ABSTRACT

Title of Document:	MECHANISMS OF DISEASE SUPPRESSION BY A HAIRY VETCH (*VICIA VILLOSA*) COVER CROP ON FUSARIUM WILT OF WATERMELON AND THE EFFICACY OF THE BIOCONTROL ACTINOVATE.
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The cover crop *Vicia villosa* suppresses Fusarium wilt of watermelon but the mechanisms of disease suppression are unknown. Possible mechanisms were examined in field, greenhouse, and *in vitro* experiments. The effects of cover crop treatments (*V. villosa*, *Trifolium incarnatum*, *Secale cereale*, *Brassica juncea*) and the biocontrol treatment Actinovate (*Streptomyces lydicus* WYEC 108) on Fusarium wilt of watermelon and its causal pathogen, *Fusarium oxysporum* f. sp. *niveum* (FON) were evaluated. In four of five field experiments there were significant elevations in soil microbial respiration. Arbuscular mycorrhizal colonization of watermelon roots following cover crop amendments of *V. villosa* and *T. incarnatum*, were significantly higher compared to bare ground. The elevation in respiration was significantly positively correlated with disease suppression of Fusarium wilt induced by *V. villosa* and *T. incarnatum* (both cover crops reduced Fusarium wilt as much as 21%). In greenhouse experiments using infested field

soil, Fusarium wilt suppression was observed in pots amended with *V. villosa* and *T. incarnatum*. However, there was an increase in Fusarium wilt of watermelon in pots that were amended with *V. villosa* and *T. incarnatum* which were also inoculated with FON when compared to plants in nonamended, inoculated pots. These leguminous cover crops may have served as a nutrient source for the pathogen. In addition, *in vitro* growth experiments showed that media amended with *V. villosa* leachate significantly stimulated the *in vitro* growth rates of FON and *Trichoderma harzianum* compared to nonamended plates. It was hypothesized that *V. villosa* stimulation of nonpathogenic *F. oxysporum* spp., which provides cross protection against FON, may have contributed to the wilt suppression. Cover crop leachate amendments did not significantly influence colony forming units of *S. lydicus*. In both field and greenhouse trials Actinovate applications either had little or no effect on Fusarium wilt of watermelon. However, *S. lydicus* significantly inhibited *in vitro* growth of FON. These studies demonstrate that both general and specific disease suppression play a role in *V. villosa* suppression of Fusarium wilt of watermelon and that *T. incarnatum* is a viable alternative biocontrol.

MECHANISMS OF DISEASE SUPPRESSION BY A HAIRY VETCH (*VICIA VILLOSA*) COVER CROP ON FUSARIUM WILT OF WATERMELON AND THE EFFICACY OF THE BIOCONTROL ACTINOVATE.

By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2013

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Acknowledgements

Special thanks to Drs. Kate Everts and Yilmaz Balci for the mentoring and advice they provided me throughout my Ph.D. program. The contributions of Drs. Daniel Roberts, Cerruti R. Hooks, and Stephan Tubene as advisory committee members were just as appreciated.

Thanks to the student interns who assisted me in this research: Bern Gross, Katie Pfluam, Kayla Pennerman, Carl VanGessel, and Chong Zhou. I enjoyed all of your unique personalities and the company you provided through the many hours of lab and field work.

I am very grateful for the expertise and assistance of Jude Maul and other USDA personnel. I found the patience and contributions of the lab technician Matt Hochmuth and the farm manager David Armentrout to be invaluable.

I am so appreciative of all the support and encouragement I received from my fellow graduate students, faculty, family, and friends: Eva Garnek, Jack Himmelstein, Megan McConnell, Dr. John Bienapfl, Dr. Karen Rane, Theresa Dellomo, Robert Eagen, Kristin Larson, Katie Unger, Rebecca Wassell, Melissa Breiner, and Justin Senseney.

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Chapter 1: Introduction

1.1.The Pathogen Causing Fusarium Wilt of Watermelon: Fusarium oxysporum *f. sp.* niveum

Fusarium oxysporum f. sp. *niveum Schlechtend*, Fr. (E.F. Sm.) W. c. Snyder & H. N. Hans, is a fungal vascular wilt pathogen of watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai). It is classified within the division Ascomycota; class *Sordariomycetes*; order *Hypocreales*; family *Nectriaceae* and the genus *Fusarium*. The genus *Fusarium* comprises a diversity of species which can be found in air, soil, or plant residue and can be isolated from many plant parts (Leslie et al., 2006). Likewise, pathogens within the genus can induce a wide range of symptoms on their hosts such as cankers, vascular wilts, seed and fruit rots, foliar lesions and root or stem rots (Smith, 2007).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON) is found in watermelon growing regions throughout the world (Egel et al., 2007). Fusarium wilt is one of the most prevalent diseases of watermelon (*Citrullus lanatus*) on the Eastern Shore of Maryland and in Delaware (Zhou et al., 2003b). When susceptible watermelon varieties are planted in heavily infested field's losses in yield due to Fusarium wilt can approach 100% (Egel et al., 2007).

There are four known races of *Fusarium oxysporum* f. sp. *niveum* (FON). Races are categorized based on their ability to cause disease on specific watermelon cultivars. Race 0 is of limited economic importance, race 1 is the most widespread of the races,

race 2, which was present in approximately 20% of surveyed watermelon fields in Delmarva is a threat because there are no existing commercial watermelon cultivars that have resistance (Zhou et al., 2003b). Race 3, which was first described in 2009, is the most aggressive race and has only been reported in Maryland (Zhou et al., 2010).

FON infects through the roots and then proliferates in the xylem. Water uptake and movement is restricted after colonization occurs. Fusarium wilt on watermelon results in yield losses by reducing fruit number and size. Marketable yield is lower because some watermelons are small, misshapen, low in sugars, or cracked or sunburned (Egel et al., 2007). Fusarium wilt of triploid (seedless) cultivars also increases production and labor costs associated with management. Increased frequency of fumigation applications is one example of costs associated with FON management. The additional acreage needed for watermelon rotation so that Fusarium wilt does not become a major problem in watermelon fields also increases costs (Egel et al., 2007).

1.2.Management Methods Available for Fusarium Wilt of Watermelon

Consumer demand for triploid (seedless) watermelons has increased since the 1990's and triploid culativars now dominate the U.S. watermelon acreage (Lucier et al., 2001). Fusarium wilt in diploid (seeded) watermelon cultivars was previously managed through genetic resistance to race 1 FON. Unlike diploid watermelons, the majority of triploid watermelon cultivars have little or no resistance to Fusarium wilt and additional methods of control are necessary (Everts et al., 2010; Everts et al., 2011).

Forecasting systems have been developed for economically important Fusarium diseases of crops like chickpea (*Cicer arietinum*) (*Fusarium oxysporum* f. sp. *ciceri*), potato (*Solanum tuberosum*) (*Fusarium sambucinum* teleomorph *Giberella pulicaris* and *F. solani* var. *coeruleum*), maize (*Zea mays*) (*F. verticilloides*) and wheat (*F. graminearum*) (De Wolf et al., 2003; Leslie et al. 2006; Schaafsma et al., 2007). Currently there is no reliable forecasting system for Fusarium wilt of watermelon and the timing of management practices depends on estimates based on previous seasons and the epidemiology of the pathogen.

Methyl bromide (MeBr) is a soil fumigant that has been used by growers to control Fusarium wilt, weeds, and other pests of several vegetable crops including watermelon (King et al., 2008). However, MeBr breaks down the stratospheric ozone layer (Gullino et al., 2005; Gareau, 2010). Therefore, in 1987, the Montreal Protocol called for a phase out of non-essential uses. However, alternative soil fumigants generally aren't as effective as Methyl Bromide at controlling FON or weeds, are expensive, and also negatively impact the environment (Ferguson et al., 1997). In order for the mandated reduction in methyl bromide to be successfully implemented, watermelon growers need economical alternatives that are effective in managing FON.

In China, Japan, and Korea watermelons are produced from grafted plants. This practice is often used to minimize Fusarium wilt yield loss in fields where watermelons are grown successively without rotation. However, grafting incurs high labor and seed costs (Davis et al., 2008). Japanese watermelons are sold at a higher price than U.S. watermelons, enabling Japanese growers to cover these production costs (Kawaide,

1985). Researchers and manufacturers are currently working on techniques and tools which would make watermelon grafting economically feasible for U.S. growers.

Zhou and Everts conducted a study in 2004 looking at the impact of thirteen different soil amendments on Fusarium wilt of watermelon and found that using a *Vicia villosa* (hairy vetch) cover crop decreased Fusarium wilt incidence as much as 63% when used in combination with highly resistant cultivars, 53% with moderately resistant cultivars and 22% with susceptible watermelon cultivars (Zhou et al., 2004). They also found an increase in watermelon yield and fruit sucrose content. However, the mechanism by which the *V.villosa* green manure suppressed Fusarium wilt is unknown.

1.3.Benefits of Cover Crops

Cover crops provide several benefits to soil health. In some cropping systems they reduce the need for synthetic chemicals by serving as an herbicide, increasing soil organic matter, or contributing nutrients to the soil (Wyse, 1994; Drinkwater, et al., 1995; Cavigelli et al., 2003; Steinmaus et al., 2008). When legumes fix nitrogen they can provide 10-25% of a crops nitrogen requirement as well as micronutrients for the next season's crop (Sustainable Agricultural Network, 2000). *Vicia villosa* is one of the most efficient leguminous cover crops for nitrogen fixation (Rosecrance et al., 2000). However, cover crops such as *Secale cereale* (rye) and *Hordeum vulgare* (barley) may compete with subsequent cash crops for nitrogen (Shrestha et al., 1998). Cover crops also help the soil retain moisture, reduce weed biomass, and decrease soil erosion (Kort et al., 1997). *Vicia villosa* cover crops significantly reduce aboveground weed biomass compared to conventional management practices (Fujii, 2001; Isik et al., 2008; Campiglia

et al., 2010a). Cover crops are regarded as components of an integrated pest management system because of their potential to reduce pesticide use and encourage beneficial insects.

1.4.Mechanisms of Cover Crop Biocontrol: General and Specific Disease Suppression

An observed reduction in disease incidence or severity in crops grown in specific soils, or in the presence of certain cover crops has been termed "disease suppression" (Alabouvette, 1999). This suppression may be general or specific. General suppression results from high diversity and activity of soil biota and is effective against a broad range of plant pathogens (Van Os et al., 2001). Specific suppression results from the presence of a specific antagonist or functional group and is effective against a single plant pathogen (Van Os et al., 2001). Many researchers measure levels of soil microbial activity and diversity as an indicator of general disease suppression (Entry et al., 2000). Tsuneo (1991) found applications of organic crop residues and manures resulted in more diverse soil microbial communities and associated this increased diversity with disease suppression of brown stem rot of adzuki beans caused by the pathogen *Acremonium gregatum*. Abawi et al., (2000) saw a similar association between changes in microbial communities and disease suppression.

1.4.1.Impact of Soil Nutrients on Disease

Adding nutrients to the soil or the plant itself impacts disease development in many ways. Organic amendments, such as cover crops that are incorporated as a green manure can improve crop health and increase microbial activity either directly or indirectly by releasing nutrients to the soil (Drinkwater et al., 1995). Förster et al. (1998) postulated that the decrease in disease incidence of pepper (*Capsicum annuum*) and

tomato (*Solanum lycopersicum*) crown rot caused by *Phytophthora capsici* following phosphite supplementation was due to phosphonates acting as antifungal compounds against these fungi. Tenuta et al. (2002) demonstrated that additions of meat and bone meal to sandy acidic soils caused a buildup of ammonia which killed microsclerotia of the plant pathogen *Verticillium dahlia*. Micronutrients such as magnesium, calcium, zinc or iron are also important in crop disease management and prevention (Ghorbani et al., 2008). In combination with a biocontrol strain of *Pseudomonas fluorescens,* low levels of zinc soil treatments reduced the ability of *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* to produce Fusaric acid, thereby decreasing incidence of root and crown rot of tomato (Duffy et al., 1997).

Nutrient applications can affect host defense responses. Spraying cucumber (*Cucumis lagenarium*) leaves with various phosphorus solutions induced systemic resistance to eight dissimilar plant diseases (Mucharromah et al., 1991). Potassium applications to oil seed rape (*Brassica napus*) decreased black spot disease severity due to amplified production of internal plant phenolics which reduced conidial germination and sporulation of *Alternaria brassicae* (Sharma et al., 1994).

An excess of available nitrogen in soil can produce succulent growth that is susceptible to disease incidence. For example excess nitrogen increased eye spot disease (*Helminthosporium sacchari*) of sugar cane (*Saccharum officinarum*) and ear rot (*Fusarium graminearum)* in maize (Antherton Lee et al., 1928; Reid et al., 2001). Nitrogen deficiencies also can result in weaker plants that are more prone to infection (Snoeijers et al., 2000).

The form of nitrogen in soil can influence disease incidence and severity. Woltz and Engelhard (1973) found that nitrate-nitrogen suppresses Fusarium wilt of chrysanthemum (*Chrysanthemum indicum* L.) caused by *Fusarium oxysporum* (Schlecht.) f. sp. *chrysanthemi.* Though the mechanism was unknown, researchers postulated it was due to an increase in soil microbial diversity resulting in general disease suppression.

1.4.2.Effects of Plant Leachates/Extracts on Disease

Cover crop leachate may suppress disease by 1) directly affecting the pathogen, 2) inducing resistance in the host plant, or, 3) by stimulating the growth of beneficial microorganisms, which are antagonists or competitors with plant pathogens. Although the mechanism of disease suppression is not completely understood cover crop leachates have repeatedly resulted in disease suppression. For example, extracts of crops such as cassia (*Cassia*), chili pepper (*Capsicum*), clove (*Syzygium aromaticum),* and essential oil of mustard (*Brassica juncea*), have suppressed wilt of muskmelon (*Cucumis melo*) caused by *Fusarium oxysporum* f. sp. *melonis* as well as wilt of chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi* (Bowers et al., 2000).

1.4.2.a.Plant Leachate/Extract Effects on the Pathogen

Wu et al. (2010) observed inhibition of FON growth with gallic acid and ferulic acid, derived from watermelon root exudates, but that these factors also caused increased production of pathogen mycotoxins and virulence factors (hydrolytic enzymes- proteinase, pectinase, amylase and cellulose) (Wu et al., 2008; Wu et al., 2009a; Wu et al., 2010). Essential oils from a bush native to northern Africa- *Lippia rehmannii* and

lemon grass (*Lippia rehmannii*) inhibited *in vitro* growth of the pathogens *Rhizoctonia solani* and *Fusarium oxysporum* (Linde et al., 2010). The essential oils and various compounds extracted from the flowers of *Cestrum nocturnum* L. and other plants such as pecan (*Carya Illinoensis*) shells, pomegranate (*Punica granatum*) husks and other organic materials directly inhibited pathogenicity and growth of organisms including *Fusarium oxysporum* and *Fusarium solani* (Al-Reza et al., 2010; Osorio et al., 2010).

FON germination and sporulation was increased in the presence of root exudates of watermelon and decreased in the presence of root exudates of rice (*Oryza sativa*) (Ren et al., 2007). Zhou et al. (2004) found that adding watermelon residue to pots in a greenhouse experiment at a rate of 5% (wt/wt) increased the soil population density of FON by 21% compared to the control and resulted in 100% disease incidence.

Brassica spp. leachates are thought to have various modes of action that help reduce disease. For example, chemicals weaken pathogen propagules so that they can be parasitized by other soil antagonists (Smolinska, 1999). The isothiocyanates produced by Indian mustard (*Brassica juncea*) and canola (*Brassica napus* L.) are thought to be directly responsible for the inhibition of pathogens such as *Gaeumannomyces graminis tritici* which causes take-all of wheat (Angus et al., 1994). Isothiocyanates arise from hydrolyzed sulfur compounds called glucosinolates which are found in most brassicas (Sarwar et al. 1998). These biocidal compounds suppress soil-borne pathogens and pests (Kirkegaard et al., 1998). Biocidal compounds may yet be identified in other cover crops which have disease suppressive effects.

