roots, and that only a rotation with 'Garry' oats and ammonium sulfate reduced root colonization by *R. fragariae*. *Brassica* species such as oilseed radish, mustard, and canola have also received attention due to the formation of

isothiocyanates that have fungicidal and broad spectrum nematicidal properties (Ettlinger and Kjaer, 1968; Snapp and Mutch, 2003). Based on findings in New York the use of sweet corn, rye, and mustard offer economic return, addition of organic matter, respectively, and a natural fumigation that can be easily incorporated into strawberry production operations.

Compost, already widely used in the nursery industry, offers another means of control. Hoitink and Fahy (1986) discuss several diseases controlled by organic matter such as the incorporation of ammoniated Douglas fir bark into soil which provided control for red stele in strawberry during the first two years of the planting and the use of composted hardwood bark to suppress several species of nematodes including *P. penetrans*. Using compost contained in a mesh tube to create a raised bed, Millner (2006) found that 'Allstar' and 'Chandler' strawberries grown in 100% compost had 16-32 times higher yield than from non-compost rows.

Considering the complexity of BRR, a multi-faceted approach incorporating chemical, host resistance, and cultural management to determine the best control strategy of BRR was taken to enable a recommendation for growers. This included a drench of Actinovate (*Streptomyces lydicus*), a pre-plant fungicide dip of ProPhyt (potassium phosphite) + Abound (azoxystrobin), and compost. The susceptible variety 'Allstar' and the tolerant variety 'Cavendish' were planted within these treatments. These sub-treatments were each evaluated within a crop rotation, fumigation, and a continuous strawberry main treatment.

Materials and Methods

In September 2004 at the Michigan State University Horticulture Farm, an experiment was established on a sandy loam soil with a four year history of strawberry production and BRR. In May 2005, 40.2 x 3.7 m strips of rye or fallow ground were worked up to a depth of 15 cm starting with the fallow ground and finishing on the areas planted with rye. Devrinol 50 DF (napropamide) was applied 10 d prior to planting at 8.96 kg/ha. Strawberry transplants (Krohne Plant Farms, Hartford, MI) were planted in areas that were previously fallow. Plants were irrigated with 2.5 cm of water following planting in 17 June 2005. Ten days later, sweet corn seed (*Zea mays* var. *rugosa* 'Jackpot') (Roger Seed, Boise, Idaho) treated with Captan, Thiram, and carboxin (Vitavax) was planted in areas previously planted to rye in rows 76.2 cm apart using a Mini Nibex (Markaryd, Sweden) so that the seeds were planted 10 cm apart. The rye/corn/mustard rotation plot measured 10.1 x 3.7 m and was replicated four times. In June 2005, 31.9 kg/ha of nitrogen was broadcast over the entire planting. In August, 57.7 kg/ha of nitrogen was applied to the entire planting. The corn was harvested and stalks removed in September 2005, with the stubble left to be incorporated. After incorporating the corn stubble, brown mustard [*Brassica juncea* (L.)] was planted 18 d after the corn harvest by hand broadcasting at 30.8 kg/ha. Lime and 19-19-19 fertilizer were applied at the rate of 36.8 kg /ha as well. Straw was placed over the strawberries in November.

On 9 April 2006, the field was rototilled starting with the rye/corn/mustard rotation plots and then the continuous strawberry plots. Using a randomized complete block design, 10.2 x 3.7-m plots were chosen to receive fumigation with Telone C-35 (1,3-dichloropropene + chloropicrin) at the rate of 39.2 kg/ha knifed in with shanks

placed at 20.3 cm spacing three days after being cultivated. Prior to planting on 25 May 2006, the area to be planted was fertilized with 19-19-19 at the rate of 44.6 kg/ha and then cultivated in the order of fumigated, rotation, and continuous in preparation for planting 2 d later. Using a randomized complete block design, the large plots were split into subplots which were further divided by variety. Each subplot consisted of two rows of ten strawberry plants, planted 45.7 cm apart in rows 83.8 cm apart. The plants within the subplots then received either: 1) a 15-min root dip of Prophyt (potassium phosphite) at the label rate of 0.95 L/3.79 L and Abound (azoxystrobin) at the label rate of 236.6 ml/3.79 L, 2) 473 ml drench per transplant hole of Actinovate (*Streptomyces lydicus*) at the label rate of 1.10 g/3.79 L, 3) planting in compost contained within a mesh polyethylene tube, or 4) no treatment. Each sub-plot was further divided into two varieties, 'Cavendish,' a tolerant variety, and 'Allstar' a susceptible control (table 23). There were a total of five plants in each of the two rows of each variety within each sub-sub-plot. There was 66.0 cm between varieties and 81.3 cm between subtreatments. Daughter plants were trained to form 50 x 270 cm beds. There were 4 blocks for each treatment.

Planting was followed by 1.27 cm of irrigation. The compost used was mature leaf-yard trimmings (MulchPlus, LaPorte, IN) blown into 20-cm diameter polyethylene netting tubes (Filtrexx, Grafton, OH) using a long flexible hose the length of the field. A drip irrigation system, 15 mil T-tape (Barry Hill Irrigation, Buffalo Junction, VA), with emitters spaced 20.3 cm apart and an emitter flow rate of 25.4 L/min -305 linear m (6.7 gal/min-1000 linear ft.) of row, was placed under the netting on the surface of the compost. The socks were planted 15 d later after thoroughly soaking the compost.

Table 23. Main treatments, sub-treatments, and strawberry varieties used in the integrated alternatives experiment conducted in a black root rot infested field in East Lansing, MI during 2006-2007.

Main plots	Sub-plots	Sub-sub-plots
Continuous strawberry	Compost	Cavendish or Allstar
	Actinovate	Cavendish or Allstar
	Untreated	Cavendish or Allstar
	ProPhyt+Abound	Cavendish or Allstar
Fumigated continuous strawberry	Compost	Cavendish or Allstar
	Actinovate	Cavendish or Allstar
	Untreated	Cavendish or Allstar
	ProPhyt+Abound	Cavendish or Allstar
Rye/Sweet Corn/ Mustard Prior to strawberry	Compost	Cavendish or Allstar
	Actinovate	Cavendish or Allstar
	Untreated	Cavendish or Allstar
	ProPhyt+Abound	Cavendish or Allstar

During June and July 2006 flowers were removed. During the summer, runners were raked into the row centers to form matted-row beds. All weed control was done by hand and irrigation was applied as needed. On 8 August 2006, mother crown counts, bed fill, and plant vigor ratings were collected from all treatments except the compost socks. Crown counts were obtained by using a frame measuring 38.1 cm x 61 cm which was centered over the row. Ratings were obtained visually using table 24. Fifteen days later, to allow for the same length of period from planting, the data were collected from the compost socks. On 23 and 25 August, total crown counts were collected from all treatments except the compost socks, which were again collected 15 d later. Total crown number includes original parent crowns. In September 2006, lime and 19-19-19 fertilizer were applied at the rates of 271.5 kg/ha and 254.5 kg/ha respectively. Soil was collected for nematode analysis in May and September from each untreated strawberry sub-plot within each main treatment, regardless of cultivar by digging a plant and 200 g of the surrounding soil. Three 'Allstar' plants were dug within each sub-treatment within the first replication for fungal assays in November before the straw was put down. These were sent to the Milner laboratory (USDA-ARS-BARC-Sustainable Agricultural Systems and Food Safety Labs, 10300 Baltimore Ave., Bldg. 001, Rm 140, Beltsville, MD USA 20705-2350) for a root necrosis rating and fungal assays. In addition, root lesions were also assessed on a scale from 1-4 on roots 2-3 mm in diameter where 1=0-25% of 2-3 mm roots w/lesions, no feeder roots black/brown; 2=25-49% of 2-3 mm roots w/lesions, a few feeder roots black/brown; 3=50-74% of roots with lesions, several clusters of feeder roots black/brown;4= >75% of roots with lesions or most feeder roots black/brown.

Table 24. Bed fill and plant vigor ratings used in the integrated management practices experiment conducted in a black root rot infested field in East Lansing, MI during 2006-2007 using strawberry cvs. Allstar and Cavendish.

Plant Vigor	**Characteristics**
5	Plants in excellent health, vigorous growth, superior runnering
4	33% plants diseased or stunted
3	50% plants diseased or stunted
2	Majority of plants diseased or stunted
1	Plants very stunted, diseased
Bed Fill	**Characteristics**
5	Runners healthy and establishing well, full bed
4	Fewer runner plants than in 5
3	Thinning evident, runner size and establishment good
2	Few runners, mother plants obvious
1	Few to no runners establishing, mother plants dead

On 4 April 2007, straw was removed. During June 2007 there were three harvests every 7 d obtained from the center meter of each sub-subplot. Berries were also counted at the time of harvest. Iron phosphate (Slug Magic) was applied (4.8 g/m^2) to prevent slug damage. It was noted that the compost socks seemed to speed up ripening, possibly due to the black fabric retaining heat. As no fungicides were sprayed to control fruit rots, berries were picked regardless of disease or damage. At the third harvest all remaining berries were picked regardless of size, maturity, or condition.

The erect petioles and leaves, along with total and parent crown counts were taken using frames measuring 38.1 cm x 61 cm. These frames were centered over the middle of the row. Erect petioles and leaves were clipped at the crown and the fresh weight obtained on 31 July 2007. They were subsequently dried for 48 h at 80°C to obtain dry weights. On 27 July, 3 'Cavendish' plants from all subplots, and 3 'Allstar' plants from only the untreated strawberry subplots were dug from replications 2 and 4 for fungal assays from the rotation, fumigation, and continuous strawberry main treatments. Before the fungal assays, the root necrosis was evaluated on a scale, provided by the Milner laboratory, of 1-5 where 1 = mostly black, dark brown, no finely branched roots, and a single crown; 2 = same as 1, except one or two finely branched roots present; 3 = half of all roots black/dark brown and unbranched, and 1 or 2 crown branches; 4 = more white, fine, branched roots present than black/brown unbranched roots, and two crown branches; and 5 = white, fine, and multi-branched roots and crown.

One plant, with surrounding soil, was removed from each variety within the untreated subplot of each block of each main treatment. Nematode presence was determined using the modified Jenkins technique and a modified root extraction process

(Jenkins, 1964; Bird, 1971). The counts of nematodes in the soil were compiled across the varieties to be similar to the process used in 2006, as no roots were collected during that year and soil was collected from each untreated sub-plot without regard to variety. The focus was on the main treatments as none of the sub-plots were expected to affect nematode populations within the field soil.

Fungal assays were conducted in the Milner laboratory at the USDA facilities in Maryland. For fungal assays, when possible, adventitious roots with obvious lesions with distinct diseased-to-healthy transition zones were used with a note made if diseased roots were unavailable. Healthy roots were not plated. Roots were prepared for isolation by removing adhering soil by rinsing roots in cold tap water, surface-disinfesting for 2 min in 0.5% NaOCl, and finally rinsing three times in sterile deionized water. After blotting them dry, 8 segments (approximately 5 to 10 mm in length) were collected from a composite of the roots of all the plants. The eight root segments are plated on water agar containing 100 ppm streptomycin and 30 ppm vancomycin or 50 ppm penicillin. Plates were incubated at 18-22°C in the dark for 2 wks and checked daily for fungal growth. Isolated fungi were subcultured on 0.50-strength PDA and incubated at 18-22°C and identified to genus using classic taxonomic procedures and growth characteristics on media.

Field Inoculum Level Assessment

The effects of the main plots on colony forming units (CFUs) of *Rhizoctonia* in the soil was of interest to determine if there was a reduction in propagule quantity.

Besides looking at infections on plants, the desire to know the disease level in the field spurred the assessment of inoculum level.

Soil Collection

Soil was collected on 10 June 2006, 19 July 2006, 17 August 2006, and 20 September 2006 using a soil probe to gather a composite sample across varieties at a depth of 15.2 to 20.3 cm from all untreated subplots. Twelve composite samples were taken back to the lab, one for each of the 12 plots, and 30 g was weighed out in the bottom of a sterile petri dish and spread evenly over the bottom. Soil was taken from the entire area, regardless of the variety planted. Soil was stored at 4°C until processing within 1 to 2 d.

Soil Inoculum Evaluation

Soil (30 g) was spread on a sterile Petri dish bottom and a pellet soil-sampler, as described by Henis *et al.* 1978, was used to plate 15 soil cores onto two semi-selective media plates, for the first soil sample (30 cores/sample), and then three plates for all subsequent samples (45 cores/sample) (Gutierrez *et al.*, 2001; Henis *et al*, 1978). The pellet soil-sampler was rinsed in distilled water, followed by alcohol, and then flamed between each sample. It was kept at a constant depth and pressed firmly into the soil with the bottom of the petri dish used to level off the pellet tubes. Plant debris and pebbles were avoided. For each sample an additional set of soil cores were randomly set aside to obtain weight for calculation of CFUs. All the additional sets from each sampling time were dried for 24 h at 105°C. Colony assessments were made after 2 to 3

d of growth. *Rhizoctonia* inoculum levels were determined by morphological identification upon plate colonization. The procedure was repeated in 2007 with samples taken the following dates: 11 May, 9 June, 9 July, 10 August, and 10 September.

Data Analysis

All statistical analyses was performed using the ANOVA and mean separation (Fisher's protected least significant difference test p=0.05) functions of the StatGraphics Plus 4.1 (StatPoint Inc., VA) statistical computer program after checking for equal variance. In the interaction analysis, model variance components were estimated due to main treatment (M), sub-treatment (S), variety (V), Block (B), M x S, M x V, S x V, M x S x V, and error. All data obtained in the field were collected from both rows, and then calculated for one square meter centered over one row. All yield data were calculated for the center meter of one row. When analyzing the data over multiple years, it was necessary to utilize SAS 9.1 (SAS Institute Inc., Cary, N.C.) using repeated measurement ANOVA in proc glm.

For the inoculum level assessment, analysis was conducted with SAS 9.1 (SAS Institute Inc., Cary, N.C.) using proc glm. In the interaction analysis, model variance components were estimated due to main treatment (M), time (T), Block (B), M x T, M x B, T x B, and error.

Results and Discussion

Year and the year x main treatment interaction were significant in the repeated measurements ANOVA of the field data (p<0.0001 and p=0.0003, respectively). The year x block interaction was also significant (p=0.0305) while year x sub-treatment was

not significant (p=0.0527). Although block was significant for the plant vigor rating (p=0.0113) and total crown number (p=0.0198) over the two years, there was not a block x main treatment interaction for total crown number. The plant vigor rating had a significant block x main treatment interaction (p=<0.0001). However, with a significant year x main treatment interaction, each year could only be examined separately. The block x subtreatment was not significant for any parameter.

The parameters measured in the current study were shown to differ between plants grown on fumigated and non-fumigated ground (Hancock *et al.*, 2001). In 2006, the plants in the fumigated main treatment all had significantly greater plant vigor, bed fill, and more total crowns than the plants in either the rotation or continuous main treatments. The plants within the fumigated main treatment continued to have significantly higher bed fill and plant vigor ratings in 2007. The plants in the sub-plots of ProPhyt + Abound and compost tended to have higher vigor, but the plants in the compost socks had the poorest bed fill because the runners could not establish directly into the socks; instead the runners established alongside the socks into the infested ground. While ProPhyt + Abound tended to have the best fill, the plants receiving this dip were not significantly different from the control (table 25).

None of the plants in any of the sub-treatments differed significantly from the plants within the untreated sub-plot in 2007 for bed fill or plant vigor. The fumigated main treatment plants continued to be superior to those plants within the other main treatments in regards to total crown number (table 25). Although not statistically different from the untreated sub-plot, the plants treated with ProPhyt + Abound tended to have more total crowns. Differences between the varieties became evident when

examining the fruit data with Cavendish being more productive and having larger fruit than Allstar. Individual berry size was greatest from plants in the compost socks. There was no difference in berry size from plants in the untreated continuous or fumigated continuous main treatments (table 25). Plants in the fumigated main treatment had the highest total berries and greatest yield. Although not statistically different from the control, plants treated with ProPhyt + Abound numerically had the greatest number of berries and higher yields. The significance observed in blocks was probably due to differences in soil fertility and microbial populations caused by previous studies. The plants placed in the compost socks and ProPhyt + Abound tended to have greater fresh biomass than the control. There was some interaction between the varieties and the main or subplots, but there was not a consistent interaction. There was no interaction between main and subtreatments.

Table 25. Effects of cultural and chemical treatments applied at planting to strawberry cv. Allstar and Cavendish on plant vigor, bed fill, biomass, and yield in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.[u]

[u] Values in columns followed by different letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons performed for main treatments, n=32, sub-treatments, n=24, variety, n=48, and block, n=24.
[v] Did not pass variance check.
[w] Scale 1 to 5 with 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.
[x] Scale 1 to 5 with 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.
[y] Statistical analysis performed after sqrt(x) transformation.
[z] Statistical analysis performed after log(x) transformation.

	2006			2007							
Main Treatment	**Bed fill rating (1-5)[zw]**	**Plant vigor rating (1-5)[x]**	**Total crown number/ m^2[y]**	**Bed fill rating (1-5)[w]**	**Plant vigor rating (1-5)[x]**	**Total crown number /m^2**	**Single berry weight (g)**	**Total berry number[zv]**	**Fruit yield/m (kg)[y]**	**Fresh biomass/m^2 (g)[v]**	**Dry biomass/ m^2 (g)**
Fumigated	3.56 b	4.41 c	7.53 b	4.53 b	5.00 b	18.73 b	5.93 b	132.33 b	0.77 a	146.06 b	49.46 b
Continuous	2.22 a	2.94 a	4.66 a	3.19 a	4.19 a	13.22 a	5.46 b	59.39 a	0.35 b	113.91 a	35.87 a
Rotation	2.41 a	3.38 b	4.67 a	3.09 a	4.16 a	12.13 a	4.26 a	49.28 a	0.22 c	93.57 a	37.94 a
Sub Treatment											
Untreated	3.21 ab	3.63 b	6.38 a	3.75 NS	4.21 NS	15.83 ab	4.72 a	106.83 a	0.57 b	123.03 ab	41.41 ab
Actinovate	2.71 b	3.00 a	5.10 b	3.33	4.50	13.35 bc	4.96 a	64.08 b	0.35 a	101.25 b	36.00 a
Compost	1.71 c	3.79 b	4.72 b	3.42	4.71	12.77 c	6.56 b	60.17 ab	0.41 ab	143.72 a	49.84 b
ProPhyt+Abound	3.29 a	3.88 b	6.26 a	3.92	4.38	16.81 a	4.63 a	90.25 a	0.46 ab	103.39 b	37.12 a
Variety											
Allstar	2.79 NS	3.60 NS	5.47 NS	3.60 NS	4.56 NS	14.41 NS	4.76 a	70.50 NS	0.35 a	109.64 NS	36.99 a
Cavendish	2.67	3.54	5.76	3.60	4.33	14.98	5.68 b	90.17	0.54 b	126.06	45.19 b
ANOVA											
Effects	Df	Significance (p)									
Main (M)	2	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0016**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0001**	**0.0015**
Sub (S)	3	**0.0000**	**0.0001**	**0.0010**	0.2399	0.3847	**0.0094**	**0.0000**	**0.0245**	0.0631	**0.0081**	**0.0116**
Variety (V)	1	0.3639	0.6604	0.3716	1.0000	0.2735	0.5503	**0.0000**	0.0596	**0.0005**	0.0924	**0.0115**
Block (B)	3	0.1673	0.1644	**0.0029**	0.1245	**0.0242**	**0.0005**	0.9609	**0.0000**	**0.0000**	**0.0000**	**0.0058**
MxS	6	0.0625	0.3220	0.2185	0.9461	0.8451	0.6317	0.5874	0.1606	0.0785	0.9431	0.8726
MxV	2	0.1379	**0.0084**	0.0692	0.3874	0.3020	0.1211	0.4923	0.2310	**0.0449**	0.4859	0.5246
SxV	3	**0.0386**	0.2483	**0.0011**	0.0783	0.7254	0.2621	0.5180	0.1239	0.2292	0.4355	0.3711
MxSxV	6	0.3078	0.7753	0.3127	0.8294	0.9022	0.3069	0.8231	0.5568	0.6366	0.4377	0.3867
Residual	69											
Total (Corr.)	95											

Table 26. Root lesion and general plant health ratings of strawberry cv. Allstar and Cavendish grown under in different cultural and chemical treatments in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.

Main Treatment	Sub-Treatment	Cultivar	2006 Root Health Rating[z]	2007 Plant Health Rating[y]
Rotation	Strawberry	Allstar	3.67	-
Rotation	ProPhyt+Abound	Cavendish	-	3
Rotation	Strawberry	Cavendish	-	3
Rotation	Compost	Cavendish	-	5
Rotation	Actinovate	Cavendish	-	2
Fumigated	Strawberry	Allstar	2.33	-
Fumigated	ProPhyt+Abound	Cavendish	-	4.5
Fumigated	Strawberry	Cavendish	-	5
Fumigated	Compost	Cavendish	-	5
Fumigated	Actinovate	Cavendish	-	4.5
Continuous	Strawberry	Allstar	1.67	-
Continuous	ProPhyt+Abound	Cavendish	-	2.5
Continuous	Strawberry	Cavendish	-	3.5
Continuous	Strawberry	Allstar	-	2
Continuous	Compost	Cavendish	-	5
Continuous	Actinovate	Cavendish	-	4.5

[x] All averages calculated from n=3.

[y] Plant health rating on scale of 1-5 for necrosis where 1 = mostly black, dark brown, no finely branched roots, and single crown; 2 = same as 1, except 1 or 2 finely-branched roots present; 3 = half of all roots are black/dark brown and unbranched, and 1 or 2 crown branches; 4 = more white, fine, branched roots present than black/brown unbranched roots, and 2 crown branches; and 5 = white, fine, and multi-branched roots and crown.

[z] Root health rating on 2-3 mm roots from 1-4 where 1=0-25% of roots with lesions, no feeder roots black/brown; 2=25-49% of roots with lesions, a few feeder roots black/brown; 3=50-74% of roots with lesions, several clusters of feeder roots black/brown;4= >75% of roots with lesions or most feeder roots black/brown.

In 2006, nematode sampling revealed that the fumigation main treatment had the lowest total plant parasitic nematode counts. There were no significant differences between season sampled or blocks (table 27). In 2007 the detection of plant parasitic nematodes was improved with root sampling. While the fumigated main treatment had the highest counts, it was not significantly different from the continuous strawberry main treatment. Over the two years, the total number of parasitic nematodes in the soil was higher in 2007 than 2006. This was also evident for total *P. penetrans* in the soil, indicating a build up over time, but there were no significant differences observed amongst the main treatments. Some initial sampling done from plants in the compost within the first block of each main treatment revealed the presence of *Trichodorus* spp., *Criconemella* spp., *Meloidogyne* spp., *Pratylenchus penetrans*, *Longidorus elongates*.

Table 27. Plant parasitic nematode populations in 100 cm^3 soil or 1 g roots from roots of strawberry cv. Allstar and Cavendish grown under different cultural and chemical treatments in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.[ru]

		2006	2007	
Main Treatment		**Total Plant Parasitic Nematodes in soil[zxs]**	**Total Plant Parasitic Nematodes in soil[y ws]**	**Total Plant Parasitic Nematodes in roots[zvt]**
Fumigated		0.50 a	107.00 NS	56.00 NS
Continuous strawberry		3.50 b	54.38	21.75
Rotation		8.25 b	101.25	44.88
ANOVA				
Effects	Df		Significance(p)	
Main (M)	1	**0.0017**	0.7714	0.9211
Season (S)	1	0.1174	-	-
Variety	2	-	0.8292	0.9421
Block (B)	3	0.8966	0.6547	0.7281
Residual	17			
Total (Corr.)	23			

[r] See Appendix C for risk ratings for parasitic nematodes on strawberries in Michigan.
[s] Total nematode averages includes:, *Trichodorus* spp., *Criconemella* spp., *Meloidogyne* spp., *Pratylenchus penetrans*, and *Longidorus elongatus*.
[t] Total nematodes include: *Meloidogyne* spp. and *Pratylenchus penetrans*.
[u] Values followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05.
[v] For pairwise comparisons, main treatment, n=8, for variety, n=12, and for block, n=6.
[w] For pairwise comparisons, main treatment, n=6, for variety, n=9, and for block, n=6.
[x] For pairwise comparisons, main treatment, n=8, for season, n=12, and for block, n=6.
[y] Statistical separation performed after sqrt(x+1) transformation.
[z] Statistical separation performed after log(x+1) transformation.

Table 28. Fungal genera isolated from roots of strawberry cv. Allstar and Cavendish grown under different cultural and chemical treatments in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.*

	2006		2007				
					ProPhyt +		
Genera present	**Control**		**Control**	**Compost**	**Abound**	**Actinovate**	**Control**
	Allstar		**Cavendish**				**Allstar**
	Lesion free	**Lesions present**	**Lesions present**				
			Fumigated				
Alternaria spp.	-	-	-	X	X	X	-
Chaetomium spp.	X	X	X	-	-	-	-
Coniothyrium spp.	-	-	X	-	-	-	-
Coniothyrium-like	-	X	-	-	-	-	-
Cylindrocarpon spp.	-	-	X	-	X	X	-
Fusarium spp.	-	X	X	X	X	X	-
Mucor spp.	X	X	-	-	-	-	-
Penicillium spp.	X	-	-	-	-	-	-
Pestalotia spp.	-	-	-	X	-	X	-
Phoma spp.	-	-	-	-	X	X	-
Pyrenochaeta spp.	-	-	-	-	X	-	-
Pythium spp.	X	X	-	-	-	-	-
Rhizoctonia spp.	-	-	X	X	X	X	-
Robillarda spp.	-	-	-	-	X	-	-
Trichoderma spp.	X	X	X	X	-	X	-
			Rotation				
Alternaria spp.	-	-	X	X	-	-	-
Cylindrocarpon spp.	-	X	X	X	X	X	-
Fusarium spp.	-	X	X	-	X	X	-
Mucor spp.	X	X	-	-	-	-	-
Penicillium spp.	-	X	-	-	-	-	-
Phoma spp.	X	X	X	X	X	-	-
Pythium spp.	X	-	-	-	X	-	-
Rhizoctonia spp.	-	X	-	-	-	X	-
Robillarda spp.	-	-	X	-	-	-	-
Trichoderma spp.	X	X	X	X	-	-	-
			Continuous				
Alternaria spp.	-	-	X	X	-	X	-
Chaetomium spp.	-	X	-	-	-	-	-
Coniothyrium spp.	-	-	-	-	-	X	-
Coniothyrium-like	X	-	-	-	-	-	-
Cylindrocarpon spp.	X	X	X	X	X	X	X
Doratomyces spp.	-	-	-	X	-	-	-
Fusarium spp.	X	-	X	X	X	X	X
Mucor spp.	X	X	X	-	-	-	-
Phoma spp.	-	X	X	X	-	X	-
Pyrenochaeta spp.	-	-	-	X	-	-	-
Rhizoctonia spp.	-	-	X	-	X	X	X
Robillarda spp.	-	X	-	-	-	-	-
Trichoderma spp.	X	X	-	-	-	X	-

* Table obtained from Milner laboratory. X indicates genera most frequently recovered.

Inoculum Level Assesment

In 2006 time and time x block were not significant in the ANOVA, but time x treatment and the block main effect were significant (p=0.0037 and 0.0188, respectively). Treatment main effect was not significant (p=0.2363). There was no block effect in 2007, but treatment and the time x treatment interaction were significant (p<0.0001 and 0.0028, respectively).

At the beginning of 2006 the fumigation and rotation main treatments had significantly fewer CFUs of *Rhizoctonia*/g soil than the untreated continuous main treatment; however, this difference was lost by the end of the first growing season, and in fact a reversal was seen (table 29).

In 2007, a general trend was observed where the fumigated main treatment had significantly fewer propagules than the other main treatments, which did not significantly differ (table 30). Again, at the end of the season the effect of the treatments blurred, possibly indicating that September is an important time for BRR and strawberry growth. This fluctuation over the season, possibly influenced by temperature has been observed by others (Martin, 1988; Scott *et al.*, 2003).

Table 29. Effect of fumigation and rotation crops on soil inoculum concentration of *Rhizoctonia* in a strawberry black root rot infested field in East Lansing, MI in 2006.*

		CFU *Rhizoctonia*/g soil			
Treatment		**June**	**July**	**August**	**September**
Continuous strawberry		2.38 a	2.25	1.08	1.00 b
Fumigated		0.75 b	1.00	1.67	2.92 a
Rotation		0.13 b	1.50	1.08	1.00 b
ANOVA					
Effect	Df		Significance (p)		
Treatment	2	**0.0003**	0.2166	0.4957	**0.0010**
Block	3	0.5295	0.3214	0.1276	**0.0163**
Error	30				

* Values followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed with n=12, except for June where n=8.

Table 30. Effect of fumigation and rotation crops on soil inoculum concentration of *Rhizoctonia* in a strawberry black root rot infested field in East Lansing, MI in 2007.*

		CFU *Rhizoctonia*/g soil				
Treatment		**May**	**June**	**July**	**August**	**September**
Continuous strawberry		13.83 a	14.42 a	14.58 a	15.00 a	14.08 NS
Fumigated		9.08 b	12.42 b	13.00 b	13.58 b	12.83
Rotation		10.25 b	13.33 b	14.58 a	14.50 c	13.92
ANOVA						
Effect	Df			Significance (p)		
Treatment	2	**0.0015**	**0.0007**	**0.0055**	**<0.0001**	0.0739
Block	3	0.3250	0.2638	0.3546	0.9557	0.3128
Error	30					

* Values followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed with n=12

It is well known that fumigation results in larger, more productive strawberry plants (Wilhelm, 1965; Hancock *et al.*, 2001). While the rotation and untreated continuous strawberry main treatments did not significantly differ for most parameters over the two growing seasons, the rotation treatment did tend to produce more total crowns and better bed fill. The plants within the rotation main treatment were significantly more vigorous than the untreated continuous plants over the two years.

The utilization of ProPhyt + Abound, although not significantly different from untreated plants, deserves further evaluation as a potential, more environmentally benign, alternative because of work in other trials (Chapter 1 and 2) and it tended to produce beds with better fill initially, indicating repeated measures like those in Chapter 6, may provide acceptable control. In addition, a ProPhyt + Abound dip provides one of the easiest alternatives to implement. This dip may work well in a weakly infested soil by initially protecting the plant as it establishes in the field. Another way is that the fungicides may help decrease any residual organisms coming in on the planting stock from the nursery. This could be determined by a thorough survey of nursery stock.

While the compost socks show potential as well, there are some downsides. The socks do not readily degrade, so eventually, there will be a disposal cost. In addition, the roots do eventually grow out of the sock into the infested soil, and all the daughter plants must establish in this soil too. Fruit production is dependent on the initial mother plants, not so much on the establishment of a perennial bed. It is worthwhile considering a larger row spacing to aid in weed control and equipment maneuvering. Also, winter injury or drought stress may be greater since the socks are lying above ground.

If BRR is approached as a complex of several independent factors, tailor recommendations should be made for a given situation. These factors include: 1) poor cultural practices, 2) fungi, 3) nematodes or grubs, 4) some combination of the previously listed causes, and 5) a strawberry planting that has reached its maximum lifespan. *Rhizoctonia fragariae* is ubiquitous, particularly in strawberry fields. However, the literature fails to describe all factors that may have been involved in any particular instance of strawberry decline. This contributes to the confusion of the actual cause, as it seems BRR encompasses any strawberry decline situation in which *Rhizcotonia*, along with several other fungi, is isolated from the strawberry roots and the cause can not be contributed to something else. This does not mean that it is the cause, but that the correct questions and information are not being asked or exchanged. In other words, the fungi only complicate a problem caused by something else.

The nurseries need to be held accountable for their crowns, growers need to be encouraged to do regular sampling, and the people evaluating the samples need to get thorough background information of the field. Only with more thorough information can the vagueness and inconsistency of this disease complex become elucidated and understood.

LITERATURE CITED

Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. J. of Nematology 3:378-385.

D'Ercole, N., Nipoti, P., and Manzali, D. 1989. Research on the root rot complex of strawberry plants. Acta Horticulturae 265:497-501.

Elmer, W. H., and LaMondia, J. A. 1999. Influence of ammonium sulfate and rotation crops on strawberry BRR. Plant Dis. 83:119-123.

Ettlinger, M. G., and Kjaer, A. 1968. Sulfur compounds in plants. Pages 59-144 *In* Recent advances in phytochemistry. T. J. Mabry, Ed. New York: Appleton-Century-Crofts.

Gutierrez, W. A., Shew, H. D., and Melton, T. A. 1997. Sources of inoculum and management for *Rhizoctonia solani* damping-off on tobacco transplants under greenhouse conditions. Plant Dis. 81:604-606.

Hancock, J. F., Callow, P. W., Serçe, S., and Schilder, A.C. 2001. Relative performance of strawberry cultivars and native hybrids on fumigated and nonfumigated soil in Michigan. HortScience 36:136-138.

Henis, Y., Ghaffar, A., Baker, R., Gillespie, S. L. 1978. A new pellet soil-sampler and its use for the study of population dynamics of *Rhizoctonia solani* in soil. Phytopathology 68: 371-376.

Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, 11:18-31.

Hildebrand, A. A., and West, P. M. 1941. Strawberry root rot in relation to microbiologiclal changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research 19 6:183-198.

Hoitink, H. A. J., Fahy, P. C. 1986. Basis for the control of soilborne plant pathogens with composts. Ann. Rev. Phytopathol. 24:93-114.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Reporter 48:692

LaMondia, J. A. 2004. Field performance of twenty-one strawberry cultivars in a black root rot infested site. Journal of the American Pomological Soc. 58:226-232.

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Martin, F. N., and Hancock, J. G. 1983. Chemical factors in soils suppressive to *Pythium ultimum*. Pages 113-116 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Martin, S. B. 1988. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78:379-384.

Millner, P. D. 2006. Control of strawberry black root rot with compost socks. Biocycle 47:1212, 56-58.

Particka, C. A., and Hancock, J. F. 2005. Field evaluation of strawberry genotypes for tolerance to black root rot on fumigated and nonfumigated soil. J. Amer. Hort. Sci. 130:688-693.

Perry, S., and Ramsdell, D. 1994. Strawberry diseases in Michigan. Ag Facts, Michigan State University Extension Bulletin E-1728:1-4.

Scott, R., Pritts, M., and Kelly, M. J. 2003. Effects of *Rhizoctonia fragariae* infection on growth and productivity of strawberry plants grown under different temperature regimes. Advances in Strawberry Research 22: 26-33.

Snapp, S. S., and Mutch, D. R. 2003. Cover crop choices for Michigan vegetables. Michigan State University Extension Bulletin E 2896: 1-6.

Strong, F.C., and Strong, M. C. 1927. Investigations on the black root of strawberries. Phytopathology 21:1041-1059.

Townshend, J. L. 1989. Population densities of four species of root-lesion nematodes (*Pratylenchus*) in the oat cultivars, Saia and OAC Woodstock. Can. J. Plant Sci. 69:903-905.

Watanabe, T., Hashimoto, K., Sato, M. 1977. *Pythium* species associated with strawberry roots in Japan, and their role in the strawberry stunt disease. Phytopathology 67:1324-1332.

Wilhelm, S. 1965. *Pythium ultimum* and the soil fumigation growth response. Phytopathology 55:1016-1020.

Wing, K. B, Pritts, M. P., and Wilcox, W. F. 1995. Biotic, edaphic, and cultural factors associated with strawberry BRR in New York. HortScience 30:86-90.