1.4.2.b. Plant Leachate Effects on Pathogen Antagonists and Intercropped or Following Plants:

Many foliar leachates and plant exudates directly affect specific microorganisms in the soil community that can be antagonistic to pathogens. The foliar leachate of raspberry (*Rubus ideaus* L.) plants had an allelopathic effect on the ectomycorrhizae that colonize black spruce (*Picea mariana*) roots (Cote et al., 1988). Increases in soil microbial respiration and bacterial populations in the soil following the application of an alfalfa (*Medicago sativa*) distillate indicated that decreases in *Sclerotium rolfsii* may have resulted from general suppression (Linderman et al., 1972).

The isothiocyantes derived from *Brassica* spp. leachate are also thought to influence microorganisms antagonistic to plant pathogens. In one study *Brassica napus* seed meal amendment induced suppression of root rot caused by *Rhizoctonia solani* on apple (*Malus domestica*). Researchers postulated that the decrease in disease was due to the isothiocyantes effects on bacterial community structure such as elevations in *Streptomyces spp*. (Cohen et al., 2005). A subsequent study that documented the changes in soil microorganism populations when using *Brassica* spp. amendments with varying amounts of isothiocyanates observed an increase in actinomyetes and fluorescent psuedomonads (Mazzola et al., 2001).

Leachate of several cover crops impact the germination of weed seeds-an example of direct influence on an essential plant function (Haramoto et al., 2007). *Vicia villosa* leachate stimulated pig weed (*Amaranthus hybridus* L.) germination while *S. cereale*

leachate inhibited germination of redroot pigweed (*Amaranthus retroflexus* L.) and common purslane (*Portulaca oleracea L.*) (Teasdale et al., 2005, Tabaglio et al., 2008).

Plant leachates play an essential role in allelopathic effects of plants and can alter the plants ability to defend itself from disease. Soil amended with undecomposed tomato leaves resulted in increased disease severity of Fusarium wilt on tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Bonanomi et al., 2007b).

1.4.3.Stimulation of Beneficial Microorganisms

Disease suppression induced by the incorporation of cover crops into the soil has been associated with changes in microbial soil communities. General suppression, due to increased microbial biomass and diversity, could be the mechanism causing this decrease in plant disease. It is also possible that the increases in the magnitude of populations of single genera or species are responsible.

Several *Trichoderma* species have been associated with disease suppression (*T. incarnatum, T. harzanum, T. viride, T. virens*) and some species have been commercialized as soil amendments (ex. Bio-fungus, Trichodex, Binab-T, Root Pro, RootShield, SoilGard) to protect crops from disease (Monte et al., 2001; Howell et al., 2005; Vinale et al., 2009). Trichoderma is widespread in soil (Vinale et al., 2009). Lewis et al., (1984) demonstrated that Trichoderma is able to proliferate when added to the soil as mycelium on a bran substrate. Biological control of damping off of peas (*Pisum sativum*), cucumbers (*Cucumis sativus*), tomatoes (*Lycopersicum esculentum*), peppers (*Capsicum annuum*) and gysophila (*Gypsophila paniculata*) caused by *Pythium aphanidermatum* was achieved by applying *Trichoderma harzanium* as seed coating

(Sivan et al., 1984). *Trichoderma* species applications significantly decreased Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris* and also increased seed germination, shoot and root length (Dubey et al., 2007).

One mode-of-action of Trichoderma-mediated suppression is parasitism of fungal hyphae (Elad et al., 1980; Howell et al., 2003). When *Trichoderma* spp. such as *T. harzianum* and *T. viride* come into contact with hyphae of another fungus, Trichoderma's mycelium coil around or grow along the hyphae, form hook-like structures and penetrate the cell wall (Haran et al., 1996; Dubey et al., 2007). Mycoparasitsm by *T. harzianum* directly affected cell walls and growth of *Crinipellis perniciosa*-a pathogen of cacoa (*Theobroma cacao* L.) and *Rhizoctonia solani,* which causes damping off of bean and tomato seedlings (Hadar et al., 1979; Elad et al., 1980; De Marco et al., 2000).

Trichoderma also produces hydrolytic enzymes that breaks down fungal cell walls (De Marco et al., 2000) such as proteases, β-glucanases (β-1, 3- and β-1, 6-glucanases), and lipases. (Hadar et al., 1979; Haran et al., 1996; De Marco et al., 2000). Trichoderma reduced Chocolate spot of bean (*Botrytis cinerea*) by decreasing the pathogens ability to produce the enzymes pectin methyl esterase, polygalacturanase, and pectate lyase (Zimand et al., 1996).

Amendment of soil by Trichoderma affects plant growth and nutrition even in the absence of a pathogen by increasing the rate of seedling emergence, increasing shoot and root dry weight and enhancing nutrient availability. This suggests that Trichoderma might produce a growth stimulant (Windham et al., 1985). Trichoderma is also able to directly colonize the outerlayers of the host plant root cortex without causing disease (Yedidia et

al., 1999). Similar to mycorrhizal relationships, this plant-fungi relationship may protect the plant from pathogen infection. Trichoderma colonization may also result in systemic resistance by inducing the production of various defense compounds such as terpenoids (Yedida et al., 1999; Howell et al., 2000).

Attributes that make Trichoderma so rhizosphere competent, such as its ability to utilize nutrients efficiently, can contribute to the biocontrol of potential plant pathogens. Trichoderma applied to cotton (*Gossypium hirsutum*) seeds rapidly metabolized cotton seed exudate in the spermosphere, compounds that normally stimulate the activity and pathogenicity of various *Pythium* spp. and *Rhizopus oryzae,* thereby decreasing disease (Howell et al., 2001). Sivan et al. (1989) found a similar mechanism of disease suppression via competition for root exudates between potential pathogens *Fusarium oxysporum* f. sp. *vasinfectum* (responsible for Fusarium wilt of cotton-*Gossypium barbadens*e L. 'Pima') or *Fusarium oxysporum* f. sp. *melonis* (responsible for Fusarium wilt of melon-*Cucumis melo* L. '56') and *Trichoderma harzianum*. Chlamydospore germination and the level of root colonization by the Fusarium wilt pathogens were inhibited by the presence of *Trichoderma harzianum* as a seed coating or soil amendment but additions of root exudates or glucose and asparagine nullified the disease suppressive effects (Sivan et al., 1989).

1.4.4. Impact of Mycorrhizal Colonization on Disease

Cover crops such as *Vicia villosa* which are colonized by mycorrhizae may increase mycorrhizae populations in the soil and subsequent colonization of the cash crop roots (Kabir, 2000; Galvez et al., 2001; Rutto et al., 2003; Sorensen et al., 2005).

Mycorrhizae colonization of plant roots improves plant health via enhanced nutrient and water uptake and also serves as a defense against potential pathogens (Bolan, 1991; Matsubara et al., 1995; Augé, 2001; McGonigle et al., 2003; Wu et al., 2006). Watermelon whose roots were colonized by mycorrhizae were shown to have significantly improved water-use efficiency and fruit yield (Kaya et al., 2003).

Nutritional enhancements provided by mycorrhizal fungi to their hosts have been linked to decreases in disease incidence. Graham et al. (1982) correlated elevations of phosphorus in wheat roots and decreases in root exudation of amino acids and reducing sugars with mycorrhizal associations and subsequent reductions in take-all of wheat caused by *Gaeumannomyces graminis*. Not only can mycorrhizae effect plant pathogens by competition for infection and colonization sites and photosynthates (Azcón-Aguilar et al., 1996) but mycorrhizal colonization can also alter the composition of host plant root exudates, which affects the survival of plant pathogens or the growth of beneficial soil microorganisms (Linderman, 1988; Andrade et al., 1997). Plant root exudates can stimulate pathogen response and pathogenicity so a decrease in the amount of plant root exudates can result in a decrease in plant disease (Graham et al., 1982). Soil borne pathogens are sometimes found in lower numbers in soils that contain roots colonized by mycorrhizae. Hwang (1992) found soil containing alfalfa and mycorrhizal roots had fewer propogules of *Fusarium oxysporum* f. sp. *medicaginis* (causing Fusarium wilt of alfalfa) and *Verticillium albo-atrum* (responsible for Verticillium wilt of alfalfa) as well as lower disease incidence when compared to a non-mycorrhizal control.

Mycorrhizal root colonization can induce local or systemic disease resistance in their host plants. Mycorrhizal colonization induced both local and systemic resistance in tomato roots against *Phytophthora parasitica* which causes Phytophthora blight (Pozo et al., 2002). Resistance to Fusarium wilt in tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici* was associated with changes in internal hormone levels of mycorrhizal tomato roots (El-Khallal, 2007). Reduction of Fusarium wilt in cow pea (*Vigna unguiculata*) was attributed to root mycorrhizae colonization and an increase in phytoalexins (Sundaresan et al., 1993).

The combined inoculation of *Trichoderma harzianum* and mycorrhizae on melon roots have functioned sygnergistically in managing Fusarium wilt (*Fusarium oxysporum* f. sp. *melonis*) of melon (*Cucumis melo* L.) (Martinez-Madina et al., 2009).

Fusarium wilt reduction in watermelon may result from an increase in mycorrhizal colonization of roots following a *V.villosa* cover crop. Finding the mechanism of *V.villosa* mediated suppression of Fusarium wilt, and determining whether it is general or specific could increase adoption of *V.villosa* as a cover crop, or assist in identifying comparable cover crops that have similar mechanisms of disease suppression. Improved understanding of the mechanism of the suppression could lead to other methods that facilitate suppression.

1.4.5.Potential of Specific Cover Crops for Disease Suppression

In this study the effects of five different cover crops on Fusarium wilt of watermelon were examined: *B. juncea, S. cereale, T. incarnatum,* and *V. villosa.* What is known about each cover crop and its potential for disease suppression varies.

Brassica juncea contains high levels of glucosinulates, even when compared to most other *Brassica* species. The glucosinulates in *B. juncea* induce high levels of biological activity (mostly antimicrobial) (Smolinska et al., 1999; Larkin et al., 2007). Though brassica cover crops suppress many diseases there are reports of an increase in Fusarium diseases following brassica incorporation or little to no effect. Six different *Brassica* species were evaluated for inhibition of the potato pathogen *Fusarium sambucinum*. Most of the species resulted in minimal disease inhibition (less than 20%) for many of the crops (Larkin et al., 2007). In an *in vitro* experiment done with isothiocyanates derived from brassica's, Fusarium exhibited only moderate inhibition (Sarwar et al., 1998). Broccoli amendments provided inconsistent *Fusarium* spp. suppression in a study done by Zasada et al. (2003). Mazzola et al. (2001) actually saw an increase in *Fusarium* spp. populations in the soil following brassica seed meal amendments. Njoroge et al. (2008) recorded elevations of *Fusarium oxysporum* populations in a watermelon field following the incorporation of *B. juncea* and *B. napus*.

All *Brassica* crops are nonmycorrhizal (Roberts et al., 2001). Using *Brassica* spp. as a cover crop can cause a decrease in mycorrhizal colonization of succeeding cash crops such as in the case of tomato roots (*Lycopersicon esculentum*) planted after a garlic mustard cover crop (*Allaria petiolat*) (Roberts et al., 2001). It is possible this could contribute to an increase in disease in plants that are dependent on mycorrhizal colonization for pathogen defense.

The allelochemicals from *S. cereale* leachates suppress weeds (Mwaja et al., 1995). Despite the inhibitive effects on weeds, *S. cereale* cover crops are not usually

disease suppressive. However, *S. cereale* residues decreased Fusarium root rot on beans by inhibiting chlamydospore germination of the pathogen *Fusarium solani* f. sp. *phaseoli* (Lewis et al., 1977). Fungistasis may have occurred due to *S. cereale's* insubstantial contribution of nutrients to the soil and consumption of available soil nitrogen (Mwaja et al., 1995). *Secale cereale* plants do form mycorrhizal associations and augmented soil mycorrhizal populations and colonization of a following maize crop (*Zea mays* L.) (White et al., 2010).

There are few reports of disease suppression following a *T. incarnatum* (crimson clover) cover crop. *Trifolium incarnatum* is susceptible to Fusarium wilt (*Fusarium oxysporum* f. sp. *trifolii*) (Pratt, 1981). *Trifolium incarnatum* is a nitrogen fixing legume (Wells, 2009), and commonly forms mycorrhizal associations, functioning as a mycorrhizal inoculant for suceeding crops (Rovira et al., 1961).

Vicia villosa cover crops have been associated with disease suppression. *Vicia villosa* amendments have reduced the inoculum densities of *Thielaviopsis basicola,* a pathogen of cotton seedlings, and also decreased the fungus' chalmydospore viability (Candole, 1998, Coumans et al., 2010). As mentioned earlier, a *V. villosa* green manure significantly suppressed Fusarium wilt of watermelon, by as much as 63% when used in combination with resistant cultivars (Zhou et al., 2004). *Vicia villosa* commonly forms mycorrhizal root associations and may increase mycorrhizal populations in the soil (Galvez et al., 1995). *Vicia villosa* is a legume, fixes nitrogen, and releases nutrients when amended to soil (Rosecrance et al., 2000; Koger et al., 2005; Teasedale et al., 2005). Pure stands of *V. villosa* cover crop with and without fertilization achieved

significantly higher marketable yield of the subsequent tomato (*Lycopersicon esculentum Mill.*) cash crop when compared to oat (*Avena sativa L.*), subclover (*Trifolium subterraneum*), a mixture of *V. villosa* and *A. sativa* and a conventionally tilled soil without mulch (Campiglia et al., 2010b).

1.5.Biocontrol Management Techniques Using Commercial Products

Biological controls to manage Fusarium wilt diseases are particularly useful for organic farmers who have fewer fungicide options. Biological control products containing competitive fast-growing microorganisms like *Penicillium, Trichoderma* or *Streptomyces* have inhibited plant pathogens and are effective on a variety of crop diseases. One example is the product RootShield, containing *Trichoderma hamatum,* which reduced Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) (Larkin et al., 1998). Another product, called Mycostop (active ingredient *Streptomyces griseoviridis*), has reduced incidence of Fusarium wilt of cyclamen (*Cyclamen persicum*) caused by *Fusarium oxysporum f. sp. cyclaminis* (Elmer et al., 2004). De Cal et al., (2009) found that the application of a conidial suspension of the fungus *Penicillium oxalicum* to seeds and seedlings of watermelon (*Citrullus lanatus*) decreased disease incidence of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* in both growth chamber and field experiments. Many different companies utilize micoorganisms like *Penicillium*, *Trichoderma* and *Streptomyces* that are known to suppress a wide range of pathogens in products that are antagonist towards plant pathogens.

If a microorganism is effective in suppressing a specific plant pathogen it may be developed into a commercial biocontrol product. Wu et al. (2009) made their own bio-organic fertilizer that incorporated the active ingredients *Paenibacillus polymyxa* and *T.*

harzianum for use on Fusarium wilt of watermelon. They attributed the subsequent disease suppression to elevations in the activities of defense related enzymes (catalase, superoxide dismutase, peroxidase and β-1,3-glucanase) in watermelon leaves, indicative of systematic acquired resistance (Wu et al., 2009b). Ling et al. (2010) found that the application of a bioorganic fertilizer product, BIO, reduced Fusarium wilt of watermelon by 59-73% in the field and 60-100% for pot experiments. The mode of action of the active ingredient of BIO, *Paenibacillus polymyxa* SQR-21 was associated with alterations in plant root exudation of phenolics, resulting in decreased FON conidial germination (Ling et al., 2011). Additionally, in field experiments in Vietnam Nga et al. (2011) used the bacteria *Psuedomonas aeruginosa* to protect watermelon from gummy stem blight, caused by the pathogen *Didymella byroniae.* Many biocontrol products like BIO are not utilized by growers because the products are not widely available or the efficacy of the product has not been demonstrated in field trials.

Actinovate is a commercial formulation of *Streptomyces lydicus* strain WYEC 108, a saprophytic soil bacteria which has been shown to reduce disease in several crops (Entry et al., 2000, Getha et al., 2005). Natural Industries (Houston, TX), the producers of Actinovate, have labeled it for management of pathogens such as Fusarium and Rhizoctonia. No studies have been conducted to evaluate the effect of Actinovate on Fusarium wilt of watermelon.

Streptomyces spp. are known to suppress disease. Soils that contained wood chips inoculated with *S. lydicus* experienced a reduction in *Verticillium dahlia*-infection of potato compared to no treatment (Entry et al., 2000). In one study, *S. lydicus* suppressed growth and infectivity of *Pythium ultimum* and *Rhizoctonia solani*. Getha et al. (2005)

found a Streptomyces strain that was antagonistic towards *Fusarium oxysporum* f. sp. *cubense* and which significantly decreased the *F.o. cubense* infection on 'Novaria' banana plantlets.

There are examples of Actinovate AG itself reducing disease but not Fusarium wilt of watermelon. Actinovate application to blueberries reduced Monilia blight (*Monolinia vacinii-corymbosi*) by 50% compared to control treatments (Teasdale, 2009). Actinovate SP application inhibited dollar spot (*Sclerotinia homescarpa*) incidence in warm- and cool-season grasses (Tomaso-Peterson et al., 2007).

Several hypotheses exist on the mechanism by which *Streptomyces lydicus'* suppresses plant disease. One potential mechanism of suppression is *Streptomyces'* production of extracellular antifungal metabolites (Yuan et al., 1995; Getha et al., 2005). Alternatively, suppression may result from production of cell wall-degrading enzymes like cellulases, amylases, chitaneses, glucanses etc. which are known to play a role in mycoparasitism (El-Tarabily, 2006; Gonzalez-Franco et al., 2009). Either direct physical obstruction of pathogen root invasion or promotion of plant growth via facilitation of soil nutrient assimilation may be involved in suppression (Doumbou et al., 2010).

Natural Industries, Inc. reports that disease prevention is due to the direct colonization of plant roots by *S. lydicus* and the ability of the *S. lydicus* to increase nutrient availability. Streptomyces which colonize plant roots may be actively preventing the onset of disease due to root colonization (Yuan et al., 1995; El-Tarabily, 2006). However, little research has been done on colonization. Researchers have not yet attempted to reisolate *S. lydicus* directly from watermelon plant roots or soil from watermelon fields following Actinovate applications. It is not known if *S. lydicus* can

colonize watermelon plant roots or have any direct or indirect effect on *Fusarium oxysporum* f. sp. *niveum* (FON).

Several studies have shown that Actinovate can be more effective against disease when used in combination with other management options. Reductions in powdery mildew (*Podosphaera xanthii*) of summer squash (*Cucurbita pepo*) and cantaloupe (*Cucumis melo*) were observed when Actinovate AG was applied in combination with the fungicide Procure 480SC (active ingredient triflumizole) than when applied alone (Zhang et al., 2011). Also, Elmer et al. (2004) found that AUDPC values for Fusarium wilt of cyclamen were lowest when Actinovate was mixed with fludioxonil and then followed by Actinovate as opposed to when the biocontrol was used by itself. There are no studies that evaluate whether Actinovate used alone, or in combination with a *V. villosa* cover crop will suppress Fusarium wilt of watermelon.

1.6.Objectives of the Dissertation

The ability of *V. villosa* to suppress Fusarium wilt of watermelon has been established. However, the mechanism of this suppression is unknown (Zhou et al., 2004; Zhou et al., 2006). Additionally, no studies have explored the ability of *T. incarnatum*, another leguminous cover crop, to suppress Fusarium wilt. Also, few studies have examined the relationships between watermelon, arbuscular mycorrhizal root colonization and disease.

The general objectives of this dissertation were to determine whether the mechanism for *Vicia villosa* cover crop suppression of Fusarium wilt of watermelon is specific or general and to assess the effectiveness of Actinovate alone and in combination with cover crops as a biocontrol of Fusarium wilt of watermelon.

The specific objectives of this thesis' studies were to:

1) Evaluate the efficacy of five cover crop treatments with and without an Actinovate biocontrol agent on Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (FON) in the field.
2) Explore general suppression via cover crop treatment effects on rates of soil microbial respiration and variation of *Fusarium oxysporum* spp. in fields where *V. villosa* and *T. incarnatum* disease suppression were observed.
3) Assess the efficacy of Actinovate AG alone and in combination with three different cover crop amendments in controlled greenhouse conditions.
4) Evaluate the *in vitro* effect of *Vicia villosa, Trifolium incarnatum,* and *Secale cereale* leachate on the mycelial growth of *Fusarium oxysporum* f. sp. *niveum* and *Trichoderma harzianum,* and the growth of *S. lydicus.*
5) Examine the percent colonization of watermelon roots by arbuscular mycorrhizae following four fall-planted cover crops, with and without an Actinovate biocontrol application.
6) Assess the *in vitro* fungicidal effects of *S. lydicus* on *F. oxysporum* f. sp. *niveum*.

1.7. Organization of the Dissertation

The first chapter of this dissertation provides the reader with background information relevant to the objectives of the study, the specific objectives, the organization of the dissertation, and discusses ultimate conclusions and recommendations based on the results of the study. Findings were compiled into three manuscripts to be submitted for peer-reviewed publication. The second chapter of this dissertation evaluates the efficacy

of *V. villosa* and other green manures (*T. incarnatum*, *S. cereale*, and *B. juncea*), as well as the product Actinovate AG, for managing Fusarium wilt of watermelon. The third chapter examines general suppression as the mechanism of *V. villosa* disease suppression of Fusarium wilt via measurements of soil microbial communities and changes in *F. oxysporum* populations. The fourth and final chapter focuses on specific disease suppression mechanisms that could play a role in *V. villosa* green manure management of Fusarium wilt, specifically enhancement of arbuscular mycorrhizal colonization of watermelon roots and the *in vitro* effects of cover crop leachates on FON and *S. lydicus*. Additionally, the *in vitro* antagonistic effects of *S. lydicus* on FON were examined.

1.8.Conclusions for Dissertation Objectives

Objective 1: Evaluate the efficacy of five cover crop treatments with and without an actinovate biocontrol agent on Fusarium wilt of watermelon caused by *Fusarium oxysporum f. sp. niveum* (FON) in the field.

Trifolium incarnatum and *V. villosa* amendments decreased Fusarium wilt of watermelon at UM-LESREC (Salisbury, MD) and UD-REC (Georgetown, DE) in 2010. The magnitude of the suppression by *T. incarnatum* was comparable to that of *V. villosa* at UM-LESREC in 2011 but was lower at UD-REC in 2011. These disease suppressive effects were only observed at the locations on the Eastern Shore of Maryland and in Delaware. The suppression was not observed in central Maryland (USDA-BARC, Beltsville, MD) where disease levels were low. *Trifolium incarnatum* was the only cover crop that significantly increased yield (16.30 x 10^2 fruit/ha) compared to bare ground plots and this was observed for only one field trial.

Prior to this study the efficacy of an Actinovate AG (active ingredient *Streptomyce lydicus* WYEC 108, Natural Industries Inc.) biocontrol in decreasing Fusarium wilt of watermelon and enhancing watermelon growth had not been tested. In our study the Actinovate product either did not reduce Fusarium wilt or the magnitude of the reduction was small. Actinovate reduced Fusarium wilt by 1.94% in 2009, as much as 7.07% in 2010 field trial and increased Fusarium wilt by 2.45% in 2011. There were no additive cover crop and Actinovate treatment effects for Fusarium wilt suppression. Actinovate significantly increased yield in one field trial, when applied in bare ground and *Secale cereale* amended plots.

Objective 2: Explore general suppression via cover crop treatment effects on rates of soil microbial respiration and variation of *Fusarium oxysporum* spp. in fields where *V. villosa* and *T. incarnatum* disease suppression were observed.

This study revealed that general suppression does play a role in *V. villosa* and *T. incarnatum* disease suppression of Fusarium wilt of watermelon. At four of the five locations where CO_2 flux was measured, respiration rates were significantly higher in *V. villosa* amended plots compared to all other cover crop and bare ground treatments. Respiration rates in *T. incarnatum* amended plots were significantly higher than nonamended plots for four of the five field trials and were higher than all other cover crop treatments for one. A significant negative correlation between Fusarium wilt severity and respiration measurements validated the hypothesis that general suppression plays a role in *V. villosa* disease suppression of Fusarium wilt of watermelon.

Additionally, increased populations of *F. oxysporum* spp. in *V. villosa* amended plots indicated the possibility that specific suppression is also playing a role in reductions of Fusarium wilt severity. Increased growth and activity of nonpathogenic *F. oxysporum* spp. and other commonly antagonistic microorganisms (ex. fluorescent *Psuedomonas* spp.) are cited as the mechanisms of disease suppression in soils naturally suppressive to FON and with induced FON suppression via a monoculture of 'Crimson Sweet' (Alabouvette et al., 1993; Larkin et al., 1993a; Larkin et al., 1993b; Larkin et al., 1996). It is possibile that increases in nonpathogenic *F. oxysporum* spp. or other antagonistic soil microbial populations are contributing to the *V. villosa* disease suppression of Fusarium wilt, in addition to the general suppression mechanism.

Objective 3: Assess the efficacy of Actinovate AG alone and in combination with three different cover crop amendments in controlled greenhouse conditions.

Surprisingly, in our studies greenhouse experiments, Fusarium wilt severity ratings were significantly higher for watermelon in pots inoculated with FON and amended with *V. villosa or T. incarnatum* than for watermelon in nonamended pots. There are several possible explanations for this Fusarium wilt severity enhancement, including that the cover crops provided a nutrient source for the FON inoculum, helping the fungus establish, and thereby overwhelming nonpathogenic *F. oxysporum* spp. or other antagonistic microbial populations that might normally play a role in *V. villosa* or *T. incarnatum* suppression of Fusarium wilt of watermelon. *Vicia villosa* disease suppression was still observed for watermelon in *V. villosa* amended pots that were not

inoculated with FON, where the background levels of FON in the field soil used in the greenhouse experiment resulted in a moderate amount of wilt.

In these greenhouse experiments Actinovate did not demonstrate the ability to significantly decrease Fusarium wilt of watermelon, nor did it enhance watermelon growth.

Objective 4: Evaluate the *in vitro* effect of *Vicia villosa*, *Trifolium incarnatum*, and *Secale cereale* leachate on the mycelial growth of *Fusarium oxysporum* f. sp. *niveum* and *Trichoderma harzianum*, and the growth of *S. lydicus.*

Vicia villosa leachate significantly stimulated FON growth rates at a pH of 3.5, with FON growing as high as 66.30 % faster on *V. villosa* amended plates than on nonamended. For one *in vitro* trial, at a pH of 6, FON grew 24.14 % faster on *V. villosa* leachate amended plates than on nonamended media. Additionally, *V. villosa* leachate significantly stimulated the growth rate of *T. harzianum* at both a pH of 3.5 and 6, with rates of growth as much as 213.32 % faster than on control plates.

Cover crops leachate amendments had no significant effect on the number of colony forming units *Steptomyces lydicus* WYEC 108 formed *in vitro*.

Objective 5: Examine the percent colonization of watermelon roots by arbuscular mycorrhizae following four fall-planted cover crops, with and without an Actinovate biocontrol application.

The percentage of watermelon roots colonized by arbuscular mycorrhizae were significantly higher following *V. villosa* and *T. incarnatum* green manures compared to

that of bareground, and plots amended with any other green manure treatment, as much as 58.47 % and 44.37 % higher, respectively.

Objective 6: Assess the *in vitro* fungicidal effects of *S. lydicus* on *F. oxysporum* f. sp. *niveum*.

Both treatments of *S. lydicus* WYEC 108-an isolate received from Natural Industries Inc. and one isolated directly from the Actinove AG product-significantly inhibited the radial growth of FON *in vitro*. *Streptomyces lydicus* WYEC 108 inhibited FON growth as much as 44.17 %.

1.9.Recommendations

Although it had already been established that a *Vicia villosa* green manure suppresses Fusarium wilt of watermelon some growers are hesitant to use it because the seed may overwinter to become a weed. This study is the first to identify that a *Trifolium incarnatum* green manure suppresses Fusarium wilt of watermelon. Other leguminous cover crops with similar characteristics, such as high biomass production and mycorrhizal associations, might also induce disease suppression. Comparing the efficacy of a range of leguminous green manures and other similar cover crops for Fusarium wilt suppression could help identify the characteristics an effective disease suppressive green manure must posses.

Other aspects of *V. villosa* management of Fusarium wilt of watermelon still need to be addressed. It has been established that *V. villosa* disease suppressive effects can carry over to the following year (Zhou et al., 2003a) but accumulative suppressive effects

from subsequent plantings of the fall cover crop in the same field have not yet been explored.

The negative correlation of significant elevations in soil respiration in *V. villosa* and *T. incarnatum* and Fusarium wilt disease established the role general disease suppression plays in *V. villosa* and *T. incarnatum* management of Fusarium wilt. However, increases in *F. oxysporum* spp. in *V. villosa* green manure plots indicate specific suppression is also contributing to the decrease in disease. The role that nonpathogenic *F. oxysporum* play in disease reductions in fields that are naturally suppressive to FON, fields with induced suppression, and in cross protection of watermelon against the FON pathogen, make these changes of significant interest (Biles et al., 1989; Alabouvette et al., 1993; Larkin et al., 1996). Although the proportion of FON to nonpathogenic *F. oxysporum* was not determined several studies have established that a form of competitive exclusion occurs between the two and that when Fusarium wilt of watermelon is reduced in concurrence with an increase in overall *F. oxysporum* spp. it usually indicates a decrease or stabilization of the former and an increase in the latter (Zhou et al., 2003b; Alabouvette et al., 1993). Future studies, testing the pathogenicity of *F. oxysporum* spp. in *V. villosa* amended plots on watermelon can validate this theory.

Results of greenhouse and *in vitro* experiments, discussed in separate chapters of this dissertation, support the hypothesis that specific suppression via *V. villosa* stimulation of nonpathogenic *F. oxysporum* spp. is complementing the general disease suppression. In the greenhouse experiments significant reductions in Fusarium wilt severity were observed for watermelon in *V. villosa* amended pots that were not artificially infested with the FON pathogen. The increases in Fusarium wilt severity of

watermelon in pots inoculated with FON and amended with *V. villosa* and *T. incarnatum* demonstrate the cover crops' ability to function as a nutrient source for *F. oxysporum* spp.. *Vicia villosa* leachate also stimulated the *in vitro* growth of FON and *T. harzianum.* As nonpathogenic *F. oxysporum* and FON have similar nutrient requirements it is likely that *V. villosa* would also effectively stimulate the nonpathogenic *F. oxysporum.*

Trichoderma harzanium's ability to compete with pathogens, enhance plant growth and decrease disease is well established. *Vicia villosa* leachate stimulation of *T. harzianum* and FON opens up the question of whether the cover crop specifically enhances the growth of just FON and *T. harzianum*, nonpathogenic *F. oxysporum*, other specific antagonists of pathogens, or just microorganisms in general. Molecular analysis could provide a more detailed picture of how *V. villosa* influences microbial soil populations of these specific fungi, other known antagonists, and the overall soil microbial community.

Both *V. villosa* and *T. incarnatum* succeeded in significantly increasing arbuscular mycorrhizal root colonization of a following watermelon crop. The extent to which this increase in mycorrhizal root colonization contributes to the disease suppression is unknown. A greenhouse experiment comparing Fusarium wilt severity of watermelon with roots inoculated with a commercial mycorrhizal formulation and uninoculated watermelon could clarify if watermelon root AM colonization plays a role in leguminous cover crop disease suppression.

Similarly, a greenhouse experiment with watermelon grown in soil treated with *V. villosa* and *T. incarnatum* leachate could support the theory that the cover crop leachate contributes to the green manure Fusairum wilt suppression. It is very possible that the

root exudates of the two cover crops are also responsible for the microbial stimulus that is resulting in the disease suppression. Additional greenhouse experiments could also explore this possibility. Many different factors likely interact in *V. villosa* and *T. incarnatum* disease suppression of Fusarium wilt of watermelon. Knowledge of the complete ecology of this pathosytem can facilitate the development of complementary or more effectual disease management approaches. Also, a better understanding of this pathosystem could encourage growers to utilize a fall-planted cover crop that is tilled in the spring as a green manure for management of Fusarium wilt of watermelon.