CHAPTER FIVE: ESTABLISHMENT OF BIOCONTROLS IN ROTATIONS

Introduction

Currently, recommendations for control of BRR consist of using crop rotation, cover crops, good aeration and drainage, and fumigation (Maas, 1998; Martin and Hancock, 1983; Perry and Ramsdell, 1994). If an existing planting develops BRR, a new site should be chosen, or the old plants plowed under and the soil cultivated for several months followed by fumigation and planting of healthy strawberries in the spring (Perry and Ramsdell, 1994). Incorporation of organic matter can encourage beneficial organisms that are antagonistic to pathogens (Perry and Ramsdell, 1994, Hoitink, 1997).

Crop rotation is known to encourage a more diverse, healthier soil microorganism community (Snapp and Mutch, 2003; LaMondia *et al.*, 2002). Continual cropping allows pathogen establishment and inoculum build-up to deleterious levels. In potato, wheat, barley, and corn, the more frequently a crop is in a rotation, the higher the decrease in yield of that crop (Schippers *et al.*, 1987). Despite this knowledge, growers often have land constraints in their strawberry U-pick operations, and rotation crops do not generally have the economic value of strawberries. Thus there is an interest in using the shortest rotation between strawberry plantings possible. Hildebrand and West (1941) found that strawberries grown after a soybean series in greenhouse experiments approached the same level of disease as sterilized soil. 'Saia' oats (*Avena strigosa* Ard.) has had some success in controlling *P. penetrans* and *R. fragariae* in a pot trial in the greenhouse (Townshend, 1989). Elmer and LaMondia (1999) found that an application of ammonium sulfate with 'Saia' oats or sorgho-sudangrass reduced *P. penetrans* populations in strawberry roots, and that only a rotation with 'Garry' oats and ammonium

sulfate reduced root colonization by *R. fragariae* in a microplot study using 'Honeoye' strawberries. *Brassica* species such as oilseed radish, mustard, and canola have also received attention due to the formation of isothiocyanates that have fungicidal and nematicidal properties (Ettlinger and Kjaer, 1968; Snapp and Mutch, 2003).

In addition, the rhizosphere is a dynamic environment. The mucilage excreted by the roots supports both beneficial and potentially harmful organisms seeking to colonize the growing root. The beneficial microbes are antagonistic to pathogens by competition for food, essential elements, and space (Whipps, 2001; Parke, 1991). These microbes enhance plant growth by suppressing major pathogens, increasing nutrient availability, decreasing toxicity levels around the plant, or a combination of these (Whipps, 2001; Parke, 1991). Some of the microbes parasitize pathogenic fungi by producing chitinases or antibiotics. For instance, *Streptomyces hygroscopicus* var. *geldonus* produces a toxin called geldanomycin which can inhibit *R. solani* (Chet *et al.*, 1991; Fravel and Keinath, 1991). One biocontrol organism that has been used in the past is *T. harzianum* Rifai. Chet and Henis (1983) reported 70% control of *R. solani* with 150 g (dry wt) per square meter in a broadcast application in carnation. Control was significantly better than that achieved in the field by establishing carnations in peat moss with 15% by volume of the *T. harzianum* preparation. Chet and Henis (1983) also report that under field conditions, seed treatment with *T. hamatum* (Bonord.) Bainier reduced cotton damping-off caused by *R. solani* by 60%, and bare patches 23 days later by 39%. It also increased density of plants by 14%. *Trichoderma harzianum* can use the *R. solani* cell wall as a sole carbon source. D'Ercole *et al.* (1989) found a reduction of post-transplant blight incidence from 24.5% in the control to 11.5% in the treatment with *T. harzianum* as a liquid dip on

'Gorella' strawberries. Studies found that T-22 (*Trichoderma harzianum*) could increase nitrogen fertilizer efficiency in corn (Harman, 2000). It causes plants to be more robust and have more extensive root systems by suppressing disease as well as stimulating plant metabolism (Harman, 2000).

There are several biological control products emerging on the market, many of which claim to control root pathogens, but have not been labeled for strawberries. These products have also not been proven to be reproducibly successful in a field situation. This experiment was conducted to evaluate whether suppressive conditions can be created prior to strawberry planting by utilizing different crop rotations in conjunction with applications of Plantshield (*Trichoderma harzianum*) or Mycostop (*Streptomyces griseoviridis*). Rotation crops were chosen by their being mentioned in either product's label, their ability to grow in Michigan, their potential to provide economic return (summer crop), and their potential to naturally fumigate the soil (kale) or build up organic matter to support a diverse microbe population.

Materials and Methods

Plots (1.8 x 1.8 m) were established in three 40.2 x 3.7 m blocks in a sandy loam soil at the Michigan State University Horticulture Farm, East Lansing, MI, which had been previously planted to wheat in September 2004. This field had a four-year history of strawberry production and a history of BRR. A randomized complete block design was used. There were 61.0 cm between each plot and 91.4 cm buffer strips on the North and South sides. The wheat was plowed under in May 2005. The area around each of the blocks was kept in a winter rye and buckwheat rotation to control weeds and reduce

contamination when walking in the field. The 61.0-cm buffers between plots were kept fallow and weed free.

On 16 June 2005, the continuous strawberries were planted 10 d after an application of Devrinol 50 DF (napropamide) to control weed emergence. The summer rotation crops of squash and sweet corn were planted 21 June 2005. Plantshield and Mycostop were applied to each rotation crop at the label rate of 2.27 kg/ha and 1.13 kg/ha, respectively, on 12 July 2005 after crop emergence with a hand pump sprayer in 0.33 L of water per plot aimed at the base of the plants followed by 0.6 cm irrigation the next day. Rotation crops not receiving either of these products served as a control. In June 2005, 32 kg/ha of 19-19-19 was applied. In August, 57.7 kg/ha of urea was applied. *Cucurbita moschata* 'Pilgrim' seeds (Horrocks Farm Market, Lansing, MI) were planted in two rows at a rate of 18 seeds per 1.8 m row and thinned to a population of 6 plants per meter row. *Zea mays* var. *rugosa* 'Checkered Choice' (W. Atlee Burpee & Co., Warminster, PA) was planted in three 1.8 m rows, 18 seeds/row, and thinned to a population of 8 plants per meter of row.

In September 2005 the above ground parts of the sweet corn and squash plants were removed from the field after harvest so that the plots could be cultivated and re-planted. Prior to planting with the winter rotation crops, the field was prepared with standard cultural practices including the addition of lime and 19-19-19 fertilizer applied at the rate of 36.8 kg /ha. The seed bed was prepared for each plot in the order of untreated, Mycostop, and Plantshield with the rototiller washed thoroughly between different product treatments using a pressure washer. The products were applied at the same rate as that for the summer crops but only in 500 ml of water per plot prior to

planting on 27 September 2005. The crops were hand broadcast at the following rates: rye (*Secale cereale*)-100.8 kg/ha, buckwheat (*Fagopyrum* sp.)-80.6 kg/ha, white clover (*Trifolium repens*)-5.6 kg/ha, and hairy vetch (*Vicia villosa* Roth.)-67.2 kg/ha. Kale (*Brassica oleracea*) was planted in two rows at 11.1 kg/ha (table 31). The kale was thinned to a population of 7 plants per meter row. All seed was obtained from Michigan State Seed Solutions (Grand Ledge, MI). Watering and manual weed control were done as needed. Straw was placed over the strawberries in 29 November 2005 at a depth of 7 cm.

The straw was removed on 9 April 2006 and the area to be planted was fertilized with 19-19-19 at the rate of 44.6 kg/ha in May. The plots were rototilled in a sequence to prevent cross contamination between the Plantshield and Mycostop treatments. Two rows of five strawberry plants were set in each plot. The rows were 83.8 cm apart, 15.2 cm from the edge of the plot and the plants were 30.5 cm apart within the rows. Initial mother plant counts were taken a week after planting. During the summer of 2006, flowers were removed and runners were manipulated to form beds measuring 45 x 160 cm. On 8 August total crown count was taken along with visual bed fill and plant vigor ratings (table 32). In September, lime and 19-19-19 fertilizer were applied at the rates of 271.5 kg/ha and 254.5 kg/ha respectively. Soil was collected for nematode analysis on 22 May and 22 September. Straw was placed over the rows in 13 November at a depth of 7 cm and removed on 21 April 2007.

Table 31. Rotations utilized in establishing biocontrols in rotations in a black root rot infested field in East Lansing, MI prior to planting strawberry cv. Allstar from 2005-2007.

Summer 2005	**Fall 2005**	**Summer 2006**
Strawberry	Strawberry	Strawberry (Fumigated)
Strawberry	Strawberry	Strawberry (Non-Fumigated)
Squash	Rye	Strawberry (Non-Fumigated)
Squash	Hairy Vetch	Strawberry (Non-Fumigated)
Sweet Corn	Buckwheat	Strawberry (Non-Fumigated)
Sweet Corn	Kale	Strawberry (Non-Fumigated)
Sweet Corn	Clover	Strawberry (Non-Fumigated)

* Each rotation received Mycostop, Plantshield, or No treatment.

Table 32. Bed fill and plant vigor rating used in establishing biocontrols in rotations in a black root rot infested field in East Lansing, MI prior to planting strawberry cv. Allstar from 2005-2007.

Plant Vigor	**Characteristics**
5	Plants in excellent health, vigorous growth, superior runnering
4	33% plants diseased or stunted
3	50% plants diseased or stunted
2	Majority of plants diseased or stunted
1	Plants very stunted, diseased
Bed Fill	**Characteristics**
5	Runners healthy and establishing well, full bed
4	Fewer runner plants than in 5
3	Thinning evident, runner size and establishment good
2	Few runners, mother plants obvious
1	Few to no runners establishing, mother plants dead

Starting 12 June 2007, yield data were collected including total berry number and weight of berries from the interior meter of both rows. Fruit were harvested every 7 d for 3 wk. Iron phosphate (Slug Magic) was applied to prevent slug damage. As no fungicides were sprayed to control fruit rots, berries were picked regardless of disease or damage. At the third harvest all remaining berries were picked regardless of size, maturity, or condition.

On 31 July, erect petioles and leaves were clipped at the crown and the fresh weight obtained. The erect petioles and leaves, along with total crown counts were taken using frames measuring 38.1 x 61.0 cm centered over the middle of each row. They were subsequently dried for 48 h at 80°C to obtain dry weights. Plant vigor and bed fill ratings were visually taken on 19 July (table 32). Plants and 200 g of surrounding soil were collected on 6 August for nematode analysis. These analyses were preformed by Fred Warner in the Michigan State University Diagnostic services. Nematode presence was determined using the modified Jenkins technique and a modified root extraction process (Jenkins, 1964; Bird, 1971).

An additional plant was also dug from rotation combinations that appeared to have fuller beds and healthier plants. This resulted in a total of 3 plants per rotation treatment, both with and without biocontrols. Fresh biomass was obtained along with a visual assessment of root quality (a qualitative scale of 1-5: 1 1= <20% of root mass is secondary roots, 2= 20-40% secondary roots, 3= 40-60% secondary roots, 4= 60-80% secondary roots, 5= >80% secondary roots) and percent necrosis. The roots from these plants were used for isolations. The crown was cut in half above the uppermost adventitious roots. The roots were washed under cold water and visually rated. After the

roots were surface sterilized in 20% bleach solution followed by two 1 min rinses in sterile distilled water, five, 6-mm root pieces with lesions were dried on sterile paper towels and placed on two separate petri plates with PDA amended with 100 µl/ml of streptomycin sulfate and penicillin-G sodium salt (10 root pieces/plant). Isolated fungi were subcultured one week later after growing at room temperature. They were subsequently identified to genus using classic taxonomic procedures and growth characteristics on media (Barnett and Hunter, 1998; Domsch *et al.*, 1980; Barron, 1968). Those fungi that could not be identified in this manner were subsequently subjected to DNA sequencing of the internal transcribed spacer (ITS) region by Timothy Miles (Sambrook *et al.*, 1989; Altschul *et al.*, 1990).

Data Analysis

All biomass and crown count data obtained in the field were collected from both rows using frames previously described, and then calculated for one row. Total crown number includes original parent crowns. Frequency of fungi recovered was calculated based on total number of fungi that grew. Percentage of lesions with no fungal growth was calculated from total lesions plated. All yield data were calculated for the center meter of one row.

Initially, the statistical analysis was performed on the rotation combinations with respective biocontrol products as one 'management treatment' because the fumigated and continuous controls were not done with either product and the products were not evaluated alone, resulting in an inability to study all interactions fully. In the interaction analysis, model variance components were estimated due to type of rotation (R), product (P), Block (B), R x P, and error after removing the fumigated and continuous strawberry

controls. Analysis was performed with the ANOVA and means separation (Fisher's protected least significant difference test p=0.05) procedures in StatGraphics Plus 4.1 (StatPoint Inc., VA) using after performing a check for equal variance. When analyzing the data over multiple years, it was necessary to utilize SAS 9.1 (SAS Institute Inc., Cary, N.C.) using repeated measurement ANOVA in proc glm.

Results and Discussion

In the repeated measures ANOVA the year and year x block effects were both significant (p<0.0001), but the year x rotation interaction was not significant (p=0.6990). It was of interest to look at the rotation and product combinations within each year as well as over both years. Block was significant for every parameter and the year x block interaction was significant for bed fill and plant vigor (p=<0.0001 and 0.0009, respectively), but not for total crown number (p=0.9402)

For the analysis of the interactions between the rotations and products, the year and year x block interaction were again significant (p<0.0001) while the year x treatment interaction was not significant (p=0.3733). The interaction year x product x rotation was also not significant (p=0.9849). Although block was significant for each parameter, there was not a block x treatment interaction for any of the measurements. Again, it was of interest to look at the rotations and products within each year.

No rotation/product combination was significantly different in the number of beginning mother plants at the start of the experiment (table 33 and 36). However, significant differences were evident between blocks. The differences derive from

planting error due to poor crown placement and deaths. This began the differences between the replicates that became more apparent over the next season.

Replanting, once it became evident that mother plants were not surviving so early in the study, was not done for several reasons. There was a concern for differences in timing among the treatments replanted versus those that were not. Also, when working with crop rotations, the changes in fungal populations may not always be beneficial for a given crop (i.e. *Verticillium* wilt after solanaceous crops) (Pritts and Handley, 1998). Although not expected, the crop rotations may have encouraged the build-up of pathogens that contributed to early decline of the strawberries. In addition, each rotation was planted to strawberries by a given pair of people randomly, so the risk of consistent errors was small.

Despite these conditions, analysis did show some promising results. The rotation combinations were not a significant source of variance in the ANOVA for the bed fill rating or plant vigor rating (table 33), although the untreated continuous strawberry consistently had the lowest average for every parameter obtained from the strawberry beds (table 33). Strawberry plants planted after the squash/rye with Plantshield or Mycostop were significantly more vigorous than the untreated control, approaching the same plant vigor as those plants in the fumigated control. In addition, these same rotation combinations tended to produce better bed fill than the untreated control. For total crown number, strawberry plants in any of the squash/rye rotations, with or without products applied, squash/hairy vetch and squash/hairy vetch with Mycostop all had more crowns than the untreated control strawberries (table 33).

Table 33. Effects of creating disease suppressive conditions using different rotations and biocontrol products on plant vigor, bed fill and total crown number of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006.[x]

Summer rotation	Fall rotation	Product	Beginning mother number	Plant vigor rating (1-5)[y]	Bed fill rating (1-5)[z]	Total crown number/m^2
Fumigated Strawberry	-	-	9.67 NS	4.00 NS	4.00 NS	8.82 a
Continuous Strawberry	-	-	7.00	2.67	2.00	3.33 e
Squash	Hairy Vetch	-	8.67	4.00	3.33	6.45 abcd
Squash	Hairy Vetch	Plantshield	6.67	3.33	3.33	5.70 bcde
Squash	Hairy Vetch	Mycostop	9.33	4.00	4.00	6.83 abcd
Squash	Rye	-	8.67	4.00	3.33	6.72 abcd
Squash	Rye	Plantshield	10.00	4.67	4.33	7.42 ab
Squash	Rye	Mycostop	9.00	4.33	4.00	6.94 abcd
Sweet Corn	Buckwheat	-	7.33	3.33	3.00	4.03 de
Sweet Corn	Buckwheat	Plantshield	9.00	2.67	3.00	5.32 bcde
Sweet Corn	Buckwheat	Mycostop	7.33	4.00	2.33	4.30 de
Sweet Corn	Clover	-	8.00	3.00	2.67	4.25 de
Sweet Corn	Clover	Plantshield	9.33	3.67	2.67	5.16 bcde
Sweet Corn	Clover	Mycostop	8.67	2.33	3.00	4.84 bcde
Sweet Corn	Kale	-	8.00	3.00	3.00	4.30 cde
Sweet Corn	Kale	Plantshield	10.00	4.00	4.00	7.37 abc
Sweet Corn	Kale	Mycostop	8.67	2.67	3.00	5.05 bcde

ANOVA

Effects	Df	Significance (p)			
Rotation (R)	16	0.7789	0.1296	0.0936	**0.0489**
Block (B)	2	**0.0017**	**0.0011**	**0.0000**	**0.0000**
Residual	32				
Total (Corr.)	50				

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference at test p=0.05. Pairwise comparisons performed with n=3.

[y] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[z] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

In 2007, treatment only accounted for significant variance in the ANOVA of total berry number. However, strawberries planted after squash/rye and sweet corn/kale with Plantshield had bed fill ratings approaching that of strawberries in the fumigated plots (table 34). Plants planted after squash/rye, with or without either product, all produced total crown numbers approaching that of strawberry plants in the fumigated plots. For total berry number, strawberries planted after squash/hairy vetch, squash/hairy vetch with Mycostop, and squash/rye with Mycostop produced more berries than the continuous strawberries. The continuous plot strawberries did not produce as many berries, but they were larger, causing there to be no difference between the controls for individual berry weight (table 34).

Plant vigor rating, bed fill rating, and total crown count were taken both years. When considering the variables over 2006 and 2007 together, the strawberries planted after the squash/rye rotations tended to have the same plant vigor and total crown numbers as the fumigated plots (table 35). The difference in years not surprising as more crowns should be evident the second year in bed establishment, especially when considering the poor initial bed conditions in some blocks. Over time, bed fill and plant vigor should be expected to improve with healthy plants, particularly in this situation where the beds had a rough establishment year, as the strawberry plants compensate and become more established.

Table 34. Effects of creating disease suppressive conditions using different rotations and biocontrol products on plant vigor, bed fill and total crown number of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2007.

[w] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons performed with n=3.

[x] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased orstunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[y] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

[z] Statistical analysis performed after log(x+1) transformation.

Summer rotation	Fall rotation	Product	Plant vigor rating (1-5)[x]	Bed fill rating (1-5)[y]	Total crown number/ m^2	Total berry number/m^2	Single berry size (g)[z]	Fruit yield/m (kg)	Fresh biomass/ m^2 (g)	Dry biomass/ m^2 (g)
Fumigated strawberry	-	-	4.67 NS	4.67 NS	19.33 NS	106.33 a	5.20 NS	0.53 NS	126.35 NS	43.92 NS
Continuous strawberry	-	-	3.33	2.33	6.83	28.50 d	10.87	0.15	45.22	16.63
Squash	Hairy Vetch	-	4.67	3.67	13.00	66.50 ab	4.46	0.31	111.65	38.01
Squash	Hairy Vetch	Plantshield	3.67	3.67	9.67	59.83 bcd	2.94	0.26	77.10	27.87
Squash	Hairy Vetch	Mycostop	3.67	3.67	14.33	67.33 ab	3.70	0.28	93.10	31.30
Squash	Rye	-	4.67	4.33	16.50	54.83 bcd	4.38	0.24	113.43	40.66
Squash	Rye	Plantshield	4.33	3.67	17.00	63.83 bcd	4.19	0.28	100.95	34.79
Squash	Rye	Mycostop	4.33	3.67	16.67	68.50 b	3.59	0.27	104.40	33.54
Sweet Corn	Buckwheat	-	4.67	3.67	11.67	44.83 bcd	4.63	0.22	67.88	24.72
Sweet Corn	Buckwheat	Plantshield	4.67	3.67	13.50	57.17 bcd	4.97	0.34	99.80	35.94
Sweet Corn	Buckwheat	Mycostop	4.33	3.67	10.33	37.67 bcd	3.74	0.15	74.42	27.86
Sweet Corn	Clover	-	4.00	2.67	10.83	36.50 bcd	3.82	0.15	57.98	20.91
Sweet Corn	Clover	Plantshield	4.67	4.00	14.67	47.17 bcd	4.52	0.21	101.60	33.22
Sweet Corn	Clover	Mycostop	3.00	2.67	9.67	32.50 cd	5.14	0.20	63.48	23.03
Sweet Corn	Kale	-	4.33	3.67	10.83	34.50 bcd	5.15	0.16	80.03	29.66
Sweet Corn	Kale	Plantshield	5.00	4.33	14.17	61.50 bcd	3.73	0.23	120.27	40.27
Sweet Corn	Kale	Mycostop	3.33	3.33	11.33	41.33 bcd	7.29	0.16	70.67	25.56

ANOVA

Effects	Df	Significance (p)							
Rotation (R)	16	0.6685	0.6385	0.3796	**0.0170**	0.5959	0.0559	0.7485	0.7582
Block (B)	2	**0.0019**	**0.0000**	**0.0000**	**0.0000**	0.8704	**0.0000**	**0.0012**	**0.0011**
Residual	32								
Total (Corr.)	50								

Table 35. Effects of creating disease suppressive conditions using different rotations and biocontrol products on plant vigor, bed fill and total crown number of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006-2007.[x]

Summer rotation	Fall rotation	Product	Plant vigor rating (1-5)[y]	Bed fill rating (1-5)[z]	Total crown number/m^2
Fumigated strawberry	-	-	4.33 NS	4.33 a	14.08 NS
Continuous strawberry	-	-	3.00	2.17 f	5.08
Squash	Hairy Vetch	-	4.33	3.50 abcde	9.73
Squash	Hairy Vetch	Plantshield	3.50	3.50 abcde	7.68
Squash	Hairy Vetch	Mycostop	3.83	3.83 abcd	10.58
Squash	Rye	-	4.33	3.83 abcd	11.61
Squash	Rye	Plantshield	4.50	4.00 abc	12.21
Squash	Rye	Mycostop	4.33	3.83 abcd	11.80
Sweet Corn	Buckwheat	-	4.00	3.33 abcde	7.85
Sweet Corn	Buckwheat	Plantshield	3.67	3.33 abcde	9.41
Sweet Corn	Buckwheat	Mycostop	4.00	3.00 cdef	7.32
Sweet Corn	Clover	-	3.50	2.67 ef	7.54
Sweet Corn	Clover	Plantshield	4.17	3.33 abcde	9.91
Sweet Corn	Clover	Mycostop	2.67	2.83 def	7.25
Sweet Corn	Kale	-	3.67	3.33 abcde	7.57
Sweet Corn	Kale	Plantshield	4.50	4.17 ab	10.77
Sweet Corn	Kale	Mycostop	3.00	3.17 bcdef	8.19

ANOVA

Effects	Df	Significance (p)		
Rotation (R)	16	0.0538	**0.0276**	0.4738
Block (B)	2	**<0.0001**	**<0.0001**	**<0.0001**
Residual	83			
Total (Corr.)	101			

[x] All means followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed on n=6.

[y] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[z] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

Comparing interactions between the products and the rotations, revealed that rotations had significant effects in 2006, but not 2007. Strawberries that followed the squash/rye or squash hairy vetch rotation tended to have better plant vigor, bed fill, and total crown number in 2006 than any of the other rotations (table 36). The success of these rotations could be due to these being the only rotations that had two well established crops prior to the strawberries. The other fall rotation crops did not establish well do to time of planting. Table 36 also indicates that the effects of rotation crops may be lost after the first year. Plants in plots receiving Plantshield had better bed fill than either Mycostop or nothing at all. Plantshield tends towards causing plants to have more mass and be healthier (table 36). Rotations and products were not significant for any parameter measured over the two years (table 37).

Due to the large number of rotation and product combinations, the most promising were chosen from data collected on the beds. These rotations (squash/rye and squash/hairy vetch) were subjected to more intensive sampling for nematodes and fungal isolations. These rotations still supported strawberry growth that tended to be better than the untreated continuous control and were most like the growth in the fumigated plots.

Table 36. Effects of creating disease suppressive conditions using different rotations and biocontrol products on plant vigor, bed fill, total crown number, biomass and yield of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.[w]

[w] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant differences test p=0.05. Pairwise comparisons performed with n=9.

[x] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[y] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

[z] Statistical separations performed after log(x) transformation.

Rotation		2006			2007							
Summer/Fall	Begin. mother number	Plant vigor rating (1-5)[x]	Bed fill rating (1-5)[y]	Total crown number/ m^2	Plant vigor rating (1-5)[x]	Bed fill rating (1-5)[y]	Total crown number/ m^2	Total berry number	Single berry wt. (g)[z]	Fruit yield /m (kg)	Fresh biomass /m^2(g)	Dry biomass /m^2 (g)
Squash/Hairy Vetch	8.22 NS	3.78 ab	3.56 ab	6.33 ab	4.00 NS	3.67 NS	12.33 NS	64.56 NS	3.70 NS	0.28 NS	93.95 NS	32.39 NS
Squash/Rye	9.22	4.33 a	3.89 b	7.03 a	4.44	3.89	16.72	62.39	4.05	0.27	106.26	36.33
Sweet Corn /Buckwheat	7.89	3.22 b	2.78 a	4.55 c	4.56	3.67	11.83	46.56	4.45	0.24	80.70	29.50
Sweet Corn/Clover	8.67	3.00 b	2.78 a	4.75 bc	3.89	3.11	11.72	38.72	4.50	0.18	74.36	25.72
Sweet Corn/Kale	8.89	3.22 b	3.33 ab	5.57 abc	4.22	3.78	12.11	45.78	5.39	0.19	90.32	31.83
Product												
None	8.13 NS	3.47 NS	3.07 NS	5.15 NS	4.47 NS	3.60 NS	12.57 NS	47.43 NS	4.49 NS	0.22 NS	86.20 NS	30.79 NS
Plantshield	9.00	3.67	3.47	6.19	4.47	3.87	13.80	57.90	4.07	0.21	99.94	34.42
Mycostop	8.60	3.40	3.27	5.59	3.73	3.40	12.47	49.47	4.69	0.26	81.21	28.26
ANOVA												
Effects	Df						Significance (p)					
Rotation (R)	4	0.6726	**0.0323**	**0.0452**	**0.0320**	0.6904	0.6456	0.1613	0.0761	0.5088	0.3128	0.6771	0.6842
Product (D)	2	0.5246	0.7176	0.4670	0.2916	0.1411	0.5359	0.6996	0.3961	0.6228	0.4425	0.5543	0.5554
Block (B)	2	**0.0045**	**0.0036**	**0.0000**	**0.0000**	**0.0054**	**0.0000**	**0.0000**	**0.0000**	0.6285	**0.0000**	**0.0011**	**0.0011**
RxD	8	0.7053	0.3482	0.6804	0.8134	0.8174	0.8976	0.8351	0.9059	0.6848	0.9127	0.8785	0.9115
Residual	28												
Total (Corr.)	44												

Table 37. Effects of creating disease suppressive conditions using different rotations and biocontrol products on plant vigor, bed fill, and total crown number of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006-2007.[x]

Summer rotation	Fall rotation	Plant vigor rating (1-5)[y]	Bed fill rating (1-5)[z]	Total crown number/m^2
Squash	Hairy Vetch	3.89 NS	3.61 NS	9.33 NS
Squash	Rye	4.39	3.89	11.87
Sweet Corn	Buckwheat	3.89	3.22	8.19
Sweet Corn	Clover	3.44	2.94	8.24
Sweet Corn	Kale	3.72	3.56	8.84
Product				
None		3.97 NS	3.33 NS	8.86 NS
Plantshield		4.07	3.67	10.00
Mycostop		3.57	3.33	9.03
ANOVA				
Effects	Df		Significance (p)	
Rotation (R)	4	0.1134	0.0532	0.2589
Product (D)	2	0.1552	0.3289	0.6923
Block (B)	2	**<0.0001**	**<0.0001**	**<0.0001**
R x D	8	0.2250	0.8763	0.9542
Residual	73			
Total (Corr.)	89			

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant differences test p=0.05. Pairwise comparisons of averages n=18. For product, n=30.

[y] Plant vigor scale of 1 to 5 where 5 = Plants in excellent health, vigorous growth, superior runnering, 4 = 33% plants diseased or stunted, 3 = 50% plants diseased or stunted, 2 = Majority of plants diseased or stunted, and 1 = Plants very stunted, diseased.

[z] Bed fill scale of 1 to 5 where 5 = Runners healthy and establishing well, full bed, 4 = Fewer runner plants than in 5, 3 = Thinning evident, runner size and establishment good, 2 = Few runners, mother plants obvious, and 1 = Few to no runners establishing, mother plants.

Again, rotations did not significantly account for variance for any individual plant parameter. For individual mother plant measurements, there were no significant differences amongst the rotations (table 38). The plants from the squash/hairy vetch rotations had averages closest to the plants from the fumigated control over all parameters. The rotations containing a fall component of kale, buckwheat, or clover were essentially a rotation of squash or sweet corn followed by a fallow field because none of the crops established to any degree before being killed by frost. This explains the lack of differences evident in many of the variables between these rotations.

Although populations did not differ among the treatments, it became apparent that nematode populations did build up over time. There were no significant differences between total parasitic nematode population in any of the rotations for either year (table 39).

There was particular interest in the root lesion nematode (*P. penetrans*) due to its implication in BRR. Sweet corn/buckwheat, sweet corn/clover, and sweet corn/kale all tended to have populations that were similar to the fumigated plot while the other rotations had more root lesion nematodes than the fumigated control (table 39). This is probably due to the fallow condition that followed the buckwheat, clover, and kale since these failed to establish and did not provide a conducive environment for survival. The needle nematode, *Longidorus elongatus*, is also damaging to strawberry roots, so, due to its presence, it was also analyzed.

Table 38. Effects of creating suppressive soils using different rotations and biocontrol products on individual plant biomass and root health of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2007.*

Summer rotation	Fall rotation	Product	Fresh foliage wt. (g)	Fresh root wt. (g)	Total fresh wt. (g)	Root quality rating	Necrosis (%)
Fumigated	-	-	24.42 NS	15.29 NS	39.71 NS	3.33 NS	30.00 NS
Squash	Hairy Vetch	-	26.78	18.78	45.57	3.00	46.67
Squash	Hairy Vetch	Plantshield	17.29	12.95	30.24	2.00	40.00
Squash	Hairy Vetch	Mycostop	22.14	16.26	31.35	2.00	60.00
Squash	Rye	-	19.50	17.57	37.07	2.33	66.67
Squash	Rye	Plantshield	11.60	8.75	20.35	1.33	63.33
Squash	Rye	Mycostop	22.14	14.65	36.79	2.00	70.00
Continuous	-	-	9.12	15.85	24.97	2.33	73.33

ANOVA

Effects	Df	Significance (p)				
Rotation (R)	7	0.7914	0.8711	0.8560	0.8561	0.0892
Block (B)	2	0.5212	0.2769	0.4275	0.1492	**0.0072**
Residual	14					
Total (Corr.)	23					

* Values followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed with n=3.

Table 39. Effects of different rotations on nematode populations, by season sampled, in 100 cm^3 soil or 1 g roots of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2006 and 2007.[x]

		Spring 2006	Fall 2006	Fall 2007			
Summer rotation	**Fall rotation**	**Total parasitic nematodes[y] (soil)**	**Total parasitic nematodes[y] (soil)**	***P. penetrans* (soil)**	**Total *P. penetrans* (roots and soil)**	***L. elongatus* (soil)[z]**	**Total parasitic nematodes[y] (soil)**
Fumigated	-	0.67 NS	2.33 NS	0.00 NS	14.33 NS	1.67 NS	25.00 NS
Continuous	-	10.33	5.33	1.67	7.00	5.33	58.33
Squash	Hairy Vetch	8.33	6.67	2.00	30.67	7.67	63.33
Squash	Rye	4.00	4.67	2.33	27.00	4.00	45.33
Sweet Corn	Buckwheat	11.33	5.00	1.67	65.67	0.00	72.33
Sweet Corn	Clover	4.33	4.00	2.33	39.00	13.00	56.00
Sweet Corn	Kale	14.67	2.00	5.33	46.67	10.67	62.00
ANOVA							
Effects	Df			Significance (p)			
Rotation (R)	6	0.1636	0.4506	0.7073	0.7786	0.5679	0.9486
Block (B)	2	0.2890	**0.0242**	0.1587	0.9066	0.2079	0.8905
Residual	12						
Total (Corr.)	20						

[w] Appendix C has risk ratings for parasitic nematodes on strawberries in Michigan.

[x] Values followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed with n=3.

[y] Total parasitic nematode numbers averages includes: *Pratylenchus penetrans*, *Trichodorus* spp., *Criconemella* spp., *Meloidogyne* spp., *Longidorus elongatus*, and *Paratylenchus* spp.

[z] Statistical analysis performed after log(x+1) transformation

Table 40. Effects of creating suppressive soils using different rotations and biocontrols on frequency of fungal genera isolated from roots of strawberry cv. Allstar in a black root rot infested soil in East Lansing, MI, in 2007.*

Genera of isolated fungi	Continuous Strawberry	Fumigated Strawberry	Squash/Rye	Squash/Rye with Plantshield	Squash/Rye with Mycostop	Squash/Hairy vetch	Squash/Hairy vetch with Plantshield	Squash/Hairy Vetch with Mycostop
Rhizoctonia	15.4	48.1	30.8	14.3	10.3	36.0	26.7	14.3
Fusarium	7.7	3.7	3.8	-	20.7	4.0	-	-
Cylindrocarpon	7.7	-	-	19.0	-	-	13.3	4.8
Phoma	65.3	25.9	46.2	47.6	20.7	32.0	26.7	57.2
Pythium	3.7	-	-	-	-	-	-	9.5
Epicoccum	-	-	-	-	3.4	-	-	-
Chaetomium	-	3.7	3.8	-	-	-	-	-
Codinaea	-	7.4	3.8	-	13.8	4.0	13.3	-
Alternaria	-	-	3.8	-	10.3	4.0	13.3	4.8
Perconia	-	-	-	4.8	-	-	-	-
Acremonium	-	-	-	-	3.4	-	-	-
Gymnoascus	-	-	-	-	-	4.0	-	-
Cordyceps	-	-	-	-	-	4.0	-	-
Tetracladium	-	3.7	-	-	-	-	-	-
Unidentified	3.8		3.8	9.6	6.8	4.0	6.7	9.6
Total fungi Isolated	26	27	26	21	29	25	15	21

*Isolated from 30 lesions per rotation/product combination. Percentage of fungal genera isolated calculated from total number of fungi isolated.

The overriding principle from this study is that strawberries, managed properly from the beginning of the planting, can handle limited disease pressure. The major issue throughout this study is the quality of the initial planting and possible desiccation of the crowns because they were not planted deep enough. This cultural factor may have weakened the plants to become more susceptible to the fungal and parasitic nematode populations. The development a decline often has origins in the cultural practices of the grower. This study provides strong evidence that, although the BRR pathogens are present, properly cultivated strawberries can still be productive and that rotations can make a difference, but one year of rotations is not sufficient. In addition, suppressive soils are difficult to develop. Multiple applications of biocontrol products should be considered to replenish the populations and increase the chances of establishment for the beneficial organisms.