This papers field and greenhouse studies demonstrated that the Actinovate AG biocontrol product is not an optimal management tool for Fusarium wilt of watermelon in Maryland and in Delaware as any of its effects on the disease were either low or nonexistent. The efficacy of Actinovate AG's active ingredient, *S. lydicus* WYEC 108, against the pathogen FON *in vitro* suggest that it is a competent antagonist when in conditions conducive to its growth. Actinovate AG might be more effective in managing plant diseases that flourish in soil environments high in pH and soil organic matter or for foliar diseases, as there can be less competition in the phyllosphere than the rhizospere. This OMRI approved product has proven its worth for other diseases and should continue to be considered as a management tool for pathogens which are problematic in conditions more favorable to survival and growth of Actinovate's active ingredient.

Chapter 2: Impact of Five Cover Crop Green Manures and an Actinovate Biocontrol Product on Fusarium Wilt of Watermelon Caused by *Fusarium oxysporum* f. sp. *niveum*

2.1.Abstract

Triploid watermelon cultivars are grown on more than 2,023 ha in Maryland and in Delaware. Triploid watermelons have little host resistance to Fusarium wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*). The effects of four different fall planted cover crops that were tilled in the spring as green manures (*Vicia villosa, Trifolium incarnatum, Secale cereale, Brassica juncea*) and bareground were evaluated alone and in combination with the biocontrol product Actinovate (*Streptomyces lydicus*) on Fusarium wilt severity, and fruit yield and quality of watermelon. Six field experiments were conducted over three years in Beltsville and Salisbury, MD and Georgetown, DE. Both *Vicia villosa* and *T. incarnatum* significantly suppressed Fusarium wilt of watermelon as much as 21%, compared to watermelon in nonamended plots. The only cover crop that significantly increased yield compared to nonamended treatments was *T. incarnatum* (129% more fruit/ha), but only for one field trial. The Actinovate product either did not reduce Fusarium wilt or the magnitude of the reduction was small. Actinovate significantly reduced Fusarium wilt by 2% in 2009, as much as 7% in 2010 and increased Fusarium wilt severity by 2.5% in 2011. Actinovate significantly increased yield for one field trial, when applied to nonamended or *S. cereale* amended plots.

2.2. Introduction

Fusarium oxysporum f. sp. *niveum* (FON) *Schlechtend*, Fr. (E.F. Sm.) W. c. Snyder & H. N. Hans is the cause of Fusarium wilt of watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai). Watermelon growers on the Eastern Shore of Maryland and in Delaware experience significant losses in yield due to the prevalence of Fusarium wilt in their fields (Zhou et al., 2003b). Losses can approach 100% if susceptible cultivars are planted in heavily infested fields (Egel et al., 2007). Yield loss is especially high in fields planted with triploid (seedless) watermelon, which currently lack resistance to Fusarium wilt (Everts et al., 2010; Everts et al., 2011). Consumer demand for seedless watermelon has increased dramatically in recent years so the need for management options for Fusarium wilt have also increased (Lucier et al., 2001; USDA-ERS, 2011).

Management options for Fusarium wilt of watermelon vary in effectiveness and cost. Grafting is an effective disease management tool, however it requires high labor and seed costs (Davis et al., 2008). Methyl bromide (MeBr), a soil fumigant historically used to manage Fusarium wilt of watermelon (King et al., 2008), has been phased out due to its destructive effects on stratospheric ozone (Ferguson et al., 1997; Gullino et al., 2005). Other soil fumigants are available for Fusarium wilt management but are generally not as successful as MeBr at controlling the disease, are costly, and also have detrimental environmental effects (Ferguson et al., 1997). Alternative biological and cultural control methods might be preferred.

In 2004, Zhou and Everts found that a *Vicia villosa* Roth (hairy vetch) green manure decreased the incidence of Fusarium wilt on watermelon as much as 42-48% (Zhou et al., 2004). Similarly, in future studies, a *V. villosa* cover crop was found

effective against Fusarium wilt of watermelon, reducing it as much as 63% (Zhou et al., 2006, Zhou et al., 2007; Keinath et al., 2010). Other cover crops such as *Brassica juncea* (Sarwar et al., 1988), *Secale cereale* (Lewis et al., 1977), and *Triticum aestivum* (Ristaino et al., 1997; Mazzola et al., 2002) reduce diseases of following cash crops such as Fusarium root rot of beans (*Fusarium solani* f. sp. *phaseoli*) and Phytophthora blight of pepper (*Phytophthora capsici*).

Actinovate AG (Natural Industries Inc., Houston, TX) is an Organic Materials Review Institute approved biocontrol product whose active ingredient is *Streptomyces lydicus* strain WYEC 108. Actinovate is labeled for protection of watermelon from Fusarium wilt and is supposed to increase plant yields. However, few studies have been conducted to evaluate Actinovate product effects on Fusarium wilt of watermelon under field conditions. Likewise there are no studies that evaluate whether Actinovate in combination with a *V. villosa* cover crop will enhance disease suppression.

The objective of this study was to evaluate the efficacy of four cover crops, *V. villosa*, *Trifolium inarnatum*, *B. juncea*, and *S. cereale* with and without an Actinovate biocontrol product on Fusarium wilt of field grown triploid watermelon.

2.3.Materials and Methods

2.3.1.Experimental Design

An experiment was conducted six times as randomized split plot block designs in three different locations over a period of three years (2009 to 2011) (Table 2.1). Field locations included the University of Delaware's Carvel Research and Education Center (UD-REC) in Georgetown (2011), the USDA's Henry A. Wallace Beltsville Agricultural Research Center (USDA-BARC) in Beltsville, MD (2009 & 2010) and the University of

Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC) in Salisbury (2009, 2010, & 2011). The main plot treatments were cover crops, and the subplots treatments were Actinovate and, in fields that were not infested, FON inoculation (Table 2.1). Whether field soil was inoculated with FON race 1 or naturally infested was different depending on the year and location (Table 2.1).

At the USDA-BARC location field soil was an amalgam of a Russett-Christiana and Downer-Hammonton complex of sandy loam. At UM-LESREC, field soil was Norfolk "A" loamy sand and at UD-REC Rosedale loamy sand. Cover crops were incorporated in the spring as a green manure. Cover crop treatments were *V. villosa*, *T. incarnatum*, *B. juncea*, and *S. cereale* but varied by year and location (Table 2.1).

2.3.2.Main Plot Treatment Establishment

Cover crops were seeded in late fall (Table 2.1) according to local seeding rates (Table 2.1). In 2011, due to low germination or winter kill the previous season cover crops were overseeded at UM-LESREC and UD-REC respectively to ensure sufficient above ground biomass (Table 2.1). The seeds of *V. villosa* and *T. incarnatum* were inoculated with a commercial *Rhizobium spp*., N-DURE (IN TX Microbials, LLC) prior to planting. All cover crop treatments were killed by a paraquat treatment (Gramoxone Extra 2.5SC, 1.2 kg ai/ha, 1.75 L/ha) in early spring (Table 2.1) and a tractor-mounted rototiller was used to disk the soil three times to a depth of approximately 15 to 20 cm. Control plots were cultivated in the same manner.

In each plot three 1 m^2 quadrats of above ground plant biomass were collected, dried and weighed prior to green manure incorporation. Biomass sample weights were used to estimate the amount of N the various cover crops contributed to their respective

plots and subsequent fertilization was based on these calculations. The fertilizer for the watermelon crop was applied all at once, immediately prior to the laying of the black polyethylene mulch. All beds were formed by a bed shaper and a single drip irrigation tube was laid under black polyethylene mulch. Beds were laid on a 1.83 m center in fields in 2009 and a 2.13 m center in 2010 and 2011.

Seeds of watermelon cv. 'Sugar Heart' were planted into trays in a potting mix (Sun Gro Redi-earth Plug and Seedling Mix; Sun Gro Horticulture, Bellevue, WA). After 4 to 5 weeks of growth watermelon seedlings were planted 0.91 m apart in each bed (Table 2.1). The pollinizer 'SP_4,' which is resistant to Fusarium wilt race 1, was transplanted between every third seedless watermelon at all locations except LESREC-UM in 2009 and USDA-BARC in 2010 when the diploid watermelon cultivar Royal Majesty was used as a pollinizer. The application of insecticides and foliar fungicides were applied as needed according to extension recommendations for the mid-Atlantic. All fields were managed conventionally except for the UM-LESREC field in 2009, which was managed with organic practices on organic certified land.

2.3.3.Subplot Treatment Establishment

The Natural Industries recommended rate of Actinovate AG foliar spray is 850.48 g Actinovate AG/946.25 L H_2O/1 ha. Therefore, 0.011 g Actinovate AG/12.2 ml H_2O per 0.156 m^2 transplant tray was applied as a suspension to watermelon seedlings one to two weeks prior to transplanting (Table 2.1). Actinovate was also applied within the week following transplanting as a soil drench around the base of the plant at 255.14 g Actinovate AG/378.5 L H_2O/4046.86 m^2; approximately 0.115 g/172 ml H_2O/1.89 m^2 per a plant.

A race 1 isolate (F-030-1) of *F. oxysporum* f. sp. *niveum* (FON), which was acquired from a wilted watermelon plant in Wicomico, Maryland in a previous study (Zhou et al., 2003b), was used for disease inoculations. Strains were maintained in a mixture of sandy soil and perlite (1:2 wt/wt) at 4°C.

Inoculum was prepared by transferring F-030-1 growing on Komadas media (Komadas, 1975) into a liquid mineral salts medium (Netzer, 1976). The culture was incubated on an orbital shaker at 128 rpm at room temperature for approximately two weeks before filtering through eight layers of cheesecloth. A Spencer hemacytometer was used to adjust the inoculum to the desired concentration.

In 2009 at both USDA-BARC and UM-LESREC seedlings were inoculated immediately after transplanting by pipetting 6 ml of FON inoculum at a concentration of 2.45×10^6 CFU/ml into a hole (approximately 3 cm in diameter and 8 cm deep) 7 cm away from the crown of the watermelon plant (Zhou et al., 2004). In 2010 at USDA-BARC, the FON inoculation method was modified to increase disease incidence. Two days after cover crop incorporation and a week prior to the laying of black plastic 60 ml of 2.85×10^8 CFU/ml FON inoculum was mixed into one gallon of H_2O and evenly banded across the center of each 36.92 m^2 bed with a watering can. A second application of 11 ml of FON inoculum at a concentration of 2.33×10^6 CFU/ml was pippeted next to each watermelon plant using the same method employed in 2009.

2.3.4. Field Evaluations

2.3.4.a.*Fusarium* spp. Stem Colonization

The presence and colonization of *F. oxysporum* in watermelon stems was also assayed. In 2010 watermelon plants were destructively harvested one week following transplanting (2 plants per bed, n=80 to 90 plants), two weeks (2 plants per bed) and three weeks (one plant per bed) after transplanting. Roots were cut into 10 mm pieces, soaked in 0.5% sodium hypochlorate for 60 seconds and plated on semi-selective isolation media to evaluate roots colonization of FON (Zhou et al., 2004). Distance of FON colonization from the hypocotyl was measured. Sections were visually inspected and the number of sites along a given length of root or stem with FON growth counted (Zhou et al. 2004). In 2011 a different technique was used to measure Fusarium root colonization to determine magnitude of stem colonization. Two plants were sampled from each plot two weeks after field planting. Five 10 mm stem pieces at 1 cm, 5 cm, 9 cm, 13 cm, and 17 cm distances from the crown node were weighed and homogenized in a blender before being filtered through eight layers of cheesecloth and plated on Komadas media as a 1:1 and 1:10 soil dilution (Zhou et al., 2006), one plate for each dilution. Colony forming units on each plate were counted a week later and converted to CFU/g of fresh stem weight.

2.3.4.b.Fusarium Wilt Ratings

Wilt severity was rated as the percentage of foliar wilt of each watermelon plant and averaged for each bed (Larkin et al., 2007). Watermelon were planted 0.91 m apart in a bed but the total number of watermelon planted per a field varied depending on year and location (n=10 to 16 watermelon plants/treatment bed) . Fusarium wilt severity was evaluated weekly after transplanting in 2009. The percentage of wilted watermelon

foliage for each plant was measured and this percentage was averaged for all watermelon in a treatment bed. In 2009 additional symptoms of stunting and chlorosis were present and included in the wilt rating. For 2009 only, if a watermelon plant was chlorotic the wilt rating was increased by 10% for that plant and if a watermelon was stunted, the wilt rating was increased by 25%. In 2010 Fusarium wilt severity was measured three times at UM-LESREC and once at USDA-BARC. In 2011 Fusarium wilt severity was evaluated weekly following transplanting. The Area Under the Disease Progress Curve (AUDCP) was calculated for each field trial except at USDA-BARC in 2010 as only one wilt rating was taken. The AUDCP was calculated as AUDPC= $\sum[(y_i + y_{i+1})/2 \times (t_{i+1}-t_i)]$ (Capdeville et al., 2002). Stems of wilted watermelon plants were plated on Komadas media to verify the presence of the Fusarium wilt pathogen (Komadas, 1975).

2.3.4.c.Plant Vigor and Yield

In 2010 watermelon vine length was measured approximately three weeks following transplanting at both locations. In 2011 vines were measured twice at UM-LESREC, once seven days after transplanting and again the following week. At UD-REC vines were measured three times at weekly intervals beginning seven days after transplanting.

All fruit were individually weighed and counted at harvest, percent Brix was assessed for three watermelon fruit per a bed (n=144 to 180 fruit sampled/field) with a hand held refractometer, and the numbers of sunburned fruit were counted. Marketable sized fruit were defined as watermelon that weighed >3.18 kg.

2.3.4.d.*Streptomyces lydicus* in the Rhizosphere

In 2010, directly after fruit harvest, seminal watermelon roots from each plot in four of the replicate blocks at UM-LESREC and USDA-BARC (n=40 to 60, respectively) were plated on Arginine-Glycerol-Salt Agar to observe any growth of *Streptomyces* spp. from the roots to determine if *Streptomyces lydicus* successfully colonized the watermelon roots (Dhananjeyan et al., 2010). As no *S. lydicus* were observed in 2010 sampling was instead done two weeks after watermelon were transplanted to the field in 2011, at both UM-LESREC and UD-REC. Seminal roots of destructively harvested watermelon plants, one for each plot (n=60), were cut into three 3 cm root sections, put into test tubes with 5 ml of sterile distilled water, soaked for five minutes and vortexed. The resulting mixture was serially diluted onto Sporulation ager (SPA) amended with nyastatin, carbenicillin and cycloheximide and Casein Agar to observe if *Streptomyces spp*. were present in the soil rhizosphere (Yuan et al., 1995).

2.3.4.e.Nutrient Analyses

Soil nutrients, dried cover crop foliage, and dried watermelon foliage for the different treatments were analyzed in 2011 to determine if nutritional differences impacted disease suppression (Ochiai et al., 2008).

In 2011 three six inch soil cores were collected from each treatment plot following tillage, May 25th at UD-REC, and June 20th at UM-LESREC, and analyzed at the University of Delaware’s Soil testing lab for pH, Buffer pH, percent OM, and in mg/kg P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, Al, CEC (meq/100g) Base Saturation % and P saturation ratio.

Cover crop biomass was collected three times from 2 m^2 in each plot, at both locations, approximately a week prior to soil incorporation.

For both UM-LESREC and UD-REC in 2011, directly before harvest, five watermelon leaves per bed that were the second or third leaf from a vines' terminal point were collected, dried, and sent for nutrient analysis to Brookside laboratories Inc. (percent nitrogen, phosphorous, magnesium, potassium, calcium, sulfur, boron, iron, manganese, copper, zinc and aluminum).

2.3.5.Statistical Analyses

Data were analyzed using the MIXED procedure with the Statistical Analysis System (version 9.2; SAS Institute, Cary, NC), which integrates random effects in the statistical model and performs covariance structure modeling (Littell et al., 1998). Treatment means were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. When disease was low the proportional wilt data was usually not normally distributed. However, because of the large sample size (n>30), this data was considered robust enough to be analyzed without transformation (Payton et al., 2006). This is supported by the Central Limit Theorem which states that the sample mean of a population same size of n> 30 will converage to a standard normal distribution (Corbett et al., 2002).