Further evidence that this disease complex is highly variable is apparent when considering that the fumigated continuous strawberry control had a high average of pathogenic fungi isolated from it, yet this treatment tended to have the highest averages over most of the bed parameters. These plants may have had an early establishment advantage. *Rhizoctonia* is a fast growing fungus that re-populates fumigated soil quickly (table 40), especially in this study where the small plots were surrounded by non-fumigated soil. This ability makes it more likely to be the only fungus in the soil to populate the roots of plants in a fumigated area, while plants in the non-fumigated soil have many different genera of fungi and other microbes that may exclude the pathogens. Although, the untreated continuous strawberries had *Rhizoctonia* and parasitic nematodes

present, along with other fungi that have been implicated in strawberry decline such as *Cylindrocarpon* spp.

Overall, a crop rotation prior to establishing strawberries is a good practice. Others have found that short rotations are not sufficient, in studies with 'Honeoye' strawberries, LaMondia *et al.* (2002) did not find that rotation crop affected pathogen recovery from roots of 2-year old strawberry crowns. LaMondia (1999) found that there were no differences in nematode populations after one year of strawberry growth, regardless of the previous crop. A one year crop rotation can not be expected to compete with fumigation, but it can provide economic benefits, allow healthy strawberry bed establishment, and be environmentally conscious. Crops that establish well, such as rye and hairy vetch, and grow for a longer time in the climate of Michigan, can enhance the soil conditions for a following strawberry crop. While there is a strong trend for the benefits of Plantshield, more work should be done, perhaps with multiple applications, to discover if this product really does limit BRR pathogens. This study also made it apparent more nematodes or fungi does not mean a decline in strawberry production if the plants become well established at planting and are kept healthy with proper cultural practices that meet the needs of the plant.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Barnett, H. L., and Hunter, B. B. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press. St. Paul, Minnesota.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins Company, Baltimore.

Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. J. of Nematology 3:378-385.

Chet, I., and Henis, Y. 1983. *Trichoderma* as a biocontrol agent against soilborne root pathogens. Pages 110-112 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Chet, I., Ordentlich, A., Shapira, R., and Oppenheim, A. 1991. Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria. Pages 229-336 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

D'Ercole, N., Nipoti, P. and Manzali, D. 1989. Research on the root rot complex of strawberry plants. Acta Horticulturae 265:497-501.

Domsch, K. H., Gams., W., and Anderson, T-H. 1980. Compendium of Soil Fungi. Academic Press Inc., New York.

Elmer, W. H., and LaMondia, J. A. 1999. Influence of ammonium sulfate and rotation crops on strawberry BRR. Plant Dis. 83:119-123.

Ettlinger, M. G., and Kjaer, A. 1968. Sulfur compounds in plants. Pages 59-144 *In* Recent advances in phytochemistry. T. J. Mabry, Ed. New York: Appleton-Century-Crofts.

Fravel, D. R., and Keinath, A. P. 1991. Biocontrol of soilborne plant pathogens with fungi. Pages 237-243 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Harman, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease 84:377-392.

Hildebrand, A. A., and West, P. M. 1941. Strawberry root rot in relation to microbiologiclal changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research 19:183-198.

Hoitink, H. A. J., Stone, A. G. and Han, D. Y. 1997. Suppression of plant diseases by composts. HortScience 32:184-187.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Reporter 48:692

LaMondia, J. A. 1999. Influence of rotation crops on strawberry pathogens: *Pratylenchus penetrans*, *Meloidogyne hapla*, and *Rhizoctonia fragariae*. Supp. Journal of Nematology, 31:4S.

LaMondia, J. A., Elmer, W. H., Mervosh, T. L., and Cowles, R. S. 2002. Integrated management of strawberry pests by rotation and intercropping. Crop Protection 21:837-846.

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Martin, F. N., and Hancock, J. G. 1983. Chemical factors in soils suppressive to *Pythium ultimum*. Pages 113-116 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Parke, J. L. 1991. Root colonization by indigenous and introduced microorganisms. Pages 33-42 *In* The Rhizosphere and Plant Growth. D. L. Keister and P. B. Cregan, Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Perry, S. and Ramsdell, D. 1994. Strawberry diseases in Michigan. Ag Facts, Michigan State University Extension Bulletin E-1728:1-4.

Pritts, M., and Handley, D. Eds. 1998. Strawberry Production Guide: For the Northeast, Midwest, and Eastern Canada. Northeast Regional Agricultural Engineering Service Cooperative Extension. Ithaca, New York.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schippers, B., Bakker, A. W., and Bakker, P. A. H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Re. Phytopahtoogy 25:339-358.

Snapp, S. S., and Mutch, D. R. 2003. Cover crop choices for Michigan vegetables. Michigan State University Extension Bulletin E 2896:1-6.

Townshend, J. L. 1989. Population densities of four species of root-lesion nematodes (*Pratylenchus*) in the oat cultivars, Saia and OAC Woodstock. Can. J. Plant Sci. 69:903-905.

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany 52:487-511.

CHAPTER SIX: SLOWING BLACK ROOT ROT IN DECLINING ESTABLISHED STRAWBERRY FIELDS

Introduction

In Michigan, strawberries are typically produced in perennial, matted-row plantings in U-Pick operations. Black root rot is a disease complex that plagues older strawberry fields and results in diminished yields to the point where the planting becomes economically unsustainable. A number of fungi and the root lesion nematode have been implicated in this disease (Hildebrand, 1934; Wing *et al.*, 1994; Maas, 1998). Black root rot often develops in fields that have been replanted to strawberries.

The recommendation is frequently made to increase the length of time when the field is planted to crops other than strawberries, or to find a new location for the strawberries (Perry and Ramsdell, 1994). However, land constraints are frequently the reason why strawberries cannot be grown in a different area on a given farm, and cessation of strawberry production is not a viable choice. In such cases, prevention or slowing of crop decline are extremely desirable. Testing of treatments that might prolong the useful life of the planting would be of value.

While the product fungicide Abound (azoxystrobin) is labeled as a drench in strawberries for control of *Rhizoctonia* root rot, this claim has not been tested in Michigan. ProPhyt is one of the phosphorous acid fungicides, which are highly systemic. Since this fungicide has good efficacy against oomycete pathogens, it may have the potential to control BRR-related decline. Furthermore, the potassium in this product may have some nutritional value as well. The phosphorus component is not available to the plant in the phosphite form, however.

While several products are labeled for use specifically against pathogenic organisms that are part of the black root rot complex, growers are continually supplied information on numerous products that are said to improve plant health, but that have little scientific support showing efficacy. In order to develop recommendations for increasing the longevity and profitability of perennial strawberry plantings, this research investigated the effectiveness of some products to slow or reverse BRR decline (fungicides and nutritional amendments). While used as a foliar fertilizer, Vigor-Cal-Phos contains phosphites, like ProPhyt, and may provide essential micronutrients (table 42) that aide in plant defenses. In 'Sweet Charlie' strawberries, a ProPhyt 4L dip treatment followed by a foliar application increased the percentage of healthy plants compared to plants that were not treated after 24 days in a North Carolina study looking to control *Phytophthora cactorum* (Louws *et al.*, 2004). Symbex 4x is designed to provide nutritional benefits as well as encouraging beneficial microbial populations. C/G is a fatty acid compound that has shown general fungicidal properties on other crops in previous studies (Schilder *et al.*, 2003).

Materials and Methods

Two commercial production sites with BRR symptoms were selected for the efficacy trial (site O in Ottawa County and site L in Leelanau County). Each site had seven different treatments in a randomized complete block design with four blocks (table 42). Each treatment plot consisted of three 3.05-m rows. All rows were treated with their respective product, with only the center row used for data collection. The treatments were applied as either a foliar spray or a drench (table 43). Sprays were

applied at a rate of 467.5 L/ha, every 2 wk, at 50 psi with a backpack sprayer. The control remained untreated. All plots received the standard practices of the cooperator. The drenches were applied at a rate of 18,700 L/ha using 19-L containers to mix the drenches and pour them over the rows. C/G was applied at 0.05% v/v rate at the first application and subsequently at 0.2%. The growers were permitted to continue their standard cultural practices over the duration of the project (table 41).

At the time of set-up, plants were removed from rows beside each replication, but not from rows within the plots, to allow for an initial pathogen evaluation. Plants were removed with 200 g of surrounding soil for analysis. Fungi were isolated from the roots using process described below. Nematode presence in soil and roots was determined by the Michigan State University Diagnostic Laboratory. Nematode presence was determined using the modified Jenkins technique and a modified root extraction process (Jenkins, 1964; Bird, 1971). An initial bed fill rating was also taken. At both locations, bed fill was assessed on a 1 to 5 scale, with 5 = runners healthy and establishing well, full bed, 4 = fewer runner plants than in 5, 3 = thinning evident, runner size andestablishment good, 2 = few runners, mother plants obvious, and 1 = few to no runners establishing, mother plants.

Plant volume was calculated from two radius measurements at right angles to one another, and a plant height measurement. Five mother plants were chosen in the center row for this measurement. Two plants were removed (June or July, depending on the site) from each treatment for fresh and dry biomass as well as a root necrosis rating. This rating was on a 1 to 5 scale with 1 = mostly black, dark brown, no finely branched roots and a single crown; 2 = same as 1, except one or two finely-branched roots present;

3 = half of all roots black/dark brown and unbranched, and 1 or 2 crown branches; 4 = more white, fine, branched roots present than black/brown unbranched roots, and > 2 crown branches; and 5 = white, fine, and multi-branched roots and crown. From each of these dug plants, 1 g of fresh root tissue was removed after the root necrosis rating, but before taking the fresh root weight, for fungal pathogen evaluation.

Table 41. Cooperator application rates and dates in the effort to slow black root rot in infested established strawberry fields in Ottawa (cv. 'Jewel') and Leelanau (cv. 'Northeaster'), MI during 2007.

	Product	Active Ingredient	Rate	Time of Application
Leelanau Co. Site				
Fungicides	Captan	Captan	1.8 qts/A	20 May
	Elevate	Fenhexamid	1.5 lbs/A	20 May
	Captan	Captan	1.8 qts/A	2 Jun
	Pristine	Pyraclostrobin and boscalid	20 ozs/A	2 Jun
Insecticides	Thiodan 3 EC	Endosulfan	1.33 qts/A	20 May
	Thiodan 3 EC	Endosulfan	1.33 qts/A	2 Jun
Fertilizers	Urea	-	60 lbs/A	21 Apr
Ottawa Co. Site				
Fungicides	Quadris	Azoxystrobin	10 oz/A	28 April
	Captec 4L	Captan	2 qt/A	28 April
	Cabrio EG	Pyraclostrobin	12oz/A	11 May
	Switch	Cyprodinil and Fludioxonil	10 oz/A	11 May
	Elevate	Fenhexamid	1.5 lbs/A	29 May
	Nova	Myclobutanil	2.5 oz/A	29 May
	Nova	Myclobutanil	2.5 oz/A	6 Aug
	Nova	Myclobutanil	2.5 oz/A	5 Sept
Herbicides	Select (+oil 1%)	Clethodim	6 oz/A	17 May
	2,4-D Amine, Weedar 64 47%	2, 4-D	1 qt/A	2 Aug
	Sinbar	Terbacil	1 qt/A	29 Aug
	2, 4-D	2, 4-D	1 qt/A	20 Oct
Insecticides	Thiodan 50 W	Endosulfan	2 lb/A	11 May
	Lorsban 4E	Chlorpyrifos	2 pt/A	11 May
	Advise (Admire)	Imidacloprid	23oz/A	1 Aug
	-	Dimethoate	0.5 pt/A	6 Aug
Fertilizers	12-12-12	-	200 lbs/A	12 July
	Calcium nitrate	-	100 lbs/A	20 April
	Potassium nitrate	-	100 lbs/A	20 Sept

Table 42. Active ingredients of products used in effort to slow black root rot in infested established strawberry fields in Ottawa (cv. 'Jewel') and Leelanau (cv. 'Northeaster'), MI during 2007.

Product	Company	Product Classification	Active Ingredients
Symbex 4x	Ago K	Fertilizer	Microbial enzymes, Calcium carbonate, Cobalt carbonate, Zinc carbonate
Vigor-Cal-Phos	Agro K	Fertilizer	Calcium phosphite, Copper phosphite
ProPhyt	Helena	Fungicide	Potassium phosphite
Abound	Syngenta	Fungicide	Azoxystrobin
Abound + ProPhyt	Syngenta and Helena	Fungicide	As above
C/G	Summerdale, Inc.	Fungicide	Pelargonic acid

Table 43. Application method, rate, and schedule for products used in effort to slow black root rot in infested established strawberry fields in Ottawa (cv. 'Jewel') and Leelanau (cv. 'Northeaster'), MI during 2007.

			Application Schedule	
Product	**Applied As**	**Applied Rate**	*Site O**	*Site L***
Symbex 4x	Drench	2 qts/acre	1,5,9	1,3
Vigor-Cal-Phos	Spray	3 qts/acre	1,2,3,4,5,6,7,8,9	1,2,3
ProPhyt	Spray	4 pts/acre	1,2,3,4,5,6,7,8,9	1,2,3
Abound	Drench	0.8 fl. oz./1000 ft. row	1,5,9	1,3
Abound + ProPhyt	Drench + Spray	As above	1,5,9 + 1,2,3,4,5,6,7,8,9	1,3 + 1,2,3
C/G	Drench	0.2% v/v	1,5,9	1,3

* Dates of applications for Ottawa Co. site: 1=23 May 2007, 2=6 June 2007, 3=20 June 2007, 4=5 July 2007, 5=18 July 2007, 6=2 August 2007, 7=15 August 2007, 8=29 August 2007, and 9=8 September 2007

** Dates of applications for Leelanau Co. site: 1=22 May 2007, 2=5 June 2007, and 3=19 June 2007.

Leelanau County Site

The trial was begun on 22 May 2007 in a field of 'Northeaster' strawberries that had been established in 2004 after a fall fumigation with Telone C35 at the rate of 25 GPA in Lake Leelanau, MI. The transplants were originally purchased from Nourse Farms (South Deerfield, MA). The matted row planting necessitated the use of an aerator (Swisher AE-48, Warrensburg, MO) by hand to ensure that the drenches would reach the root zone. The aerator was used at the first application only because of the concern of damaging fruit in subsequent applications. The soil was aerated to a depth of 5-8 cm. Applications were made on the schedule given in table 43. The strawberries were harvested on 21 June 2007 and again on 28 June 2007. A bed fill rating and plant volume measurement were also obtained at the second harvest date. In June, after the last harvest, two plants, chosen from the five plants that were used for the plant volume measurement, were dug from the central meter of the plot to assess fresh foliage, fresh root, dry foliage, and dry root mass, and obtain a root necrosis rating. The trial at this location was ended in early July because the field was plowed up by the grower because of poor yield.

Ottawa County Site

On 23 May 2007 a trial was established in a field of 'Jewel' strawberries in Hudsonville, MI that had been established in 2005 following a plow-down of sorghum sudan grass. The transplants were purchased from Krohne Plant Farms, Inc. (Hartford, MI). The cooperator utilized raised beds with drip tape to establish the strawberry rows at this location, making aeration unfeasible. Applications were made according to the

schedule given in table 43, starting when the strawberry fruit was thumb-sized. Harvests occurred on 13 June, 19 June, and again on 27 June 2007. The first plant volume measurement and a bed fill rating were also obtained at the third harvest date. Bed fill ratings were also taken on 23 July and at the end of the experiment on 14 September 2007. A second plant volume measurement was taken 23 July. On 30 July 2007, two representative plants were dug to assess fresh foliage, fresh root, dry foliage, and dry root mass, and obtain a root necrosis rating. These plants were taken from the center row, but not the central meter so as not to interfere with harvest data the second year of the study.

Yield Estimation

The interior meter of the center row was marked and all ripe berries were picked at each harvest date. Berries were counted at the time of picking and weighed on location. The berries were picked without regard to size or condition.

Plant Material Processing

From each of the dug plants, 1 g of fresh root tissue was removed after the root necrosis rating, but before taking the fresh root weight, for fungal isolations. All plants were stored at 4°C until processing. The first plant of each treatment, from Leelanau Co. site, was processed on 5 July 2007 after removing all flower stalks, runners, and dead leaves, in addition to knocking as much soil off of the plant as possible before the roots were washed under cold running water. The rating was taken, and then fresh weights were obtained by cutting the crown in half just above the uppermost adventitious roots. The foliage and roots were then dried for 48 h at 80°C to obtain dry weights. The second

plant of each treatment was processed 10 June 2007 using the same procedure. The process was repeated for the Ottawa Co. site, with the exception that all plants were processed on the same day in August.

The roots were surface sterilized with 20% bleach solution for 2 min and then rinsed for 1 min in two sequential sterile water baths. The roots were dried on sterile paper towels, and ten lesions were selected to be placed on water agar, at five 6-mm root pieces per plate. Plates were left on the laboratory bench and checked daily for growth for 8 d. If growth was observed, fungi were sub-cultured onto potato dextrose agar and subsequently identified morphologically upon plate colonization (Barnett and Hunter, 1998; Domsch *et al.*, 1980; Barron, 1968). Those fungi that could not be identified in this manner were subsequently identified by sequencing the DNA of the internal transcribed spacer (ITS) regions by Timothy Miles (Sambrook *et al.*, 1989; Altschul *et al.*, 1990).

Nematode samples were taken at the Ottawa site again at the end of the experiment in September. Nematode presence was determined using the modified Jenkins technique and a modified root extraction process by Fred Warner in the Michigan State University Diagnostic Services laboratory (Jenkins, 1964; Bird, 1971). A nematode sample was taken again in October to ensure that the populations had been properly evaluated at the Ottawa site. Plants and surrounding soil dug for the September sampling were removed from the outside rows so as not to interfere with future data collection. Only a composite soil sample from the top 15-20 cm within the center row was evaluated for nematodes in October.

Data Analysis

All statistical analyses was performed using the ANOVA and mean separation functions (Fisher's protected least significant difference test at p=0.05) of the StatGraphics (StatPoint Inc., VA) statistical computer program after checking for equality of variance. When analyzing the bed fill data over multiple sampling times, it was necessary to utilize SAS 9.1 (SAS Institute Inc., Cary, N.C.) using repeated measurement ANOVA in proc glm. This procedure was also used for the analysis of plant volume data at the site in Ottawa county.

Each site was analyzed separately due to differences in variety, age of planting, the duration of the experiment at each location, the presence of nematodes at one site, and the difference in production and management practices.

The frequency of fungi recovered was calculated based on the total number of fungi that grew. The number of roots showing no growth was calculated based on the total number of roots.

Rsults and Discussion

Both strawberry sites were in serious decline; in fact, the Leelanau Co. site was taken out of production in 2007. The initial pathogen assessment revealed that the Ottawa Co. site suffered from high levels of needle nematodes (*Longidorus elongatus*) as well as several fungal root pathogens (*Fusarium* and to a lesser extent *Rhizoctonia, and Cylindrocarpon*), whereas the Leelanau Co. site had predominantly fungal pathogens (mostly *Rhizoctonia fragariae*) and no nematodes (tables 44 and 45).

Table 44. Preliminary fungal isolations from 40 strawberry root pieces from infested established strawberry fields in Ottawa (cv. 'Jewel') and Leelanau (cv. 'Northeaster'), MI prior to study evaluating products in an effort to slow black root rot during 2007.

	% Root Pieces Colonized*	
Genera Isolated	**Ottawa Co.**	**Leelanau Co.**
Rhizoctonia fragariae	-	50.0
Pythium sp.	26.9	-
Fusarium sp.	50.0	4.6
Cylindrocarpon sp.	11.5	9.1
Other**	11.5	36.4
Colonized root pieces	65.0	55.0
No colonization	40.0	45.0

* Multiple fungi would colonize the plate from a singe root piece.

** Other fungi included unknown fungi and *Phoma* spp.

Table 45. Preliminary nematode presence at the Ottawa site (cv. 'Jewel') prior to the study to evaluate products in an effort to slow black root rot in infested established strawberry fields during 2007 and the risk rating for needle nematode at this location.

Number of Nematodes in Soil (100 cc)					Nematodes in root tissue (1 g)
Pratylenchus penetrans	*Longidorus elongatus*	*Xiphinema americanum*	*Criconemella* spp.	*Trichodorus* spp.	*Pratylenchus penetrans*
0	33	4	0	0	4
0	36	2	0	0	0
8	145	0	3	0	8
0	140	0	0	0	0
4	45	0	0	1	3

* There were no parasitic nematodes found at site L. Samples obtained from plants surrounding area to be used in the study.

** Risk ratings for parasitic nematodes on strawberries in Michigan available in Appendix C.

At the Leelanau county site both time and time x block were significant ($p<0.001$) in the repeated measures ANOVA, but time x treatment was not ($p=0.6361$). While block was significant for the bed fill rating, upon further analysis the block x treatment interaction was not significant ($p=0.3670$). It was of interest to look at the bed fill rating over the entire season.

At the Ottawa county site time, time x block, and time x treatment were significant in the repeated ANOVA ($p=0.0001$, <0.0001, and 0.0458 respectively). Block ($p<0.0001$) and treatment ($p=0.0458$) were significant in the bed fill rating ANOVA. The block x treatment interaction was significant at this site ($p<0.001$). With the significance of the time x treatment interaction, it was not possible to look at the bed fill rating over the entire season.

There were no significant differences among treatments for any of the bed fill ratings. Abound did tend to produce the highest average ratings at both sites. The final bed fill rating at the Ottawa site was higher for beds receiving Abound or Abound + ProPhyt, but did not significantly differ from the untreated control. The block significance at the Ottawa site was driven by the block closest to the field which seemed to decline further, possibly due to poorer irrigation towards the edge of the field. At the site in Leelanau Co. the lack of differences between treatments for each bed fill rating is not surprising since the plants did not have an opportunity to set new daughter plants during the experiment (table 46). However, at the Leelanau site, over the season, beds receiving any treatment did tend to improve, with C/G improving the most when compared to the untreated control.

ProPhyt, C/G, and Symbex 4x tended to produce higher yields in Leelanau, but higher yields were observed in almost all treatments at both sites (table 47). There were no significant differences at either sites among the treatments for average berry weight or total berries (table 47).

Table 46. Effects of fungicide or fertilizer treatments on bed fill of cv. 'Jewel' or 'Northeaster' strawberries in a study to slow black root rot decline conducted in Leelanau and Ottawa counties in Michigan in 2007.[y]

	Initial bed fill (1-5)[z]		Harvest bed fill (1-5)		July bed fill (1-5)		Final bed fill (1-5)		Bed fill over entire season (1-5)	
Treatment	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*
Untreated	1.50 NS	1.50 NS	1.75 NS	1.75 NS	1.75 NS	-	1.50 NS	-	-	1.63 NS
Abound	2.00	1.75	2.50	2.50	3.25	-	3.00	-	-	2.13
Abound + ProPhyt	1.75	1.50	2.00	2.00	3.25	-	3.00	-	-	1.75
ProPhyt	1.75	2.00	2.00	2.00	3.00	-	2.25	-	-	2.00
C/G	2.00	2.50	2.25	2.25	2.00	-	2.25	-	-	2.38
Symbex 4x	1.75	1.75	2.25	2.25	2.50	-	2.50	-	-	2.00
Vigor-Cal-Phos	1.75	1.75	2.00	2.00	3.00	-	2.00	-	-	1.88

ANOVA

Effect	Df	Significance (p)							Df	p value
Treatment	6	0.7877	0.4926	0.9611	0.9611	0.3028	-	0.1312	-	6	0.6361
Block	3	0.0771	0.2801	0.1285	0.1285	0.0799	-	**0.0006**	-	3	**0.0158**
Residual	18									102	
Total (Corr.)	27									111	

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant differences test p=0.05. Pairwise comparisons of averages perfomed with n=4. For repeated measurement over the season, n=8.

[z] Bed fill was assessed on a 1 to 5 scale, with 5 = runners healthy and establishing well, full bed, 4 = fewer runner plants than in 5, 3 = thinning evident, runner size andestablishment good, 2 = few runners, mother plants obvious, and 1 = few to no runners establishing, mother plants.

Table 47. Effects of fungicide or fertilizer treatments on yield and plant volume of cv. 'Jewel' or 'Northeaster' strawberries in a study to slow black root rot decline conducted in Leelanau and Ottawa counties in Michigan in 2007.[y]

Treatment		Fruit yield/m (kg)		Single berry wt. (g)		Total berry number		Beginning plant volume cm^{3x}		Ending plant volume cm^3	
		Site O	*Site L*[z]	*Site O*	*Site L*[z]	*Site O*	*Site L*[z]	*Site O*	*Site L*	*Site O*	*Site L*
Untreated		0.69 NS	0.50 NS	3.88 NS	4.71 NS	167.25 NS	104.00 NS	3387.45	2540.80	987.35	-
Abound		1.12	0.63	5.12	5.15	205.50	124.25	5344.05	3597.05	3356.80	-
Abound + ProPhyt		0.64	0.68	3.95	5.75	149.75	119.50	5728.5	2259.90	3016.55	-
C/G		0.92	0.84	4.33	5.00	209.50	167.50	3441.55	3096.10	1963.55	-
ProPhyt		0.82	0.99	4.15	5.86	179.00	162.25	5399.70	3600.60	3082.85	-
Symbex 4x		0.87	0.84	4.32	5.07	183.50	167.00	3436.45	3921.15	2298.45	-
Vigor-Cal-Phos		0.83	0.73	4.54	5.06	181.75	144.25	2739.55	2754.80	2669.45	-
ANOVA											
Effects	Df	Significance (*p*)									
Treatment	6	0.6660	0.3054	0.5650	0.5244	0.7548	0.1825	0.4095	0.0725	0.3604	-
Block	3	**0.0120**	0.4016	**0.0392**	0.5921	**0.0023**	0.0753	0.2178	**0.0061**	0.0677	-
Residual	18										
Total (Corr.)	27										

[x] Plant volume calculated by taking average of two radius measurements and calculating the volume using the formula: $4/3(\pi r^3)$

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant differences test p=0.05. Pairwise comparisons of harvest parameter averages perfomed with n=4. Plant volume pairwise comparisons performed with n=20.

[z] Statistical analysis was performed after log(x) transformation

In Ottawa, use of the fungicides Abound, ProPhyt, and Abound + ProPhyt tended to have higher plant volumes when compared to the untreated control. For the second plant volume measurement in Ottawa Co., all treatments had higher average plant volumes when compared to the control. In Leelanau all treatments but ProPhyt + Abound tended to have a higher average plant volume than the untreated control (table 47).

The Abound treatment numerically had the highest average fresh foliage weight, but was not significantly different from any other treatment at both sites. Abound provided the only difference from the untreated control at the Ottawa site for average fresh root weight (table 48). There were no significant differences among the treatments for dry foliage weight at either site. In Ottawa county the only significant improvement in dry root weight was provided by Abound, when compared to the untreated control. At the site in Ottawa Co. use of C/G, Abound, and ProPhyt resulted in numerically less root necrosis. All treatments differed from the control in Leelanau Co., with Abound producing roots with the least necrosis. Abound was the only significantly improved treatment from the untreated control for total fresh and dry plant weights in Ottawa. There were no significant differences between treatments for either parameter in Leelanau Co., but Abound and Abound + ProPhyt tended to be higher.

Table 48. Effects of fungicide or fertilizer treatments on plant weights and root necrosis in cv. 'Jewel' or 'Northeaster' strawberries in a study to slow black root rot decline conducted in Leelanau and Ottawa counties in Michigan in 2007.[x]

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant differences test p=0.05. Pairwise comparisons of averages perfomed with n=6.

[y] Rating was on a 1 to 5 scale with 1 = mostly black, dark brown, no finely branched roots and a single crown; 2 = same as 1, except 1 or 2 finely-branched roots present; 3 = half of all roots black/dark brown and unbranched, and 1 or 2 crown branches; 4 = more white, fine, branched roots present than black/brown unbranched roots, and > 2 crown branches; and 5 = white, fine, and multi-branched roots and crown.

[z] Statistical analysis was performed after log(x) transformation.

Treatment	Fresh foliage wt. (g)		Fresh root wt. (g)		Dry foliage wt. (g)		Dry root wt. (g)		Root necrosis rating (1-5)[y]	
	Site O	*Site L*	*Site O*	*Site L*	*Site O*[z]	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*
Untreated	5.40 NS	25.55 NS	36.83 ab	75.41 NS	2.02 NS	7.89 NS	14.03 ab	31.54 NS	1.00 NS	1.50 d
Abound	17.32	40.83	100.31 c	64.17	6.44	13.43	37.64 c	29.92	2.13	4.00 a
Abound + ProPhyt	14.84	24.60	66.41 b	81.19	5.35	7.43	25.89 bc	33.60	1.50	2.63 bc
C/G	8.53	35.89	55.86 ab	58.59	3.22	11.55	20.81 ab	27.33	2.13	2.50 c
ProPhyt	7.98	36.46	52.76 ab	52.16	3.44	12.44	23.35 ab	23.20	2.25	3.25 abc
Symbex 4x	7.56	29.14	60.82 ab	57.10	2.91	9.71	23.52 ab	26.17	1.63	3.13 bc
Vigor-Cal-Phos	5.62	26.97	31.20 a	67.16	7.04	8.94	11.98 a	31.51	1.50	3.38 ab
ANOVA										
Effects	Df					Significance (*P*)				
Treatment	6	0.1373	0.2407	**0.0067**	0.8586	0.3337	0.1860	**0.0128**	0.9679	0.0825	**0.0002**
Replication	3	**0.0345**	0.8697	**0.0076**	0.5586	**0.0221**	0.9733	**0.0152**	0.5958	0.0549	0.3232
Residual	18										
Total (Corr.)	27										

Table 48. cont'd

Treatment	Total fresh wt. (g)		Total dry wt.(g)	
	Site O	*Site L*	*Site O*	*Site L*
Untreated	42.23 ab	100.96 NS	16.05 a	39.43 NS
Abound	117.63 c	105.00	44.08 b	43.35
Abound + ProPhyt	81.26 bc	105.79	31.23 ab	41.03
C/G	64.39 ab	94.48	24.03 a	38.88
ProPhyt	60.75 ab	88.62	26.79 a	35.64
Symbex 4x	68.38 ab	86.24	26.43 a	35.88
Vigor-Cal-Phos	36.81 a	94.13	19.02 a	40.44
ANOVA				
Effects	Significance (*p*)			
Treatment	**0.0091**	0.9853	**0.0382**	0.9961
Replication	**0.0078**	0.6285	**0.0071**	0.6781

Table 49. Frequency of fungal genera isolated from diseased roots of cv. 'Jewel' or 'Northeaster' strawberries from fungicide or fertilizer treatments in a study to slow black root rot decline conducted in Leelanau and Ottawa counties in Michigan in 2007.*

Fungal Genera	Fungi that Colonized (%)													
	Untreated		Abound + ProPhyt		Symbex 4x		C/G		Abound		Vigor-Cal-Phos		ProPhyt	
	Site O	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*	*Site O*	*Site L*
Acremonium	-	7.0	1.9	4.2	-	-	-	2.2	-	5.4	-	3.5	3.3	-
Alternaria	9.3	14.0	-	8.3	4.8	6.5	-	8.9	-	10.8	-	12.3	3.3	4.2
Botrytis	-	-	-	2.1	-	-	-	-	-	-	-	-	-	-
Cephalosporium	-	-	-	-	-	-	-	-	-	-	-	1.8	-	-
Chaetomium	-	-	-	2.1	4.8	-	-	-	-	-	2.4	-	-	-
Cladosporium	-	-	-	-	-	-	-	2.2	-	-	-	-	-	-
Cylindrocarpon	11.6	4.7	7.4	14.6	7.1	8.7	8.9	6.7	20.5	16.2	16.7	12.3	5.0	2.1
Epicoccum	-	-	-	-	-	-	-	2.2	-	-	-	-	-	-
Fusarium	41.9	-	50.0	2.1	26.2	-	33.3	-	29.5	8.1	21.4	5.3	31.7	-
Gliocladium	-	-		2.1	-	-	-	-	-	-	-	-	-	-
Humicola	-	-	-	-	-	-	-	-	-	-	-	-	1.7	-
Mucor	-	-	-	-	-	-	2.2	-	-	-	-	-	-	-
Nectria	2.3	-	-	-	2.4	-	2.2	-	2.3	-	7.1	-	-	-
Penicillium	-	-	-	-	-	-	2.2	-	-	-	2.4	1.8	1.7	-
Phoma	11.6	37.2	27.8	29.2	21.4	32.6	13.3	44.4	20.5	29.7	7.1	22.8	16.7	62.5
Phomopsis	-	-	-	-	2.4	-	-	-	-	-	-	-	-	-
Pythium	2.3	-	-	-	16.7	-	2.2	2.2	4.5	-	4.8	-	6.7	-
Rhizoctonia	11.6	23.3	3.7	16.7	7.1	32.6	13.3	15.6	11.4	13.5	35.7	29.8	21.7	16.7
Sphaerodes	-	4.7	-	2.1	-	-	-	-	-	2.7	-	-	-	2.1
Streptomyces	4.7	-	-	-	2.4	-	-	2.2	2.3	-	-	-	-	-
Tetracladium	-	9.3	-	16.7	2.4	19.6	4.4	8.9	-	13.5	-	10.5	-	12.5
Trichoderma	2.3	-	-	-	-	-	13.3	4.4		-	2.4	-	1.7	-
Unidentified	2.3		9.4	-	2.4	-	4.4	-	9.1	-			6.6	
No Growth	46.3	46.3	32.5	40.0	47.5	42.5	43.8	43.8	45.0	53.8	47.5	28.8	25.0	40.0

* Fungi frequency calculated based on total fungi isolated from 80 root pieces per treatment from both locations.

Table 50. Average number of nematodes present in 100 cm^3 of soil and 1 g of roots at the Ottawa county site in a study evaluating products in an effort to slow black root rot in Hudsonville, MI in September and October 2007.

	September			
Treatment	**Soil**			**Root**
	Pratylenchus penetrans	*Longidorus elongatus*	*Criconemella* spp.	*Pratylenchus penetrans*
Untreated	2.75	7.00	11.50	8.50
C/G	2.50	6.25	3.25	2.50
	October			
Untreated	5.50	1.0	0.75	0.0

Except for Vigor-Cal-Phos and ProPhyt, the plants dug out at the Ottawa county site had an overall lower recovery of *Rhizoctonia* than those plants dug from the site in Leelanau Co. (table 49). *Pythium* sp. was predominately found in roots from Ottawa Co. The lowest recovery of *Rhizoctonia* occurred from plants treated with Abound + ProPhyt in Ottawa and Abound in Leelanau. Other fungi often associated with BRR were isolated from both sites, including *Cylindrocarpon* and *Fusarium* spp. As has been observed before with BRR complex, *Fusarium* was found at the Ottawa site where there were high parasitic nematode populations. This may indicate that some *Fusarium* spp. readily colonize roots after nematodes damage them.

This experiment had a few limitations. The experiment should have been started in April when the strawberries first began to grow for the season. The lack of suitable sites and time constraints prevented early set-up. An earlier application of products may have targeted the first root growth of the season. It also would have been easier to get the drenches into the root zone as there would have been less foliage.

Two weeks after the first application, it was noted that plants receiving drenches had visually more vigorous growth and higher fruit quality than the untreated control. The 2007 growing season was very dry. Some measurements, particularly those taken after harvest, were influenced by the irrigation schedule. This schedule was determined by the cooperator, and was not recorded. It was not possible to aerate the site in Ottawa Co. like was done in Leelanau Co. because of the drip tape under the hills. Fortunately, the soil was sandy enough to allow application of the drenches. An important consideration is that the site in Leelanau Co. did not receive as many applications as the

site in Ottawa Co. over the season because the evaluation ended when the grower plowed down the field.