2.4. Results

2.4.1. Cover Crop Foliar Analysis

Differences in cover crop nutrient concentrations were not consistent across field locations and there were no obvious trends that linked these variations to Fusarium wilt disease suppression (Table A1.1).

2.4.2. Fusarium Wilt Severity

There were no significant interactions in the effect of the cover crop and Actinovate biocontrol treatments on Fusarium wilt severity (data not shown).

2.4.2.a. Cover Crop Effects

There was low disease pressure at UM-LESREC in 2009 and minimal cover crop biomass at UM-LESREC in 2010 which resulted in no, or very low wilt severity (less than a percent difference) among treatments (Table 2.1 & Table 2.2). *Vicia villosa* and *T. incarnatum* disease suppression was seen at both UM-LESREC and UD-REC in 2011 (Table 2.3). Watermelon plants in *V. villosa* amended plots and *T. incarnatum* amended plots had significantly less disease (24.24% to 11.37% and 25.83% to 7.53% less, respectively) than plants in nonamended plots or in plots amended with any other cover crop treatment at UM-LESREC in 2011. Watermelon in *V. villosa* and *T. incarnatum* amended plots also had a lower AUDPC (56.27 and 68.04 respectively) compared to watermelon planted in *B. juncea* (81.82) or bare ground (84.57) amended plots (Figure 2.1). Fusarium wilt severity of watermelon in *B. juncea* amended plots was consistently the highest or second highest numerically, although they were never significantly higher than the watermelon in nonamended or *S. cereale* amended plots (Table 2.3).

Although the two leguminous cover crops suppressed Fusarium wilt to a similar extent in 2011 at UM-LESREC this was not the case for UD-REC where *T. incarnatum* did not suppress Fusarium wilt more than *S. cereale* or *B. juncea*. Six weeks after transplanting to the field the watermelon grown in *V. villosa* amended plots had significantly less disease than watermelon in any other treatment plots except for that of *T. incarnatum* amended plots. Watermelon in *T. incarnatum* plots also had significantly less Fusarium wilt than plants in bare ground plots (Table 2.3). The watermelon wilt severity was also lowest in *V. villosa* and *T. incarnatum* plots the following week. However, the *P* value was 0.0535 and therefore not considered significant (Table 2.3). The AUDPC of watermelon in *V. villosa* amended plots was significantly less (56.27) than watermelon in *B. juncea* (81.82) and bare ground (84.57) treated plots (Figure 2.2).

Cover crop treatments did not significantly influence Fusarium wilt at USDA-BARC where disease was nominal but the same trend in *V. villosa* and *T. incarnatum* disease suppression was still numerically apparent in 2009 and 2010 (Table 2.3).

2.4.2.b.Actinovate Biocontrol Effects

Actinovate treatments reduced Fusarium wilt for two field trials and increased Fusarium wilt for one field trial. Six weeks after transplanting, at UM-LESREC in 2009, watermelon treated with Actinovate had 1.94% less Fusarium wilt than watermelon in untreated plots (Table 2.4). At UM-LESREC in 2010 the AUDPC and Fusarium wilt severity measured four weeks after transplanting was lower for watermelon in Actinovate treated plots than for watermelon in control plots. In contrast, Actinovate biocontrol applications increased Fusarium wilt severity 2.45% for the wilt reading taken three

weeks after transplanting at UD-REC in 2011 (Table 2.4). Ultimately Actinovate treatment effects were minimal and inconsistent.

2.4.3. Plant Vigor and Yield

Significant cover crop treatment effects on marketable watermelon yield were only observed at UM-LESREC in 2011 where *T. incarnatum* amended plots had marketable watermelon yield that was higher (14.67 x 10^2 to 20.68 x 10^2 more fruit/ha) than that of all other amended or nonamended plots (Table 2.5).

There was a significant Actinovate and cover crop treatment interaction for marketable watermelon for only one field trial. Beds amended with *S. cereale* or bare ground and treated with Actinovate had significantly higher marketable yield compared to their respective untreated or uninoculated beds at USDA-BARC in 2009 (Table A1.2). The only other field trial where Actinovate demonstrated significant positive effects on marketable watermelon yield was LESREC in 2011 when Actinovate significantly increased marketable watermelon yield (9.33 x 10^2 more fruit/ha) compared to beds that were not treated with Actinovate (Table 2.5).

Neither cover crop nor Actinovate affected marketable watermelon weight, or FON growth from watermelon stems for any field trial (data not shown). In addition, no cover crop or Actinovate treatment effects were seen in more than one field trial for sugar content (Table A1.3 and A1.4), watermelon vine length (Table A1.5), or the number of sunburned watermelon (Table A1.6).

2.4.4.Watermelon Foliar Analysis

Cover crop and Actinovate treatments did not consistently effect nutrient concentration of watermelon foliage nor was there any correspondence with disease suppressive trends observed in the field data (Table A1.7).

2.5.Discussion

2.5.1.Temperature, pH and Soil Texture

Fusarium wilt severity varied by location and year. Fusarium wilt severity was low at USDA-BARC while it was high in fields on the Eastern Shore of Maryland and in Delaware where significant differences in disease suppression were observed. The variation in Fusarium wilt disease pressure observed in our experiments may have resulted in part from differences in the soil environment. Fusarium wilt of watermelon is most prevalent in sandy, slightly acidic soils (pH 5-6) (Zitter et al., 1996). The field at USDA-BARC had a higher amount of organic matter and a more neutral pH compared to fields located on the Eastern Shore of Maryland and in Delaware which are very sandy, low in organic matter (>1%), and have low pH levels. Amir et al., (1993) demonstrated that Fusarium wilt suppression is not completely independent of soil texture and that clay textured soils can encourage Fusarum wilt disease suppression. Hoper et al., (1995) also found that Fusarium wilt disease suppression was influenced by soil texture, as well as pH. These environmental differences likely contribute to the prevalence of Fusarium wilt of watermelon on the Eastern Shore of Maryland and Delaware and could explain why it is less common in central Maryland (Zhou et al., 2003b)

Fusarium wilt severity is also dependent on inoculum and weather. Fusarium wilt severity is greatest at temperatures around 25-27º C and low to moderate moisture (Zitter et al., 1996). Field trials at UM-LESREC and USDA-BARC in 2010 were extremely hot and resulted in stunting and yellowing of plants, and less foliar wilt. The average daily peak soil surface temperature (top 50.8 mm) at UM-LESREC in July of 2010 was 33.14 º C. A study specifically looking at temperature found that the temperature significantly impacted Fusarium wilt of tomato with low disease incidence at cool temperatures, severe wilt at warm temperatures (27º C) and only moderate wilt at high temperatures (Larkin et al., 2002).

Additionally, at UM-LESREC in 2010 there was >3 meters of snow over the winter which reduced cover crop biomass in many of the plots. In 2011 at UD-REC and UM-LESREC cover crops were overseeded in the early spring to ensure sufficient biomass coverage, the weather was moderate, and watermelon were planted in fields with soil conditions conducive to Fusarium wilt and which already had high levels of FON.

Suppression of Fusarium wilt using a *V. villosa* cover crop has been evaluated in geographical climates and soil conditions different from that of the Eastern Shore of Maryland and in Delaware, but thus far no definitive conclusions have been drawn. Keineth et al. (2010) conducted a study in South Carolina where Fusarium wilt of watermelon was too low to detect any significant *V. villosa* suppressive effects for the 2008 field season. Due to low levels of disease at USDA-BARC no conclusions can be drawn about the effectiveness of *V. villosa* as a disease management tool in central MD. However, *V. villosa* and *T. incarnatum* green manures showed a small, but nonsignificant, Fusarium wilt reduction in the two trials done at USDA-BARC.

Achieving any measure of reduction in Fusarium wilt of watermelon can potentially impact marketable watermelon yield, providing financial benefits to the grower.

2.5.2. *Vicia villosa* and *Trifolium incarnatum* Disease Suppression

Vicia villosa and *T. incarnatum* reduced Fusarium wilt of watermelon at UD-REC and UM-LESREC in 2011. The ability of a *V. villosa* cover crop to suppress Fusarium wilt of watermelon has been observed in previous studies done on the Eastern shore of Maryland (Zhou et al., 2003b; Zhou et al., 2004, Zhou et al., 2006); however, no previous studies have reported the disease suppressive effects of a *T. incarnatum* green manure on Fusarium wilt of watermelon or any other disease.

Despite many demonstrations of a *V. villosa* green manure suppressing Fusarium wilt of watermelon, it has not been widely adopted because the seed may overwinter to become a weed (Jacobsen et al., 2010). Our findings indicate that both *V. villosa* and *T. incarnatum* cover crops significantly reduce Fusarium wilt of watermelon on the Eastern shore of Maryland and Delaware. The magnitude of the suppression by *T. incarnatum* was comparable to that of *V. villosa* at UM-LESREC in 2011 but was lower at UD-REC in 2011.

2.5.3. Marketable Watermelon Yield

Marketable watermelon yield varied by location. However, these differences were not likely to be attributed to fertilizer. Fertilizer was applied to plots based on cover crop biomass and its estimated nitrogen contribution. *Vicia villosa* and *T. incarnatum* plots received less nitrogen fertilizer. Fields at USDA-BARC had higher marketable watermelon yields, which could be attributed to greater soil organic matter and lower disease pressure.

Trifolium incarnatum improved marketable watermelon yield at UM-LESREC in 2011 compared to other amended or nonamended plots. Additionally, *V. villosa* and *T. incarnatum* cover crops increased marketable yield in 2009 at USDA-BARC, although not significantly. It has been previously demonstrated that both *V. villosa* and *T. incarnatum* can increase watermelon fruit yield (Rangappa et al., 2002; Zhou et al., 2004; Keinath et al., 2010). It is also well established that cover crops can improve soil health by increasing soil organic matter, nitrogen fixation, reducing soil erosion, and improving soil water filtration (Wyse, 1994; Drinkwater et al., 1995; Rosecrance et al., 2000; Cavigelli et al., 2003; Hartwig et al., 2002; Kort et al., 1997; Steinmaus et al., 2008; Campiglia et al., 2010a). Together, Fusarium wilt reductions and soil health improvements contribute to increased watermelon yield. *Vicia villosa* and *T. incarnatum* suppression of Fusarium wilt of watermelon and the soil health benefits associated with using a leguminous green manure makes them valuable tools for growers.

2.5.4.*Brassica juncea* Effects on Fusarium Wilt

Brassica juncea amended plots had the highest Fusarium wilt severity at USDA-BARC in 2010, UM-LESREC in 2011 and at UD-REC in 2011. Although these differences were not always significant, Fusarium wilt severity of watermelon in *B. juncea* amended plots was never significantly lower than in bare ground plots. The results of our study are similar to that of Mazzola et al. (2001) who saw an increase in *Fusarium spp.* populations in the soil following brassica seed meal amendments. Smolinska et al. (2003) observed *Brassica spp.* isothiocynate Benzyl ITC increased sporulation of *F. oxysporum* isolate 9051C. Njoroge et al. (2008) recorded elevations of *F. oxysporum spp.* populations in watermelon fields following the incorporation of *B. juncea*. For one field

season Njoroge et al. (2008) also observed that Fusarium wilt of watermelon in *B. juncea* amended plots had 10% more disease than plants in control (bare ground) plots.

The allelopathic effects of brassica cover crops on watermelon have not been extensively examined but *Brassica spp.* secondary compounds could potentially be increasing watermelon stress, reducing their defense response. Further research on the direct and indirect effects of *Brassica spp.* on soil populations of FON would help elucidate *B. juncea's* association with increased Fusarium wilt of watermelon.

2.5.5.Actinovate Biocontrol Efficacy

Several biocontrol products have suppressed Fusarium wilt of watermelon. Bio-organic fertilizers with *Paenibacillus polymyxa* as an active ingredient effectively decreased incidence of Fusarium wilt of watermelon (Wu et al., 2009b; Ling et al., 2010; Ling et al., 2011).

However, Actinovate biocontrol either did not reduce Fusarium wilt of watermelon or else the reduction was minimal and inconsistent. This is similar to results in other pathosystems where Actinovate did not suppress disease. For example, Actinovate treatment did not reduce anthracnose (*Colletotrichum obiculare*) incidence or defoliation in watermelon, corm rot (*Fusarium oxysporum* f. sp. *gladioli*) of Gladiolus (*Gladiolus x hortulanus*), Fusarium wilt incidence on tomato (*Fusarium oxysporum f. sp. lycopersici*), or Fusarium root rot (*Fusarium spp.*) of sweet potato (*Ipomoea batatas*) (Elmer, 2001; Elmer et al., 2000; Damicone et al., 2006; Henn, 2009).

In the trials reported in this paper Actinovate negatively impacted vine length at one location, improved marketable watermelon yield in two of the six field trials, and decreased marketable watermelon yield in one of the six field trials. Other studies that

tested Actinovate's growth and yield promoting abilities found similar results. Biomass of spinach treated with Actinovate AG + Micro 108 Seed Inoculant was no different than spinach in control plots (Cummings et al., 2008). Tomato treated with Actinovate had yields that were no different than that of control plots (Vallad et al., 2011). Actinovate SP + Latron BI956 treatments did not significantly impact watermelon yield (Damicone et al., 2006).

The mechanism of Actinovate disease suppression has been linked with the ability of its active ingredient, *S. lydicus*, to colonize crop roots (Yuan et al., 1995; Tokala et al., 2002). We were unable to isolate *Streptomyces lydicus* from the watermelon plant roots despite repeated attempts. *Streptomyces* spp. grow best in higher pH conditions around 25° C and are able to utilize complex organic materials as an energy source (Hiltunen et al., 2008). As soil pH levels are low on the Eastern Shore of Maryland and in Delaware, *S. lydicus* growth could have been inhibited, preventing the colonization of watermelon roots.

The combination of two disease management tactics has been shown to result in greater disease suppression. In 2004 Zhou and Everts found that a *V. villosa* green manure decreased the incidence of Fusarium wilt of watermelon 63%, 53% and 22% with a highly resistant, moderately resistant, and susceptible watermelon cultivar, respectively (Zhou et al., 2004). Watermelon in plots amended with *V. villosa* and treated with Urea (875 lb/A) had 32% less Fusarium wilt of watermelon than in bare ground plots and 10% less than watermelon in plots only amended with *V. villosa* (Zhou et al., 2002). However, according to the six field trials conducted for this experiments the

combination of the green manures and Actinovate biocontrol treatment achieved no significant additive effect on the suppression of Fusarium wilt.

2.6. Conclusion

Both *V. villosa* and *T. incarnatum* suppressed Fusarium wilt of watermelon but the suppression was dependent on environmental conditions as significant cover crop effects were only observed in fields on the Eastern Shore of Maryland and Delaware, not Beltsville, MD. The soil health benefits of *T. incarnatum* are similar to that of *V. villosa* but *T. incarnatum* is also killed more easily and less likely to overwinter under mid Atlantic conditions.

Due to the nominal and inconsistent effects of Actinovate on Fusarium wilt of watermelon in this study we do not recommend it for use against the FON pathogen. Actinovate did not have any additive effects on Fusaium wilt in combination with any of the cover crop treatments. However, the combination of *V. villosa* or *T. incarnatum* green manures with biocontrol products whose ability to colonize the watermelon rhizosphere and suppress Fusarium wilt of watermelon are already established could provide improved management of Fusarium wilt.