The difference in specific (fungi vs. nematodes) pathogens present between the two sites may help explain differences in treatment effects between the sites. Nematodes alone can be very damaging, particularly with the populations found at the Ottawa Co. site (Chapman, 1956; Brown *et al.*, 1993). The potential for an interaction between the nematodes and the fungi also exists (Powell, 1971). The performance of the fungicides would be expected to make a greater impact at site the site in Leelanau Co. At the Ottawa Co. site, the addition of a nematicide may have improved the control of BRR. The data indicates that there was an improvement over the season at both sites for some treatments because of the reduction of fungal pathogens that may have been compounding the damage caused by the nematodes in Ottawa Co. (table 50).

Late spring to early summer applications of fungicides or foliar fertilizers improved root health and yield in strawberry fields declining due to black root rot. A drench of Abound was generally the most effective at improving root health, although foliar sprays of ProPhyt also worked well. While bed fill was not significantly different between the treatments, plant growth tended to be visually better in all treatments compared to the untreated plants. C/G is in the process of being developed as a fungicide and may be suitable for organic production.

Considering the success of the treatments incorporating Abound, it becomes necessary to consider resistance management, otherwise the use of this fungicide will be short-lived. It is always important to consider how growers will apply this on their own farm. In this instance a grower could apply Abound through trickle irrigation like that

used at the site in Ottawa county. One or two applications per season may provide some measure of control, if the application is timed appropriately. In addition, alternating between ProPhyt and Abound may provide control while managing resistance. Other growers could make use of a sweep cultivator to furrow along the rows and contain the drenches applied with a sprayer. This experiment will continue at the site in Ottawa in 2008. Larger-scale trials on farms with products applied through the irrigation system may be conducted in the future.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Barnett, H. L., and Hunter, B. B. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press. St. Paul, Minnesota.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams and Wilkins Company, Baltimore.

Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. J. of Nematology 3:378-385.

Brown, D. J. F, Dalmasso, A., and Trudgill, D. L. 1993. Plant Parasitic Nematodes in Temperate Agriculture. Nematode Pests of Soft Fruits and Vines. K. Evans, D. L. Trudgill, and J. M. Webster, Eds.. CAB, International.

Chapman, R. A. 1956. Plant parasitic nematodes associated with strawberries in Kentucky. Plant Dis. Reporter 40:179-183.

Domsch, K. H., Gams, W., and Anderson, T-H. 1980. Compendium of Soil Fungi. Academic Press Inc., New York.

Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Can. J. Research, Sec. C. 11:18-31.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Dis. Reporter 48:692.

Louws, F. J., Driver, J. G., and Leandro, L. 2004. Evaluation of fungicide pre-plant dip and spray applications to manage Phytophthora crown rot on strawberry plugs. American Phytopathological Society. Fungicide and Nematicide Tests, 60:SMF041

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Perry, S., and Ramsdell, D. 1994. Strawberry diseases in Michigan. Ag Facts, Michigan State University Extension Bulletin E-1728: 1-4.

Powell, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annual Review of Phytopathology 9: 253-274.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schilder, A. M. C., Gillett, J. M., and Sysak, R. W. 2003. Evaluation of fungicides for control of foliar diseases and black rot in grapes. American Phytopathological Society. Fungicide and Nematicide Tests, 59:SMF027

Wing, K. B., Pritts, M. P., and Wilcox, W. F. 1994. Strawberry BRR: A Review. Advances in Strawberry Research. 13:11-19.

CHAPTER SEVEN: EVALUATING RHIZOCTONIA VIRULENCE IN-VITRO

Introduction

While *Rhizoctonia* spp. are a vast group of fungi that are grouped largely by their lack of distinctive taxonomic feateures, with teleomorphic states in both the basidiomycetes and ascomycetes, the three groups associated with plant diseases includes: *R. solani*, the binucleate species, and the isolates with a *Waitea* teleomorph (Vilgalys and Cubeta, 1994).

Rhizoctonia spp. have a highly variable growth rate. Under certain conditions, some species produce sclerotia-like tufts consisting of short, broad cells that function as chlamydospores. The hyphae display characteristic right-angle branching with dolipore septa and a moniliform resting cell (Maas, 1998). The branches are slightly constricted and cross walls are present near the junction. Infrequently, the perfect stage, *Thanatephorus cucumeris* (A.B. Frank) Donk, is formed in the multinucleate species, or *Ceratobasidium* spp. in the binucleate species (Ogoshi and Ui, 1983; Burpee *et al.*, 1980).

Ogoshi and Ui (1983) developed anastomosis groups (AG) for Japanese isolates, while Burpee *et al.* (1980) developed groups for North America. Ogoshi (1985) then compared the two different groups and found that Burpee's seven groups corresponded to several groups in the Ogoshi system. Ogoshi's anastomosis groups AG-A, AG-G, and AG-I have been implicated in BRR of strawberries (Martin, 1988). *Rhizoctonia fragariae* is represented in groups AG-A, AG-G, and AG-I. Isolation frequency and virulence vary between and within each group and by site (Husain and McKeen, 1963; Burpee *et al.*, 1980; Martin, 1988; Mass, 1998; Martin, 2000). Martin (2000) found that isolates within AG-I were particularily virulent and that AG groups differed between

locations, and even within a single location depending on the time of year. Each of these AG's have different host ranges that should be considered before incorporating into a rotation prior to strawberry (Martin, 1988)

Due to the variability observed in disease development in field situations, and even in more controlled greenhouse environments, an efficient and expedient way to evaluate isolate pathogenicity was sought to ensure an optimum isolate was used in future trials. Therefore, an in-vitro virulence assay was evaluated.

Materials and Methods

Isolate Selection and Staining

Seven isolates were randomly chosen from the cultures maintained in Dr. Annemiek Schilder's laboratory at Michigan State University, East Lansing, MI. The only prerequisite was that the isolates had been recovered from strawberry roots. Prior to the evaluation, the isolates were sequenced for identification by Dr. Mursel Catal, Michigan State University, East Lansing, MI. They were also stained with acridine orange to determine the nuclear complement with the assistance of Dr. Mursel Catal (Sambrook *et al.*, 1989; Altschul *et al.*, 1990; Sándor *et al.*, 2000). All isolates were obtained from strawberry plants in Michigan, except Rhzsp06-121, which was isolated from strawberries in Oregon, and Rhzsp06-115 which originally came from the Driscoll Strawberry Institute in California. Both Rhzsp06-121 and Rhzsp06-115 were provided by Dr. Gerry Adams, Michigan State University, East Lansing, MI (table 60).

Root Preparation

Daughter plants were propagated in sterile, 2 NS grade sand from 'Allstar' strawberry plants obtained from Krohne Plant Farms (Hartford, MI). Roots were surface sterilized in 20% bleach solution for 5 min to eliminate any organisms that may inhibit infection. The daughter plants were approximately 6 months old. The roots were rinsed twice in sterile water for 1 min, and left in final sterile water wash until ready for plating on 100 x 15 mm petri plates containing water agar. These plates were prepared by placing an agar plug of an isolate in the middle of the water agar plate. Then, after being dried on a sterile paper towel, root pieces were placed between the agar plug and the side of the petri dish. The plugs were surrounded by five 6-mm, secondary root pieces placed 25 mm from the plug. There were a total of 15 root pieces per isolate. The experiment was repeated in May 2007 using the same procedure with the exception that the root pieces came from three different daughter plants that were approximately 6 wk old. In June 2007, the experiment was repeated again, using plants that were approximately 12 wk old.

Inoculum Preparation

A 6-mm agar plug from ten different *Rhizoctonia fragariae* isolates grown on potato dextrose agar (PDA) for 5 d was taken and placed in the middle of separate water agar plates. A sterile PDA agar plug served as a control. The plates were not sealed with parafilm.

Evaluation

After inoculation for 12 to13 d at 25°C on a laboratory bench under ambient light, each root piece was evaluated for necrosis on a scale of 1-4 (1-<25% necrosis, 2-25-50% necrosis, 3-50-75% necrosis, 4->75% necrosis).

Analysis

All statistical analyses was preformed with a one way ANOVA and mean separations (Fisher's protected least significant difference test at p=0.05) in StatGraphics Plus 4.1 (StatPoint Inc., VA) after checking for equality of variance.

Results and Discussion

The water agar allowed the isolates to grow and establish on minimal nutrition. After approximately 5 d, each isolate had colonized the entire plate. With the low nutrient level of water agar, the theory was that the fungus would penetrate the strawberry root in order to sustain itself. This penetration would be evident as the tissue was degraded and darkened, which did occur when compared to the control.

Table 60. Percent necrosis caused by *Rhizoctonia fragariae* isolates from strawberries in an in-vitro evaluation of virulence and anastomosis group at Michigan State University, East Lansing, MI.[x]

			Root Necrosis Rating (1-4)[w]			
	Isolate	Anastomosis Group[y]	First Experiment	Second Experiment	Third Experiment	Over All Three Experiments
1	Control	-	1.00 NS	1.00 NS	1.00 NS	1.00 a
2	Rhfr03-038	AG-A	2.80	1.67	2.00	2.16 bc
3	Rhfr03-047	AG-A	2.47	1.40	2.53	2.13 bc
4	Rhfr03-057	AG-G	2.93	1.60	2.00	2.18 bc
5	Rhfr03-077	AG-I	2.53	1.53	-	2.15 bc
6	Rhfr03-083	AG-G	2.67	1.60	2.80	2.36 bc
7	Rhfr05-096	AG-A	2.80	1.60	2.80	2.40 bc
8	Rhzsp05-108	AG-A	2.40	1.20	2.80	2.13 bc
9	Rhzsp06-115[z]	-	2.67	1.93	2.87	2.49 c
10	Rhzsp06-121[z]	-	2.47	1.13	2.00	1.87 b

ANOVA Effect	Significance (p)			
Isolate	**0.1248**	**0.0876**	**0.0816**	**0.0001**
Experiment	-	-	-	**0.0000**
Df				
Between groups	9	9	8	-
Within groups	20	20	18	-
Isolate	-	-	-	9
Experiment	-	-	-	2
Residual	-	-	-	75
Total (Corr.)	29	29	26	86

[w] Roots pieces were evaluated after inoculation for 12 to 13 d at 25°C, on a laboratory bench for necrosis on a scale of 1-4 (1-<25% necrosis, 2-25-50% necrosis, 3-50-75% necrosis, 4->75% necrosis).

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test p=0.05. Pairwise comparisons performed with n=15 for each experiment and n=45 over all three. For isolate Rhfr03077, n=30 when analyzing over all three experiments.

[y] Anastomosis groups determined by Dr. Mursel Catal using ITS sequencing and nuclear staining with acridine orange.

[z] Isolates originally from Dr. Gerry Adams laboratory at Michigan State University, East Lansing, MI. Anastomosis group not determined for these isolates.

All isolates sequenced and stained were identified as *Rhizoctonia fragariae*. In all experiments, with respect to the amount of necrosis they caused on the root pieces, the isolates were not significantly different from one another or the control. Isolate Rhfr03-077 was not evaluated a third time because there were not enough root pieces to allow a full assessment of the isolate. Isolates 115 caused the most necrosis over the three experiments (table 60).

Virulence did not differ amongst isolates, but only a small number were tested. The only difference in procedure over the three experiments was the age of the plant. The first experiment utilized the oldest plants, followed by the third evaluation, and the second evaluation had the youngest plant tissue. The results observed may be a result of the older plants having the potential to receive more root damage as they were moved in their pans in the greenhouses or damage received upon removal from the sand. Older plants may also have more naturally occurring damage and openings in their roots that allow penetration as well. This would be indicative to observations made in the field where older plantings are more likely to have this disease develop.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Burpee, L. L., Sanders, P. L., and Cole Jr., H. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia 72:689-701.

Husain, S. S., and McKeen, W. E. 1963. *Rhizoctonia fragariae* sp. nov. in relation to strawberry degeneration in southwestern Ontario. Phytopathology 53:532-540.

Maas, J. L. 1998. Compendium of Strawberry Diseases. 2nd Ed. American Phytopathological Society.

Martin, F. N. 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. Phytopathology 90:345-353.

Martin, S. B. 1988. Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78:379-384.

Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Fitopatol. Brasileira 10:371-390.

Ogoshi, A., and Ui, T. 1983. Anastomosis groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Pages 57-58 *In* Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, Eds. American Phytopathological Society. St. Paul.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sándor, E., Karaffa, L., Paul, G. C., Pócsi, I., Thomas, C. R., and Szentirmai, A. 2000. Assessment of the metabolic activity of Acremonium chrysogenum using acridine orange. Biotechnology Letters 22:693-697.

Vilgalys, R., and Cubeta, M. A. 1994. Molecular systematics and population biology of *Rhizoctonia*. Annu. Rev. Phytopathol. 32:135-155.

APPENDIX A

INOCULATION PROCEDURE EVALUATION

Introduction

Previous experiments (Olatinwo and Schilder, 2002; Sabaratnam and Schilder unpublished data) had varying success in achieving BRR symptoms in greenhouse experiments. The inherent variability in disease pressure made it difficult to create BRR artificially, and consistently, in the greenhouse for further study. Evaluation of past techniques, and additional techniques (Martin, 2000), was undertaken to aid in future efficacy trials.

Materials and Methods

Plant Material

One or two 'Allstar' strawberry plants were propagated from mother plants from Krohne Plant Farms, Inc. (Hartford, MI). Plants were placed in 10 x 10-cm black plastic pots filled with sterilized 2 NS-grade sand that had been autoclaved in a metal bin at 121°C for at least 16 h. The establishing daughter plants were kept in place on the sand using paper clips so that they would not touch the table top. The plants were kept outside on benches between greenhouses at Michigan State University, East Lansing, MI. Strawberry plants used in the experiment were selected for uniformity in size and root mass.

Inoculum Techniques and Preparation

Two inoculation techniques were evaluated, with the pathogens either grown on bran or as a mycelial suspension. The bran inoculations were at the rates of 0.75% and 1.5% wt/wt using the *Rhizoctonia* spp. (isolate Rhfr03038) and the *Pythium* sp. (isolate Pyth03007), both available from Dr. Annemiek Schilder's laboratory at Michigan State University, East Lansing, MI. These isolates were originally obtained from strawberry roots in Michigan. Both were chosen due to previous work showing that they were virulent (Sabaratnam and Schilder, unpublished). These inoculation techniques were done in both autoclaved and steamed sandy-sandy loam with both pathogens (table 61). On 14 September 2005, previously steamed sandy-sandy loam soil was autoclaved at 121°C for at least 16 h. Sandy-sandy loam soil (steamed at least 45 min at 82°C) is available from the Michigan State University greenhouse. Standard clay pots (13 x 13 cm) were used and placed on individual plastic trays. Each pot contained approximately 1 kg of oven dry soil.

Table 61. Inoculation method, soil treatment, and pathogen inoculated in greenhouse experiment to develop an inoculation protocol for creating black root rot conditions in greenhouse experiments using strawberry cv. Allstar in East Lansing, MI in 2005.

Inoculation	Soil Condition	Pathogen
0.75% bran*	Autoclaved	None
0.75% bran	Autoclaved	*Pythium*
0.75% bran	Autoclaved	*Rhizoctonia*
0.75% bran	Steamed	None
0.75% bran	Steamed	*Pythium*
0.75% bran	Steamed	*Rhizoctonia*
1.50% bran	Autoclaved	None
1.50% bran	Autoclaved	*Pythium*
1.50% bran	Autoclaved	*Rhizoctonia*
1.50% bran	Steamed	None
1.50% bran	Steamed	*Pythium*
1.50% bran	Steamed	*Rhizoctonia*
Mycelium	Autoclaved	None
Mycelium	Autoclaved	*Pythium*
Mycelium	Autoclaved	*Rhizoctonia*
Mycelium	Steamed	None
Mycelium	Steamed	*Pythium*
Mycelium	Steamed	*Rhizoctonia*

* Wt/wt of bran mixed with soil.

The bran inoculum was created by putting 3 to 4 PDA-Amp plugs of each isolate into separate flasks that contained 169 g oat bran/100 ml distilled water that had been autoclaved for 30 min. The fungus was allowed to grow at 25°C for one week before being mixed with a sterile rod. Growth continued at the same temperature for another week, after which the bran was allowed to air dry in a laminar flow hood. The procedure was similar to the one used by Martin (2000) except that the bran was not ground and passed through sieves. The drying process took several days, and the bran was stored overnight on a laboratory bench. After drying, the bran was broken to a consistent size (<2.5 cm). There were three pots per soil x inoculation x pathogen combination placed in a completely randomized design. Each pathogen x soil combination was thoroughly mixed with the soil (1 kg oven dried/pot) in separate 52 cm x 64 cm x 6.4 cm autoclaved aluminum pans.

For the mycelium inoculation, each fungus was grown separately in sterile potato dextrose broth using fungal agar plugs to inoculate the broth. The cultures were allowed to grow for seven days and then passed through a sterile Buchner funnel to collect the mycelium on sterile Watman filter paper. The mycelium of each fungus was pressed dry with sterile paper towels and re-suspended in sterile deionized water, to create a 100 ml/pot suspension. The suspension was added, as the strawberries were planted, at the rate of 20 ml/pot. Sterile water served as the control. All 54 plants were allowed to grow for six weeks before evaluation. Watering was done by hand using a plastic beaker as needed, to prevent cross contamination.

Evaluation

The plants were removed from their pots and their roots were washed under running water. All three plants from each inoculation x soil x pathogen combination were placed on a plastic tray with water to keep them moist. Figure 1 shows the qualitative rating scale used to rate roots from 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots. Percent of the root surface that was necrotic was also visually assessed.

Pathogen Recovery

Five root pieces from the edges of five lesions, for each treatment, were taken for plating on water agar. These were kept in petri dishes with moist Watman paper at 4°C until processing. The root pieces were surface sterilized for 2 min in 20% bleach solution and rinsed in two sterile water baths for 1 min each, dried on sterile paper towels and then placed on water agar. Fungi growing from the root pieces were subcultured onto V8 media (163 ml V8 juice, 1.8 g $CaCO_3$, 15 g agar/1 L) and subsequently identified using morphological characteristics after plate colonization. Those fungi that could not be identified in this manner were subsequently processed for DNA sequencing from the internal transcribed spacer (ITS) region by Dr. Mursel Catal (Sambrook *et al.*, 1989; Altschul *et al.*, 1990).

Figure 1. Rating scale used for root quality ratings for strawberry black root rot studies conducted in infested fields or greenhouse trials at Michigan State University, East Lansing, MI during 2005-2007. This image is presented in color.

Data Analysis

All statistical analyses were performed using the ANOVA and mean separation procedure (Fisher's protected least significant difference test at p=0.05) in StatGraphics Plus 4.1 (StatPoint Inc., VA) after initially checking for equal variance. In the analysis the mycelial suspension procedure was removed because the lack of necrosis and resulting excellent root quality, did not allow for a valid variance check. In the analysis, model variance components were estimated due to bran level (B), soil treatment (S), pathogen (P), B x S, B x P, S x P, B x S x P, and error.

Results and Discussion

It seemed that the autoclaving process had its own impact on the plant with autoclaved soil having a low root quality rating and high percent necrosis (table 62). The B x S and B x S x P interactions were always significant. However, pathogen presence was not different from the absence of a pathogen in root quality or percent necrosis (table 62). In addition, the bran inoculum may have been contaminated with bacteria which may have altered the true inoculum density and could have had a negative impact on the strawberry roots.

Treatments with mycelial suspension of *Rhizoctonia*, *Pythium*, or none had average root quality ratings of 5, regardless of soil, except for *Rhizoctonia* in steamed soil which had an average root quality rating of 4.33. In addition, the mycelial suspension of *Rhizoctonia* had 5.0% and 3.7%, *Pythium* had 5.0% and 0.0%, and no pathogen had 6.7% and 0.0% average root necrosis in autoclaved and steamed soil, respectively.

Table 62. Effects of inoculation, soil treatment, and pathogen on root health of cv. Allstar strawberries in a greenhouse experiment to develp an protocol for creating black root rot conditions in East Lansing, MI in 2005.[x]

	Root quality rating (1-5)[z]	Root necrosis (%)[y]
Bran Quantity		
0.75%	3.83 a	15.83 NS
1.50%	3.17 b	38.00
Soil Condition		
Autoclaved	2.39 a	42.83 a
Steamed	4.61 b	11.00 b
Pathogen		
Rhizoctonia	3.25 NS	28.75 NS
Pythium	3.83	25.08
None	3.42	26.92

ANOVA

Effect	Df	Significance (p)	
Bran Quantity (B)	1	**0.0451**	0.0613
Soil Condition (S)	1	**0.0000**	**0.0000**
Pathogen (P)	2	0.3138	0.2968
Block	2	0.7569	0.7211
BxS	1	**0.0213**	**0.0028**
BxP	2	0.0754	0.8883
SxP	2	0.6703	0.1602
BxSxP	2	**0.0335**	**0.0241**
Residual	22		
Total (Corr.)	35		

[x] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons performed with n=3.

[y] Statistical analysis was performed after log(x) transformation.

[z] Qualitative rating scale used to rate roots from 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots.

Rhizoctonia sp. was recovered from roots of plants in every inoculation technique x soil combination that had *Rhizoctonia* as the inoculated pathogen. In addition, *Rhizoctonia* was recovered from the roots of plants from the mycelium x steamed soil combination in which only *Pythium* sp. had been inoculated. *Pythium* sp. was re-isolated from treatments that were and were not inoculated with *Pythium* as shown in table 63. *Fusarium* spp. was found to be the most common contaminant, being isolated from roots several of the inoculation techniques x soil combinations, regardless of whether the pots were inoculated with *Pythium* sp., *Rhizoctonia* sp., or neither. Other fungi isolated from root pieces included *Aureobasidium* spp., *Arthrobotrys* spp., *Cephalosporium* spp., and *Stachybotrys* spp.

Table 63. Fungal genera recovered from roots of cv. Allstar strawberries in a greenhouse experiment to develp a protocol for creating black root rot conditions in East Lansing, MI in 2005.

			Fungal Presence*			
Inoculation	**Soil**	**Pathogen**	***Rhizoctonia***	***Pythium***	***Fusarium***	**Other**
0.75%	autoclaved	Control	-	-	X	X
0.75%	autoclaved	Pythium	-	X	-	X
0.75%	autoclaved	Rhizoctonia	X	-	-	-
0.75%	steamed	Control	-	X	X	-
0.75%	steamed	Pythium	-	X	-	-
0.75%	steamed	Rhizoctonia	X	-	-	-
1.50%	autoclaved	Control	-	X	X	X
1.50%	autoclaved	Pythium	-	-	X	-
1.50%	autoclaved	Rhizoctonia	X	-	-	-
1.50%	steamed	Control	-	-	X	-
1.50%	steamed	Pythium	-	X	-	-
1.50%	steamed	Rhizoctonia	X	-	-	-
Mycelium	autoclaved	Control	-	X	-	X
Mycelium	autoclaved	Pythium	-	-	-	X
Mycelium	autoclaved	Rhizoctonia	X	-	-	-
Mycelium	steamed	Control	-	-	-	X
Mycelium	steamed	Pythium	X	-	-	-
Mycelium	steamed	Rhizoctonia	X	-	-	-

* Plated root tissue from five lesions for each treatment.

The largest concern was that the *Pythium* sp., upon identification with DNA sequencing of the ITS regions, turned out to be *Geomyces pannorum*, completely invalidating any conclusions that could be drawn from the *Pythium* inoculations and in fact confounding the autoclaved versus steam soil conclusions. *Geomyces* is a ubiquitous saprophyte. Another isolate Pyth03-008, available from Dr. Annemiek Schilder's laboratory, Michigan State University, East Lansing, MI, was identified as *Pythium ultimum* var. *ultimum*. From this experiment, it was determined that it was sufficient to autoclave soil for less time, in smaller batches and that bran inoculum needs to be evaluated more thoroughly. The autoclave processed used in this experiment may have inhibited nutrient availability and released toxic compounds into the soil due to the length of time.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Martin, F.N. 2000. *Rhizoctonia* spp. recovered from strawberry roots in central coastal California. Phytopathology 90:345-353.

Olatinwo, R. O., and Schilder, A. M. C. 2002. Transplant root dips with biocontrol agents reduce strawberry black rot. *Phytopathology* 92:S61.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

APPENDIX B

INOCULATION OPTIMIZATION

Introduction

After the inoculation procedure evaluation experiment, further assessment was needed. Again, a previous inoculation procedure was re-evaluated (Olatinwo and Schilder, 2002) and improvements made upon new techniques that had been used in the previous experiment. The purpose of this experiment was to further evaluate and find the optimum bran quantity that would cause disease. Weed blocking fabric was used to examine the prevention moss on the soil surface of pots.

Materials and Methods

Plant Material

During March and May 2007, daughter plants were propagated from 'Allstar' mother plants (Krohne Plant Farms, Inc., Hartford, MI) into 52 x 64 x 6.4-cm aluminum pans filled with 2 NS-grade sand kept in a greenhouse at Michigan State University, East Lansing, MI. The pans and sand were autoclaved for 4 h at 15 psi. The mother plants were forced to produce runners in the greenhouse under lights set at a 12 h day length.

Pathogen Evaluation in Planting Stock

Prior to the experiment, five, 6-mm long root pieces (10 pieces/plant) of three randomly selected daughter plants were placed on water agar. Root pieces were selected for possible lesion formation. Root pieces were sterilized for 2 min in 20% bleach

solution, followed by two 1-min rinses in sterile deionized water. The root pieces were dried on sterile paper towels before placing on the media. Evaluation was performed after plate colonization. No fungal growth was observed.

Inoculum Preparation

The *Rhizoctonia fragariae* inoculum was grown from 2 plugs of PDA-Amp agar placed on autoclaved oat bran in flasks with 0.6 ml/g of sterile distilled water. After one week of growth at 25°C the bran was stirred with a sterile rod; after the second week the bran was air dried in a laminar flow hood for two days. The bran was stored covered on a laboratory bench overnight. The bran was mixed the morning of each day to aide in drying. The bran was ground with a mini prep plus food processor (Cuisinart, Stamford, CT) after drying for 22 h, except for the unground bran treatment which was simply broken to an approximate consistent size. A 6.30 mm sieve was used to ensure that the bran was less than 0.6 cm in size. The procedure was used by Martin except that the bran was not passed through nested sieves (Martin, 2000). Each pot held approximately 1 kg of soil.

Initial Pathogen Evaluation in Soil

Five grams of soil taken from a single pot in each of the four autoclaved batches of soil was mixed with 125 ml sterile deionized water in sterile 125 ml flasks to create a 1:25 dilution. One milliliter of this dilution was plated onto semi-selective media (water agar, 100 mg/L streptomycin sulfate and penicillin-G sodium salt, and 800 µl/L sodium hydroxide) or PDA for a total of two plates per batch of autoclaved soil. A sterile bent

glass rod was used to spread the soil dilution over the plate evenly, and the plates were evaluated 4 d later. No fungal growth was observed.

Inoculation

On 16 May 2007, 13 x 13-cm standard clay pots containing the greenhouse mixed sandy-sandy loam were autoclaved for 4 h. The following day each pot was individually mixed with the necessary wt/wt inoculum in sterile aluminum pans. Each bran quantity was evaluated with and without *Rhizoctonia*. Control pots had autoclaved bran. The PDA plate inoculation consisted of three, 15-d-old *Rhizoctonia* cultures grown on 20 ml PDA plates. The cultures were started from 6-mm discs from a culture kept on PDAamp. These plates were cut into quadrants and placed in half filled pots. The pots were filled and strawberries planted. Control pots had only sterile PDA media placed in the pots. Both the control and *Rhizoctonia* plates were kept unwrapped on a laboratory bench at 25°C. Weed blocking fabric (15-year commercial landscaping fabric) was placed around plants in certain treatments, as indicated in table64. This material was cut to cover the entire surface of each pot with a hole cut in the middle to allow for planting and was evaluated for its effect on the experiment. Treatments were replicated five times with each replication consisting of one pot placed on an individual inverted plastic tray. Flowers and runners were removed if they developed. Watering was done carefully to prevent cross contamination.

Evaluation

All plants were evaluated 8 wk after planting. Roots were visually rated using a qualitative scale of 1-5 (Scale of 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots (Appendix A, Fig.1). Fresh weight of the foliage and roots were obtained separately by splitting the crown horizontally just above the uppermost adventitious roots. These were then dried in a gravity convection oven set at 80°C for 48 h and dry weights were obtained. Total fresh and dry weights were obtained by adding the foliage and root weights together.

Data Analysis

All data were analyzed using the ANOVA and mean seperations (Fisher's protected least significant difference test at p=0.05) in .StatGraphics Plus 4.1 (StatPoint Inc., VA) after checking for equality of variance.

Results and Discussion

The unground bran treatments served as a comparison since unground bran had been used in the 2005 and 2006 greenhouse efficacy experiments (chapter 2). In all other treatments of this trial utilizing the bran carrier, the bran was ground.

The treatments having 1.50% bran with and without fabric allowed the evaluation of the surface cover to see if that assisted in disease development. The grinding did not make a significant difference in plant weights when compared to the unground bran treatments (table 64). The fabric did not influence disease development when considering the parameters measured. It did become clear from this trial that 3% bran

could provide a significant difference between plants inoculated with and without *Rhizoctonia*. Only with 3% bran was there a difference between the plants placed in pots inoculated with *Rhizoctonia* versus plants placed in non-inoculated pots that had only the bran. In addition, plants in the non-inoculated pots did not differ from plants in pots that contained no bran (table 64). This was consistent for every parameter measured. The PDA plates made a difference between plants in inoculated and noninoculated pots. Perhaps if the experiment had been run longer the PDA plates would also work.

The grinding procedure was adopted for the 2007 greenhouse efficacy experiment. Grinding does create a more consistent inoculum and more propagules to infest the pot. In addition, it appears that bran can have a negative influence on strawberry growth if it gets too high as evidenced by the 5.00% bran inoculation level. Consideration should be made in evaluating additional carriers and amounts to maximize the inoculation protocol.

Table 64. Effects of varying amounts of bran and inoculation with *Rhizoctonia fragariae* on plant biomass and root health of cv. Allstar strawberries in a greenhouse study to develop an inoculation protocol for black root rot in East Lansing, MI in 2007.[y]

Inoculum Carrier	Fabric	Inoculum	Fresh foliage wt. (g)	Fresh root wt. (g)	Dry foliage wt. (g)	Dry root wt.[z] (g)	Total plant fresh wt. (g)	Total plant dry wt. (g)	Root quality rating (1-5)
No bran	Yes	-	12.76 cd	16.18 bcd	4.24 bcde	2.39 bcd	28.95 cd	6.64 bcd	5.00 c
No bran	No	-	12.05 cd	15.85 bcd	3.69 abc	2.48 bcd	27.90 cd	6.17 bcd	5.00 c
0.75% bran	Yes	-	16.22 de	18.16 bcde	5.64 def	2.60 cd	34.38 de	8.24 cde	5.00 c
0.75% bran	Yes	*Rhizoctonia*	17.30 e	19.60 bcde	5.96 ef	3.30 cd	36.90 de	9.26 de	4.20 c
1.50% bran	Yes	-	17.09 de	18.02 bcde	5.82 ef	2.55 bcd	35.11 de	8.36 cde	5.00 c
1.50% bran	Yes	*Rhizoctonia*	19.79 de	25.74 e	6.51 f	3.70 d	45.53 e	10.21 e	5.00 c
3.00% bran	Yes	-	12.61 cd	14.06 bc	4.13 bcde	1.66 b	26.67 cd	5.79 bc	4.20 c
3.00% bran	Yes	*Rhizoctonia*	3.78 ab	3.31 a	1.85 a	0.67 a	7.09 ab	2.52 a	2.60 b
5.00% bran	Yes	-	2.69 a	1.60 a	1.91 a	0.57 a	4.29 a	2.47 a	1.00 a
5.00% bran	Yes	*Rhizoctonia*	3.80 ab	3.47 a	1.82 a	0.80 a	7.28 ab	2.62 a	1.80 ab
Unground bran (1.50%)	No	-	20.51 e	22.48 de	6.71 f	3.51 d	42.99 e	10.22 e	5.00 c
Unground bran (1.50%)	No	*Rhizoctonia*	17.24 de	19.33 bcde	5.38 cdef	3.20 cd	36.57 de	8.58 cde	5.80 c
PDA plates	Yes	-	11.79 cd	20.56 cde	3.88 bcd	2.91 cd	32.36 cde	6.79 bcd	5.00 c
PDA plates	Yes	*Rhizoctonia*	8.49 bc	11.81 b	2.86 ab	1.69 bc	20.30 bc	4.55 bc	4.20 c

ANOVA

Effect	Df	Significance (p)						
Treatment	13	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Block	4	0.4387	0.9223	0.3868	0.8868	0.8052	0.8883	0.6367
Residual	52							
Total (Corr.)	69							

[x] Qualitative rating scale used to rate roots from 1-5: 1= <20% of root mass is secondary roots, 2= 20-40% of root mass is secondary roots, 3= 40-60% of root mass is secondary roots, 4= 60-80% secondary roots, 5= >80% of root mass is secondary roots.

[y] Values in columns followed by differing letters are significantly different according to Fisher's protected least significant difference test at p=0.05. Pairwise comparisons performed with n=5.

[z] Statistical analysis performed after log (x) transformation.

APPENDIX C

RISK RATINGS FOR MAJOR PARASITIC NEMATODES OF STRAWBERRIES IN MICHIGAN

Table 65. Risk ratings of major parasitic nematodes found on strawberries grown in Michigan. (Data furnished by Fred Warner, Michigan State University Diagnostic Services)

Samples collected in fall prior to a strawberry crop (100 cc soil)

Risk rating	*Pratylenchus penetrans*	*Meloidogyne* spp.	*Longidorus elongatus*	*Xiphinema americanum*	*Criconemella* spp.	*Trichodorus* spp.
0	0	0	0	0	0	0
1	1-5	1-5	1	1-5	1-20	1-5
2	6-15	6-20	2-5	6-15	21-50	6-15
3	16-30	21-50	6-15	16-30	51-100	16-30
4	31-50	51-100	16-30	31-50	101-250	31-50
5	>50	>100	>30	>50	>250	>50

Samples collected in the fall prior to a strawberry crop (1.0 g root tissue and 100 cc soil)

Risk rating	*Pratylenchus penetrans*	*Meloidogyne* spp.	*Longidorus elongatus*	*Xiphinema americanum*	*Criconemella* spp.	*Trichodorus* spp.
0	0	0	0	0	0	0
1	1-10	1-20	1	1-5	1-20	1-5
2	11-25	21-50	2-5	6-15	21-50	6-15
3	26-50	51-100	6-15	16-30	51-100	16-30
4	51-100	101-200	16-30	31-50	101-250	31-50
5	>100	>200	>30	>50	>250	>50

Samples collected in a strawberry crop (1.0 g root tissue and 100 cc soil)

Risk rating	*Pratylenchus penetrans*	*Meloidogyne* spp.	*Longidorus elongatus*	*Xiphinema americanum*	*Criconemella* spp.	*Trichodorus* spp.
0	0	0	0	0	0	0
1	1-10	1-20	1-5	1-10	1-20	1-10
2	11-25	21-50	6-15	11-25	21-50	11-25
3	26-50	51-100	16-30	26-50	51-100	26-50
4	51-100	101-200	31-50	51-100	101-250	51-100
5	>100	>200	>50	>100	>250	>100

* Risk Rating: 0=none, 1=low, 2=low-moderate, 3=moderate to high, 4=high, and 5=severe. A rating ≥3 would result in the recommendation of control measures.