Table 2.1 Management and design of field experiments to evaluate tilled cover crop and Actinovate[a] biocontrol application on Fusarium wilt severity and watermelon yield for six different field trials in Maryland and in Delaware

Location, Year	USDA-BARC[b] 2009	USDA-BARC 2010	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	UD-REC 2011
Main plot treatment	*Vicia villosa, Trifolium incarnatum, Secale cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground
Sub plot treatment	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate	FON, FON + Actinovate	FON, FON + Actinovate
Cover crop seeding rates	*V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha	*V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, *B. juncea* 6.73 kg/ha.	*V. villosa* 50.44 kg/ha, *S. cereale* 134.50 kg/ha,	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha
Cover crop seeding dates	9/24/2008	9/22/2009	10/16/2008	9/25/2009	10/15/2010; 3/9/2011[c]	10/25/2010; 3/15/2011
Cover crop tillage	5/23/2009	5/23/2010	5/15/2009	5/24/2010	5/19/2011	5/25/2011
Dates of Actinovate applications	Foliar 06/03/2009; Soil drench 06/29/2009	Foliar 06/16/2010; Soil drench 06/29/2010	Foliar 06/02/2009; Soil drench 06/29/2009	Foliar 06/08/2010; Soil drench 06/15/2010	Foliar 05/20/2011; Soil drench 06/03/2011	Foliar 05/26/2011; Soil drench 06/07/2010
Watermelon transplant date	06/17/2009	06/18/2010	06/19/2009	06/12/2010	06/03/2011	06/07/2011

[a]Actinovate AG is a biocontrol product (Natural Industries Inc.) which is labeled for management of Fusarium wilt of watermelon

[b]UM-LESREC=University of Maryland Lower Eastern Shore Research and Education Center located in Salisbury. USDA-BARC=United States Department of Agriculture Beltsville Agricultural Research Center located in Beltsville Maryland and UD-REC=University of Delaware's Carvel Research and Education Center located in Georgetown.

[c]In 2011 cover crops were overseeded in the early spring to ensure plots had sufficient cover crop biomass

[d]Soil moisture was either measured using a soil moisture probe (FieldScout TDR-300 Spectrum Technologies, East Plainfield, IL, USA) or by weighing and drying soil samples taken by a soil auger.

Table 2.2 Variance analysis for the effects of cover crop and Actinovate AG application main effects on watermelon Fusarium wilt severity and the area under the disease progress curve (AUDPC) in field experiments conducted at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture at the Beltsville Agricultural Research Center (USDA-BARC) and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010 and 2011

Location	Week 1[a]	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	AUDPC
Main effect									
UM-LESREC 2009									
Cover Crop[b]	-[c]	0.0016	0.6832	0.6743	0.8041	0.4853	0.5593	-	0.6459
Actinovate[d]	-	0.7363	0.1392	0.1396	0.3596	0.0096	0.7203	-	0.1533
USDA-BARC 2009									
Cover Crop	-	-	0.3475	0.7066	0.4363	0.5578	0.3569	-	0.3858
Actinovate	-	-	0.9085	0.5346	0.3829	0.4297	1.0000	-	0.8924
UM-LESREC 2010									
Cover Crop	0.5496	-	-	0.1619	-	0.0887	-	-	0.2024
Actinovate	0.0670	-	-	0.0379	-	0.0603	-	-	0.0373
USDA-BARC 2010									
Cover Crop	-	-	-	-	-	-	-	0.1386	-
Actinovate	-	-	-	-	-	-	-	0.3707	-
UM-LESREC 2011									
Cover Crop	0.4118	0.1152	0.1445	0.0013	0.0056	<0.0001	0.0458	-	<0.0001
Actinovate	0.0924	0.7092	0.6713	0.9771	0.5115	0.6459	0.5030	-	0.5247
CARVEL-REC 2011									
Cover Crop	0.3888	0.4779	0.2418	0.4201	0.1641	0.0175	0.0535	-	0.0251
Actinovate	0.1319	0.1898	0.0206	0.7908	0.6214	0.6861	0.3333	-	0.8020

[a]The number of weeks after watermelons were transplanted to the field.

[b]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.

[c]The symbol – indicates the measurement was not taken for that date and location or, for BARC in 2010, that no wilt was observed.

[d]Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at a rate of 0.011 g/12.2 ml H_2O per 0.156 m^2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H_2O/1.89 m^2 per a plant.

Table 2.3 Severity of Fusarium wilt of watermelon following tilled cover crop or bare ground at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC) and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010 and 2011

Location	Weeks after Transplanting								
Cover crop[a]	1	2	3	4	5	6	7	8	AUDPC
USDA-BARC 2009									
Vicia villosa	-[b]	-	1.30 a[c]	0.00 a	0.42 a	0.42 a	0.00 a		1.50 a
Trifolium incarnatum	-	-	0.42 a	0.42 a	0.00 a	0.42 a	0.42 a		1.20 a
Secale cereale	-	-	0.71 a	0.21 a	0.00 a	1.25 a	0.83 a		2.22 a
Bare ground	-	-	2.92 a	0.63 a	0.00 a	0.21 a	0.00 a		4.17 a
P>F			0.3475	0.7066	0.4363	0.5578	0.3569		0.3858
LESREC 2009									
V. villosa	-	0.97 b	0.92 a	0.053 a	1.10 a	0.28 a	1.40 a	-	3.50 a
S. cereale	-	0.49 a	6.20 a	0.38 a	1.10 a	1.10 a	1.70 a	-	12.10 a
Bare ground	-	0.97 b	6.80 a	0.40 a	0.56 a	0.56 a	2.50 a	-	10.00 a
P>F		0.0016	0.6832	0.6743	0.8041	0.4853	0.5593		0.6459
USDA-BARC 2010									
V. villosa	-	-	-	-	-	-	-	12.12 a	-
T. incarnatum	-	-	-	-	-	-	-	13.82 a	-
S. cereale	-	-	-	-	-	-	-	18.50 a	-
B. juncea	-	-	-	-	-	-	-	26.91 a	-
Bare ground	-	-	-	-	-	-	-	20.64 a	-
P>F								0.1386	
LESREC 2011									
V. villosa	0.64 a	5.63 a	8.71 a	27.58 b	41.86 b	40.68 b	35.90 b	-	124.55 b
T. incarnatum	0.00 a	2.08 a	4.92 a	31.36 b	47.20 b	31.14 b	43.48 ab	-	119.16 b
S. cereale	1.12 a	4.38 a	13.83 a	46.44 a	60.95 a	54.81 a	51.02 a	-	189.43 a
B. juncea	0.32 a	6.25 a	14.48 a	48.56 a	66.10 a	56.97 a	54.62 a	-	196.66 a
Bare ground	1.44 a	6.46 a	7.01 a	48.26 a	57.31 a	52.05 a	52.61 a	-	184.09 a
P>F	0.4180	0.1152	0.1445	0.0056	0.0056	<0.0001	0.0458		<0.0001
UD-REC 2011									
V. villosa	0.60 a	1.49 a	14.00 a	13.74 a	11.50 a	6.68 c	12.12 b	-	56.27 b
T. incarnatum	0.60 a	1.49 a	16.03 a	15.97 a	15.19 a	9.49 bc	15.61 ab	-	68.04 ab
S. cereale	1.79 a	8.93 a	15.18 a	16.42 a	13.53 a	12.91 ab	19.35 ab	-	72.91 ab
B. juncea	3.57 a	13.39 a	16.69 a	18.16 a	16.34 a	12.98 ab	22.46 a	-	81.82 a
Bare ground	2.38 a	8.93 a	18.18 a	19.97 a	17.34 a	15.19 a	23.53 a	-	84.57 a
P>F	0.3888	0.4779	0.2418	0.4201	0.1641	0.0175	0.0535		0.0251

[a]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[b]The symbol – indicates the measurement was not taken for that date and location or for USDA-BARC in 2010 that no wilt was observed.

[c]Means in a column followed by the same letter are not significantly different at $\alpha=0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 2.4 The effect of Actinovate[a] AG on severity of Fusarium wilt of watermelon at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC) in 2009 & 2010 and the University of Delaware's Carvel Research and Education Center (UD-REC) in 2011

Location, Year	Week after Transplanting								
Treatment	1	2	3	4	5	6	7	8	AUDPC
UM-LESREC 2009									
Nontreated	-[b]	2.25 a[c]	0.28 a	0.01 a	0.83 a	0.00 b	2.22 a	-	3.36 a
Fusarium	-	1.94 a	13.25 a	0.84 a	1.67 a	1.94 a	1.39 a	-	19.37 a
Fusarium + Actinovate	-	2.64 a	0.40 a	0.02 a	0.28 a	0.00 b	1.94 a	-	2.99 a
P>F		0.7363	0.1392	0.1396	0.3596	0.0096	0.7203		0.1533
UM-LESREC 2010									
Fusarium	4.88 a	-	-	9.75 a	-	10.31 a	-	-	28.07 a
Actinovate	2.44 a	-	-	2.68 b	-	3.81 a	-	-	9.95 b
P>F	0.067			0.0379		0.0603			0.0373
UD-REC 2011									
Fusarium	2.62 a	1.01 a	14.80 b	16.26 a	15.12 a	11.17 a	19.46 a	-	72.09 a
Actinovate	0.95 a	0.36 a	17.25 a	16.64 a	14.44 a	11.74 a	17.79 a	-	73.32 a
P>F	0.1319	0.1898	0.0206	0.7908	0.6214	0.6861	0.3333		0.802

[a]Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at 0.011 g/12.2 ml H2O per 0.156 m2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H2O/1.89 m^2 per a plant.
[b]The symbol – indicates the measurement was not taken for that date and location.
[c]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 2.5 Effects of tilled cover crop and Actinovate AG biocontrol application on marketable watermelon yield (no./ha)[a] at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture at the Beltsville Agricultural Research Center (USDA-BARC) and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010 and 2011

Cover Crop[b]	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	USDA-BARC 2009	USDA-BARC 2010	UD-REC 2011
Vicia villosa	8,353 a[c]	6,261 a	1,416 b	3,020 a	7,400 a	6,159 a
Trifolium incarnatum	-[d]	-	2,883 a	3,104 a	6,355 a	6,745 a
Secale cereale	8,073 a	8,120 a	1,089 b	2,829 a	7,736 a	7,486 a
Brassica juncea	-	7,074 a	815 b	-	6,840 a	6,536 a
Bare ground	8,324 a	7,427 a	1,253 b	2,840 a	8,598 a	6,572 a
P<F	0.2917	0.2247	0.0018	0.3417	0.5514	0.9323
Inoculation Treatment[e]						
No treatment	8,259 a	-	-	1,581 ab	7,310 a	-
Fusarium oxysporum f. sp. *niveum* (FON)	8,180 a	3,558 a	1,024 b	1,500 b	7,714 a	7,032 a
Actinovate + FON	8,317 a	2,996 a	1,957 a	1,671 a	7,131 a	6,374 a
P<F	0.6834	0.0549	0.0033	0.0080	0.7392	0.4863

[a] Marketable watermelon yield is quantified as the mean number of watermelon fruit per hectare that weighed more than 3.18 kg. The watermelon cultivar Sugar heart was used for all field trials.

[b]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.

[c]Means in a column followed by the same letter are not significantly different at $\alpha=0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

[d]The symbol – indicates the absence of the specific cover crop treatment for the given time and location or that there was a loss in sufficient replications.

[e]Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at a rate of 0.011 g/12.2 ml H_2O per 0.156 m 2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H2O/1.89 m2 per a plant. FON was inoculated in 2009 at USDA-BARC and UM-LESREC at 6 ml of 2.45 x 10^6 CFU/ml by pippet into a hole (approximately 3 cm in diameter and 8 cm deep) 7 cm away from the crown of each watermelon plant immediately after watermelon transplanting. In 2010 at USDA-BARC two days after cover crop incorporation and a week prior to the laying of black plastic, 60 ml of 2.85 x 10^8 CFU/ml FON inoculum was mixed into one gallon of H2O and evenly banded across the center of each 36.92 m^2 bed (where watermelons would later be transplanted to) with a watering can. In addition 11 ml of FON at 2.33 x 10^6 CFU/ml was added next to each watermelon plant using the same method employed in 2009. Fields used at UM-LESREC in 2010 and 2011 and UD-REC in 2011 were naturally infested.

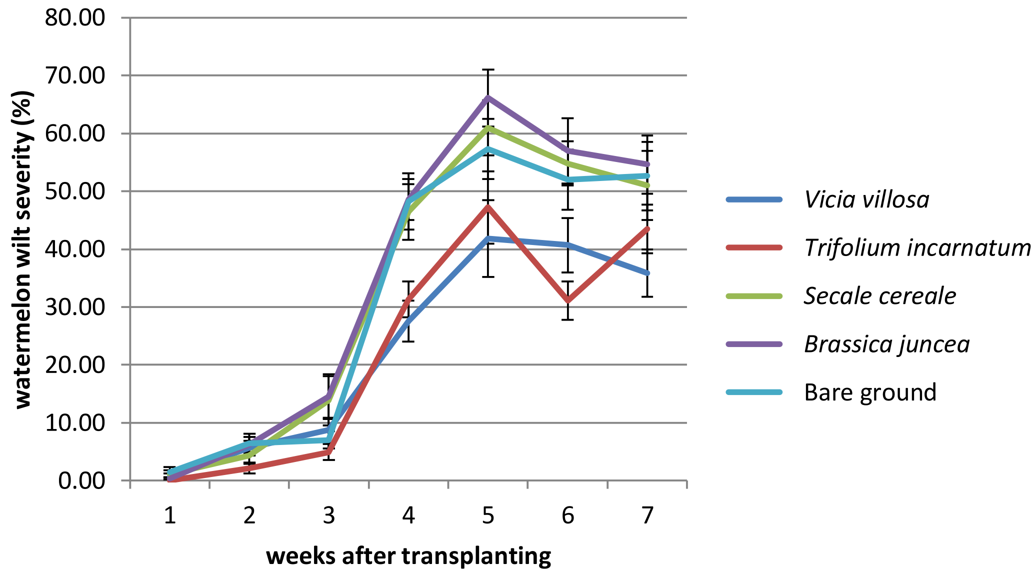

Figure 2.1 Disease Progress Curve for Fusarium wilt of watermelon following a fall planted cover crop that was tilled in the spring or bare ground at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC) in Salisbury, 2011.

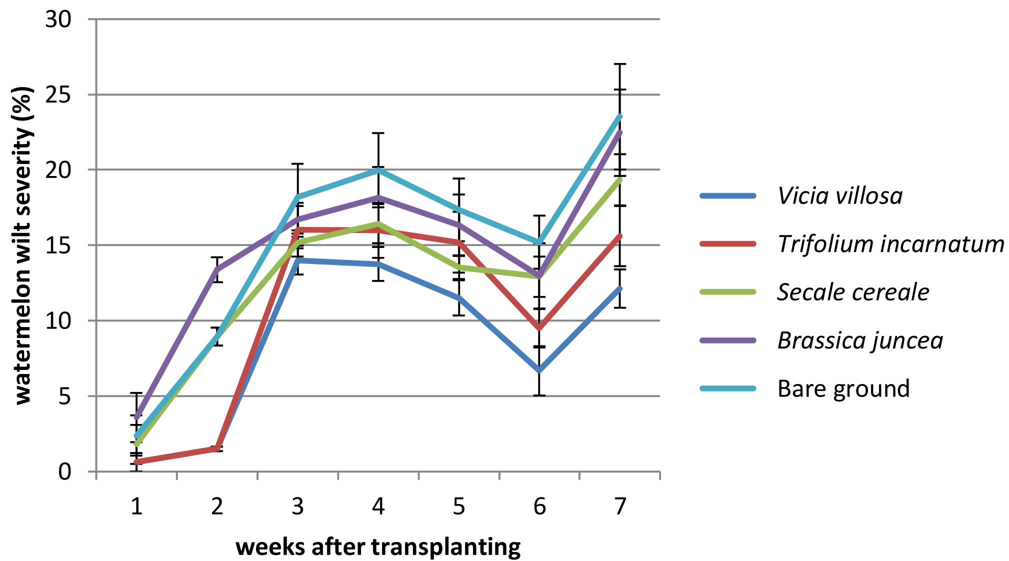

Figure 2.2 Disease Progress Curve for Fusarium wilt of watermelon following a fall planted cover crop that was tilled in the spring or bare ground at the University of Delaware Research and Education Center (UD-REC) in Georgetown, 2011.

Chapter 3: General Suppression of Fusarium Wilt of Watermelon by Cover Crop Green Manures in Maryland and Delaware

3.1.Abstract

A fall planted *Vicia villosa* cover crop incorporated in spring as a green manure suppresses Fusarium wilt of watermelon. Experiments were conducted to determine whether the the mechanism of disease suppression is general or specific, and to evaluate the efficacy of the biocontrol Actinovate. For this purpose, the effects of cover crop green manures (*V. villosa*, *Trifolium incarnatum, Secale cereale, Brassica juncea*) on soil respiration and *F. oxysporum* spp. were assessed in five field experiments. Actinovate was evaluated in greenhouse experiments alone and in combination with four different cover crop treatments (*V. villosa, T. incarnatum,* and *S. cereale*) and no cover crop in soil infested or non-infested with FON. Significant elevations in soil microbial respiration both preceeded and followed *V. villosa* and *T. incarnatum* green manure incorporation compared to plots with no cover crop, and was significantly negatively correlated with Fusarium wilt, suggesting that general suppression was present. However, *Fusarium oxysporum* spp. significantly increased in *V. villosa* amended plots, indicating that specific suppression may also contribute to disease reductions. Actinovate did not consistently suppress Fusarium wilt when used alone, nor with any of the cover crop treatments in the two greenhouse trials.

3.2.Introduction

Watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai) is a major fresh market vegetable grown in Maryland and Delaware (McCann et al., 2007). Production has shifted from diploid (seeded) to triploid (seedless) watermelon (Lucier et al., 2001). However, there currently are no triploid watermelon cultivars with resistance to all races of Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (FON) *Schlechtend*, Fr. (E. F. Sm.) W. c. Snyder & H. N. Hans (Everts et al., 2010; Everts et al., 2011). FON is widespread on the Eastern Shore of Maryland and in Delaware (Zhou et al., 2003b). Management options other than resistant culativars are also available to farmers but they vary in cost and effectiveness. Less effective alternatives include long crop rotations, soil solarization, and elevation of soil pH (Zitter et al., 1996; Eschel et al., 2000). Methyl bromide was previously used as a soil fumigant in watermelon fields. Following its loss, less succesful soil fumigants have been utilized (Ferguson et al., 1997; Gullino et al., 2005). The product, Actinovate AG (Natural Industries, Inc., Houston, TX), which has the active ingredient *Streptomyces lydicus* WYEC 108, is another tool available to producers. It has reduced several plant diseases and is labeled for use on Fusarium wilt of watermelon (Elmer et al., 2004; Tomaso-Peterson et al., 2007; Teasdale, 2009). Another, yet less commonly utilized alternative is green manures, in particular *Vicia villosa* which suppressed Fusarium wilt of watermelon by as much as 69% (Zhou et al., 2002; Zhou et al., 2004; Zhou et al., 2007; Keinath et al., 2010). Although the ability of *V. villosa* to suppress Fusarium wilt of watermelon has been established the mechanism of the disease suppression is still unknown.

Disease suppression can be categorized as general or specific. General suppression is defined as suppression that arises from the overall activity of soil biota and has efficacy against a wide range of soilborne pathogens (van Os et al., 2001; Weller et al., 2002). This phenomenon has been observed in several different pathosystems. General suppression occurred with the pre-emergence damping off (*Rhizoctonia solani*) of impatiens (*Impatiens balsamina*) using a composted swine waste amendment, which elevated microbial activity (Diab et al., 2003). Specific suppression is when the activity of an antagonist or a defined group of microorganisms suppresses disease caused by a single pathogen (van Os et al., 2001; Weller et al., 2002). Kraus et al. (2001) observed specific suppression when a decomposed pine bark mixture was inoculated with *Trichoderma hamatum* 382 and *Chryseobacterium gleum* and resulted in significantly reduced Rhizoctonia damping-off of radish and Rhizoctonia root and crown rot of poinsettia.

Incorporation of cover crops or other organic soil amendments often increase activity and diversity of soil microbes associated with suppression (Tsuneo et al., 1991; Rothrock et al., 1995; Bonanomi et al., 2007a). Increases in soil microbial activity can be measured as an increase in soil respiration. Bonanomi et al. (2010) found that substrate respiration and microbial biomass were principal quality factors for measuring the likelihood of disease suppression. Alabouvette et al. (1985) measured the rate of soil biota respiration via evolution of CO_2 of soils from fields naturally suppressive to Fusarium wilt of watermelon to that of nonsuppressive soils. He found respiration rates in suppressive soils were 2-4 fold that of nonsuppresive soils and proposed that the mechanism of disease suppression in these soils, due to excessive competition for

nutrients, was a form of general suppression. Nonpathogenic *Fusarium oxysporum* spp. play a role in the suppression of FON in these soils as well (Alabouvette et al., 1993).

In this study, we investigated the effects of *V. villosa*, *Trifolium incarnatum*, *Secale cereale*, and *Brassica juncea* cover crop treatments on the rates of soil microbial respiration and fluxes of *Fusarium oxsyporum* spp. in fields where *V. villosa* and *T. incarnatum* disease suppression were observed. In addition, we compared the efficacy of Actinovate AG alone and in combination with three different cover crop amendments in controlled greenhouse conditions.

3.3.Materials and Methods

3.3.1.Field Trials

Field experiments were established at the United States Department of Agriculture, Beltsville Agricultural Research Center (USDA-BARC) in 2009 & 2010, at the University of Maryland Lower Eastern Shore Research and Education Center in Salisbury (UM-LESREC) in 2009, 2010 & 2011, and at the University of Delaware Carvel Research and Education center in Georgetown (UD-REC) in 2011.

Each field was set up as a split plot block design with cover crops as the main plot treatments and Actinovate application as the subplot treatments (Table 3.1). *Vicia villosa*, *Trifolium incarnatum*, *Secale cereale*, *Brassica juncea* and bare ground were the main plot treatments, however not all cover crop treatments were planted for every field trial (Table 3.1). An additional subplot treatment was inoculation with FON unless the field was already infested with FON (Table 3.1).

3.3.1.a.Soil Respiration

Soil respiration was measured in plots infested with FON and amended with the various cover crop regimes or bare ground using a EGM-4 gas analyzer with a SRC-1 chamber from PP Systems (110 Haverhill Road, Suite 301 Amesbury, MA 01913, USA) (Korhonen et al., 2009).

The EGM-4 gas analyzer has a closed chamber that measures CO_2 flux within the chamber at a flow rate of approximately 350 ml/min. The beveled collar seals were placed in the plots at least 24 hours prior to measurements. Collars were pushed 4 cm into the soil and approximately 7 cm of the collars remained above the soil line. Respiration was measured from at least three collars placed randomly in each plot and then averaged per a plot. Treatments in three to six replicates were measured in each trial (n=45-90). A soil temperature sensor was connected to the EGM-4 data logger to record soil temperature in plots while soil respiration data was collected. Respiration was measured in areas of the field where Actinovate was not applied as a soil drench in order to only measure cover crop treatment effects.

Soil respiration measurements were recorded for five field trials, two at USDA-BARC, two at UM-LESREC and one at UD-REC. The first field trial at USDA-BARC in 2009, respiration was measured before tillage and during the watermelon growing season. The largest magnitude of treatment differences for elevations in soil respiration was directly following tillage. Therefore CO_2 flux was primarily recorded directly following tillage in subsequent experiments.

3.3.1.b.Soil Dilutions

The effect of cover crop treatment on *Fusarium oxysporum* spp. in the soil (FON was not differentiated from non-pathogenic *F. oxysporum*) was evaluated for two years (two locations per a year) by soil dilution onto selective media. Soil cores were taken once towards the end of the growing season from every plot at each location (n=60/field location) (Table 3.1) in 2010. The following year a baseline sample was collected in March (Table 3.1) when cover crop growth was minimal. Subsequent samples were collected again near the end of the growing season (Table 3.1). One soil core was sampled from three locations within each subplot. The three soil cores from each plot were thoroughly mixed and a 5 g subsample (dry weight equivalent) was placed in 45 ml of autoclaved 0.1% water agar. This solution was mixed on a rotary shaker at 150 rpm, vortexed, and 1 ml of the soil suspension was spread evenly on to plates of Komadas media (Komadas, 1975) for a 1:10 soil dilution. Additionally, a 1 ml aliquot of the suspension was transferred to test tubes with 9 ml of 0.1% autoclaved water agar, vortexed, and 1 ml of the resulting suspension was pippeted to Komadas media plates for a 1:100 soil dilution (Zhou et al., 2004). The resulting colonies of *Fusarium oxysporum* spp. were counted five days after plating.

Baseline *Fusarium oxysporum* spp. colony forming units (CFUs) were subtracted from the counts in 2011. Pink and purple pigmented CFUs were also recorded separately to see if there were any selective effects on *Fusarium oxysporum* spp. populations. Pigmentation is a characteristic other studies have used to identify and track changes in multiple *F. oxysporum* spp. sampled from the same field sites (Mandeel et al., 1991; Skovgaard et al., 2001; Smith et al., 2001; Leslie et al., 2006).

The CFU/g were adjusted to dry weight equivalent prior to statistical analysis. Soil water content was measured by sampling soil from each plot with a 7.62 cm wide soil auger to a depth of 15.24 cm, drying out 5 g of soil from each sample at 105° C for approximately four days, and then weighing the samples once more. Treatment effects on soil water content were also measured at other times during the field season (Table 3.1) using the same soil drying technique or by differences in electrical conductivity using a FieldScout TDR-300 (Spectrum Technologies, East Plainfield, IL, USA).

3.3.2. Greenhouse Experimental Design and Management

The capacity of *Streptomyces lydicus* WYEC 108, the active ingredient of the biological control product Actinovate, to colonize watermelon roots or the rhizosphere in natural field soil and the biocontrol products ability to suppress disease when used alone or in combination with three different green manure amendments was evaluated in two greenhouse pot trials in the fall of 2011, the first at UM-LESREC and the second at UD-REC.

The experiment was arranged as a completely randomized factorial design (CRD) with nine replicates per treatment (n=216). Treatments were soils amended or nonamended with cover crop residue and FON and/or Actinovate application. A Fort Mott loamy sand from Salisbury, MD (homogenized with a soil mixer) was used for potting soil in both experiments.

Watermelon cv. 'Sugar Heart' was seeded in 128 cell trays and grown in Sun Gro Redi-earth Plug and Seedling Mix (Sun Gro Horticulture, Bellevue, WA) in ambient greenhouse temperatures (20-31 ºC) for two and a half weeks. Actinovate was suspended in water and applied to 10 day old seedlings at 0.08 g/0.09 L H_2O/m^2 (0.012g/13.24 ml

$H_2O/0.14m^2$ tray). Seedlings were gently repotted 7 days after the first Actinovate application and an Actinovate soil drench was applied to the 29 cm^2 pots at 0.06 g/0.09 L H_2O/m^2 (0.017 g/26.34 ml H_2O/29 cm^2 per a pot) the same day.

A race 1 isolate (F-030-1) of *F. oxysporum* f. sp. *niveum* (FON), which was acquired from a wilted watermelon plant in Wicomico county Maryland in a previous study, was used for disease inoculations (Zhou et al., 2003b). The isolate had been maintained in a mixture of sandy soil and perlite (1:2 wt/wt) at 4°C.

Inoculum was prepared by transferring F-030-1 growing on Komadas media into a liquid mineral salts medium (Netzer, 1976; Zhou et al., 2006). The culture was incubated on an orbital shaker at 128 rpm at room temperature for approximately two weeks before filtering through eight layers of cheesecloth. The spore suspension was amended to the soil to attain a 2,500 CFUs/g concentration (Zhou et al., 2004). The soil was mixed thoroughly and incubated in plastic bags with filtered air exchange at room temperature for a week and a half to allow for the formation of chlamydospores.

Cover crop biomass was amended a week and a half after FON inoculation. Cover crop treatments were soil amendments of 1) *V. villosa* 2) *S. cereale* 3) *T. incarnatum* and 4) no amendment. Soil then received a) FON inoculation and Actinovate application, b) FON inoculation and no Actinovate, c) no FON inoculation and Actinovate application, or d) no inoculation.

Cover crops were collected from fields at UM-LESREC and USDA-BARC in early spring (May 18th and 19th, 2011), dried in the greenhouse, and then chopped into pieces approximately 4-6 cm long to represent rough tillage. Cover crop amendment rates

were determined by averaging aboveground biomass present in 2009 and 2010 field seasons.

The *V. villosa* green manure amendment was incorporated into the potting soil treatment at three different rates; 2722 kg/ha (x1), 3685 kg/ha (x2), and 5670 kg/ ha (x3) to represent average cover crop biomass at UM-LESREC, a median level, and USDA-BARC, respectively. *T. incarnatum* was incorporated into the soil at rate of 5103 kg/ha and *S. cereale* was incorporated at a rate of 3969 kg/ ha. Biomass was mixed into the soil by hand, watered, and allowed to decompose for two weeks. Soil treatments were then transferred into sterilized plastic pots (29 cm^2). A single Sugar Heart seedling was transplanted in to each pot and greenhouse conditions were maintained at 20 to 31° C. Plants were mechanically watered twice a day for five minutes at 7:30 am and 3:00 pm at UM-LESREC and hand watered twice a day at UD-REC.

3.3.2.a.Greenhouse Measurements

Six of the nine treatment replicates were evaluated for wilt severity and vine length (n=144). Wilt severity was evaluated weekly for six weeks on a scale of 0 to 3 where 0=no wilt (0%), 1= 1-33% wilted foliage, 2=34-63% wilted foliage, 3=64-100% wilted foliage. Watermelon plants that died of wilt were plated on Komadas media to confirm the presence of the FON. Vine length, which was the length of the longest watermelon vine from the crown of the plant to the vine tip, was measured weekly for each pot for six weeks.

Three of the treatment replicates (n=72), for each treatment, were destructively harvested two weeks after watermelons had been transferred to pots to observe if

Streptomyces lydicus had established in the rhizosphere. Seminal roots of watermelon plants were cut into three 3-cm long root sections, placed into test tubes of 5 ml DI water, soaked for five minutes and vortexed before being serially diluted onto SPA Agar (amended with carbenicillin, nyastatin and cyclohexamide) and Casein Agar (Yuan et al., 1995).

3.3.3.Statistical Analyses

Data were analyzed using the MIXED procedure with the Statistical Analysis System (version 9.2; SAS Institute, Cary, NC), which integrates random effects in the statistical model and performs covariance structure modeling (Littell et al., 1998). Treatment means were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. The majority of the wilt data was not normal due to the nature of the evaluations. As the sample size for each trial was larger than 30 the data was considered robust and no transformations were employed (Payton et al., 2006). This is supported by the Central Limit Theorem which states that the sample mean of a population with the size of n> 30 will converage to a standard normal distribution (Corbett et al., 2002).

A correlation analysis was performed for two of the variables measured in the field experiment-wilt severity and respiration. The MIXED procedure was used and means were separated at a $P \leq 0.05$ and by using Pearson's correlation analysis. This analysis was only performed for UM-LESREC and UD-REC in 2011 where the wilt suppression by the leguminous cover crops was most apparent and for dates where cover crop treatment effects on respiration and wilt severity were significant.

3.4.Results

3.4.1.Field Trials

3.4.1.a.Respiration

Significant differences in soil respiration among cover crop treatments occurred prior to tillage, and immediately following tillage, but not later (Table 3.2). At four of the five locations where CO_2 was measured respiration rates were significantly higher in *V. villosa* amended plots compared to all other cover crop and bare ground treatments. Three weeks prior to tillage at USDA-BARC in 2010, one day following tillage at UM-LESREC in 2010, and one and two days after tillage at UM-LESREC and UD-REC in 2011 *V. villosa* amended plots had significantly higher rates of CO_2 respiration than in any of the other green manure treatments or bare ground. Additionally, respiration in *V. villosa* amended plots was significantly higher than that of bare ground at USDA-BARC in 2010, one day after tillage.

Respiration rates in *T. incarnatum* amended plots were significantly higher than nonamended plots for one or more dates in four of the five field trials and were higher than all other cover crop treatments for one. Plots amended with *T. incarnatum* had significantly higher rates of CO_2 respiration five days prior to tillage and five days after tillage compared to all other cover crop and bare ground treatments at USDA-BARC in 2009. For measurements taken three weeks, and ten days prior to tillage at USDA-BARC in 2010, as well as one day after tillage the flux of CO_2 was higher in *T. incarnatum* amended plots compared to bare ground. Respiration was significantly higher in *T. incarnatum* amended plots than in bare ground plots for all measurements taken at UM-LESREC in 2010 and 2011.

For one or more dates in all five field trials *B. juncea* and *S. cereale* amended plots had rates of respiration that were significantly lower than that of one of the leguminous amended plots. Respiration was never significantly higher in *B. juncea* or *S. cereale* amended plots compared to that of leguminous plots. The respiration in *B. juncea* amended plots was significantly higher than bare ground respiration for two field trials, and was significantly lower than all other green manure amended plots for both measurements taken at UM-LESREC in 2011. Although soil respiration measurements in *S. cereale* amended plots were significantly higher than bare ground for three field trials it was also significantly lower than all other green manure amended and nonamended plots at UD-REC in 2011.

Both *V. villosa* and *T. incarnatum* amended plots had significantly higher rates of respiration than bare ground plots for four field trials. Respiration rates were higher in leguminous green manure plots than other cover crop treatments for all five field trials. Neither *V. villosa* nor *T. incarnatum* ever had rates of respiration significantly lower than that of *B. juncea*, *S. cereale* nor nonamended plots.

Respiration was negatively correlated with wilt severity at UM-LESREC in 2011 and UD-REC in 2011. Wilt severity measured six weeks after watermelon were transplanted to the field at UD-REC in 2011 was significantly negatively correlated to respiration measured in that same field, the morning after cover crop tillage ($P = 0.0328$). The AUDPC ($P = 0.0107$) and wilt measurements taken four ($P = 0.0010$) and five weeks ($P = 0.0163$) after transplanting at UM-LESREC in 2011 were significantly negatively correlated with respiration measured one day after tillage in that field. All wilt measurements at UM-LESREC, in 2011 where significant cover crop treatment effects

were observed, four (P = 0.0004), five (P = 0.0001), six (P = 0.0025) and seven (P = 0.0118) weeks after transplanting, as well as the AUDPC (P = 0.0053), were significantly correlated with respiration measured two days after cover crop incorporation. All of the Pearson's correlation coefficients were negative, demonstrating that there is an inverse relationship between wilt severity and respiration-specifically that the higher a plots' respiration the lower the wilt severity rating.

3.4.1.b.Soil Dilutions

At USDA-BARC in 2010 *V. villosa* amended plots had numerically more *Fusarium oxysporum* spp. colony forming units (CFUs) per gram of soil (37.95 to 253.71 more CFU/g soil) than the other plot treatments. There was high variability, and no significant differences between treatments were observed for this field trial (Table 3.3). However, at UM-LESREC in 2010 *V. villosa* amended plots had significantly higher *Fusarium oxysporum* spp. CFU/g of soil (228.74) compared to all other cover crop and nonamended plots (P = 0.0086).

To reduce error and to allow measurement of discrete differences between treatments the number of purple and the number of pink pigmented CFUs were recorded separately and totaled at LESREC in 2011. The number of pink pigmented CFUs/g soil were significantly greater (274.39 CFUs/g) in plots amended with a *V. villosa* cover crop (P = 0.0126) than in nonamended plots at LESREC in 2011. There were no significant treatment effects observed for purple pigmented CFUs or the sum of both the pink and purple CFUs (Table 3.3).

The *V. villosa* amended plots at UD-REC in 2011 contained more purple pigmented CFUs/g soil (1093.63) than bare ground (70.37), S. *cereale* (358.77), and *T. incarnatum* (475.81) amended plots ($P = 0.0318$). Additionally, for the sum of the two pink and purple CFU pigments, more CFUs/g of soil were found in *V. villosa* amended plots than in bare ground and *S. cereale* amended plots ($P = 0.0262$). In this case bare ground plots had significantly less CFU/g soil than plots amended with any cover crop other than *S. cereale* (Table 3.3).

For two of the four field trials the CFU/g of soil of *Fusarium oxysporum* spp. was significantly higher in *V. villosa* amended plots compared to all other treatments. For three of the four field trials *Fusarium oxysporum* spp. CFUs were higher in *V. villosa* amended plots compared to bare ground. Although *V. villosa* amended plots repeatedly had higher CFU/g of *F. oxysporum* compared to other treatments *T. incarnatum* amended plots did not.

There were no significant Actinovate treatment effects on the number of *Fusarium oxysporum* spp. CFUs/g soil for any of the four field trials soil dilutions were done.

3.4.1.c.Soil Temperature and Water Content

Soil temperature was measured for three field trials. Significant differences in temperature between cover crop treatments were observed for all three field trials but there was no clear pattern between the trials nor did the dates with differences in soil temperature correlate with differences in soil respiration (Table A3.1).

There were no significant differences in the soil water content between any of the cover crop or bare ground treatments (data not shown).

3.4.2. Greenhouse Experiment

3.4.2.a. Vine Length

In both greenhouse experiments FON inoculations significantly reduced the rate of watermelon vine growth in pots amended with leguminous cover crops while bare ground pots inoculated with FON were not similarly affected (Table 3.4). Additionally, in the presence of no FON inoculation and leguminous cover crops, watermelon vines grew faster compared to plants in nonamended pots or those amended with *S. cereale* (Table 3.4).

Actinovate treatments had no significant main or simple effects on the rate of watermelon linear vine growth for the first pot experiment. In the second pot experiment only the main effects of the Actinovate treatment significantly impacted the linear rate of watermelon vine growth. In this case watermelon treated with Actinovate had significantly slower rates of vine growth compared to plants that did not receive the treatment (data not shown).

3.4.2.b. Fusarium Wilt

Because field soil was used, some Fusarium wilt was observed on watermelons which were not inoculated with FON for both greenhouse trials. By the end of both experiments Fusarium wilt was reduced in noninoculated, *V. villosa* amended pots compared to watermelon in noninoculated, *S. cereale* or nonamended pots (Table 3.5).

As the greenhouse experiments progressed Fusarium wilt increased on plants in pots inoculated with FON and amended with leguminous cover crops resulting in disease ratings that were significantly higher than that of their noninoculated counterparts (Table 3.5). This was not the case for plants in nonamended pots or, for the first experiment, those amended with *S. cereale* where wilt was high in both inoculated and noninoculated pots (Table 3.5).

Watermelon in pots inoculated with FON and amended with a leguminous cover crop had significantly more wilt than FON inoculated, nonamended pots for both experiments. According to wilt ratings measured six weeks after transplanting in the first greenhouse trial and the AUDPC of the second greenhouse trial plants in FON inoculated pots amended with *V. villosa* x1 had significantly less wilt than plants in FON inoculated pots amended with *T. incarnatum*, or *S. cereale*.

Actinovate biocontrol treatment effects were not consistent within or between pot experiments. In the first greenhouse experiment, two weeks after transplanting, Actinovate treatment decreased Fusarium wilt across all cover crop treatments. Actinovate also decreased disease in the first experiment in *V. villosa* amended pots four weeks after transplanting. However, for that same reading, *T. incarnatum* in combination with Actinovate enhanced wilt compared to several other treatments. In the second pot experiment a three way interaction occurred between Actinovate, FON, and cover crop treatments. Here Actinovate significantly increased wilt ratings of plants inoculated with FON and amended with either *T. incarnatum* or *S. cereale*. Results from these two greenhouse experiments indicate that Actinovate's ability to suppress wilt alone or in combination with a cover crop are inconsistent to negligible. The only significant pattern

that was observed across both trials was that Actinovate treatment can induce increased wilt, particularly when in combination with a *T. incarnatuum* green manure (data not shown).

3.5.Discussion

3.5.1.Field Trials

This study is the first to evaluate the corresponding effects of a *V. villosa* green manure amendment on soil respiration and *F. oxysporum* soil populations on watermelons. In our study there were elevations in overall microbial activity for plots amended with *V. villosa* and *T. incarnatum*. Increases in *F. oxysporum* were also seen in *V. villosa* amended plots. Other studies on *V. villosa* green manure or on Fusarium wilt disease suppression have found similar results. For example, a *Vicia villosa* green manure increased the absolute numbers of all microbial groups and influenced soil microbial community composition more than manure or compost amendments in a tomato crop system (Carrera et al., 2007; Buyer et al., 2010). A positive relationship was observed in another study between *V. villosa* cover crop incorporation, the suppression of Fusarium wilt of watermelon, and elevations in soil bacterial populations (Zhou et al., 2007). The mechanism of disease suppression of soils naturally suppressive to Fusarium wilt of watermelon and those with induced suppresiveness via a monoculture of the watermelon cultivar 'Crimson Sweet' has been attributed to general suppression due to an increase in overall soil microbial activitiy as well as a more specific suppression by antagonistic microbial populations-most specifically nonpathogenic *Fusarium oxysporum* spp. (Alabouvette et al., 1985; Alabouvette et al., 1993; et al., 1993a; Larkin et al., 1993b; Larkin et al., 1996). The absence of significantly increased *F. oxysporum* spp.

populations in our field *T. incarnatum* plots could account for why the cover crop is not as effective at reducing Fusarium wilt as *V. villosa*.

It is probable that the increase in *F. oxysporum* in *V. villosa* amended plots was composed mainly of nonpathogenic *F. oxysporum* spp. FON saprophytic growth and chlamydospore germination declined in fields naturally suppressive to Fusarium wilt (Alabouvette et al., 1993). Many studies have demonstrated that pre-inoculating watermelon roots with nonpathogenic *F. oxysporum* spp. can decrease Fusarium wilt of watermelon, a form of cross-protection (Biles et al., 1989; Freeman et al., 2002). According to the competitive exclusion principle, different species with the same ecological niche are not able to coexist for long (Baker et al., 1974). The competitive coexistence of FON and nonpathogenic *Fusarium oxysporum* in the soil is supported by a 2003 study done by Zhou et al., (2003b) who found that as FON increased in fields conducive to Fusarium wilt of watermelon so did the ratio of FON relative to the total population of *F. oxysporum* spp. in the field.

Because pathogenic and nonpathogenic *F. oxysporum* occupy a similar niche and have comparable nutrient requirements and preferences, *V. villosa* compounds that stimulate nonpathogenic *F. oxysporum* would also stimulate FON. It was found that the antagonistic actions of nonpathogenic *F. oxysporum* Fo47b10 against *F. oxysporum* f. sp. *dianthi*, the pathogen responsible for Fusarium wilt of carnation (*Dianthus caryophyllus* L.) was mainly due to the competition of the *Fusarium* spp. for glucose (Lamanceau et al., 1993). Additionally, the antagonism was largely dependent on the ratio of nonpathogenic to pathogenic *F. oxysporum* populations-the higher the ratio, the greater the antagonism (Lamanceau et al., 1993).

3.5.2.Greenhouse Trials

3.5.2.a.Cover Crop Effects

Due to the background level of FON in the field soil used in greenhouse trials, *V. villosa* and *T. incarnatum* suppression of Fusarium wilt of watermelon was observed in the pots that did not receive the FON inoculation. In the pots inoculated with FON and amended with *V. villosa* and *T. incarnatum* there was an increase in Fusarium wilt severity. The *V. villosa* and *T. incarnatum* amendments may have stimulated FON growth. For laboratory or field experiments plant material is often used to help stabilize and encourage growth of pathogen inoculum. For example, a mixture of soil, rolled oats and dried bean leaves can be used to incubate inoculum of *F. oxysporum* spp. (Mandeel et al., 1991). The cover crop biomass amendments may have given the FON a competitive advantage, overwhelming the nonpathogenic *F. oxysporum's* suppressive effects. It is interesting to note that in both trials watermelon in pots inoculated with FON and amended with larger amounts of biomass, namely *V. villosa* x2 and x3 treatments, had significantly higher wilt severity ratings than watermelon in all other FON inoculated, cover crop amended pots, indicating this extra biomass functioned as a nutrient source for the pathogen. In contrast, plants in FON inoculated, *V. villosa* x1 amended pots had significantly less wilt than pots inoculated with FON and amended with *S. cereale* or *T. incarnatum*.

Other explanations could exist for the results seen in the FON inoculated pots such as excessive inoculum levels that could negate the cover crops suppresiveness (Termorshuizen et al., 2008). Zhou et al. (2007) found that high inoculum densities of FON can overcome the suppressive effects of *V. villosa.*

Only dried aboveground biomass of *V. villosa* was used for these greenhouse trials. It is possible that the roots of *V. villosa* are necessary for attaining the level of microbial activity that contributes to the disease suppressive effects seen in the field. In a 2010 study Buyer et al., observed that *V. villosa* roots increased soil microbial biomass significantly more than *V. villosa* shoots.

Changes in the soil microbial community that are necessary for achieving Fusarium wilt suppression might not occur if the leguminous cover crop is not physically grown in the potting soil prior to incorporation. In one study soil microbial respiration observed in *V. villosa* and *T. incarnatum* green manure plots prior to tillage was significantly higher than respiration in bare ground plots (unpublished data). The failure of watermelon cultivars other than ‘Crimson Sweet’ to induce suppresiveness of Fusarium wilt of watermelon was attributed to variables like composition or amounts of root exudates which promoted different types of rhizophere microflora populations (Larkin et al., 1993a).

3.5.2.b.Actinovate Biocontrol Effects

There were no synergistic effects between cover crop amendments and the Actinovate biocontrol treatment in either of the greenhouse experiments. As in previous field studies (unpublished data), Actinovate alone was not effective in reducing Fusarium wilt of watermelon. Additionally, a promotion in watermelon growth was not observed in these trials and were absent in the field trials as well (unpublished data). We were unable to isolate *Streptomyces lydicus* from the watermelon roots, or soil rhizosphere. It is possible that the soil conditions on the Eastern Shore of Maryland and in Delaware are

not conducive to the growth of *S. lydicus* as the low soil pH is not amenable to many kinds of bacteria. For Entry et al., (2000) substrate significantly impacted the effectiveness of the *S. lydicus* biocontrol. The authors used a wood chip-polyacrylamide core around plant roots to alter the rhizosphere environment so that it was more favorable to the growth of a *Streptomyces lydicus* WYEC 108 biocontrol. Due to the amendment changes in the soil environment the *S. lydicus* was able to successfully decrease *Veriticillium dahlia* incidence on potato (*Solanum tuberosum* L.) (Entry et al., 2000).

3.6.Conclusion

This study confirms that the mechanism of general suppression plays a role in *V. villosa* suppression of Fusarium wilt of watermelon. However it also raises the possibility that specific suppression is contributing to Fusarium wilt suppression. Respiration measurements were significantly negatively correlated with wilt severity measurements where Fusarium wilt suppression via the leguminous cover crops was observed. The higher the microbial activity, measured by soil respiration, the lower the wilt severity. The increases in microbial activity and elevations in *F. oxysporum* in *V. villosa* amended soils are parallel to conditions found in soils with induced or natural FON suppression. We hypothesize that general suppression in addition to a more specific form of suppression by means of elevated populations of antagonistic microbes and nonpathogenic *F. oxysporum* are responsible for the *V. villosa* disease suppression of Fusarium wilt. A study utilizing PFLA analysis to observe *V. villosa* effects on soil populations not only found that *V. villosa* increased overall soil microbial biomass but that the cover crops stimulation of soil fungi was proportionally greater than its effects on bacterial communities (Buyer et al., 2010). To gain a more complete picture of *V. villosa*

disease suppression, factors like microbial competition, direct antagonism, and/or induced host resistance must be explored. Future studies looking at more specific changes in the microbial community could provide additional insight on the mechanisms of *V. villosa* and *T. incarnatum* disease suppression of Fusarium wilt of watermelon. Although a PLFA could help detect substantial changes in the soil microbial community structure, a molecular approach would be a more powerful method for discerning these microbial dynamics (Abadie et al., 1998; Buyer et al., 2001)

Table 3.1 Management and design of field experiments to evaluate tilled cover crop and Actinovatea biocontrol application on Fusarium wilt severity and watermelon yield for six field trials in Maryland and in Delaware

Location, Year	USDA-BARC[b] 2009	USDA-BARC 2010	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	UD-REC 2011
Main plot treatment	*Vicia villosa, Trifolium incarnatum, Secale cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground	*V. villosa, T. incarnatum, B. juncea, S. cereale,* Bare ground
Sub plot treatment	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate	FON, FON + Actinovate	FON, FON + Actinovate
Cover crop seeding rates	*V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha	*V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, *B. juncea* 6.73 kg/ha.	*V. villosa* 50.44 kg/ha, *S. cereale* 134.50 kg/ha,	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha	*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha
Cover crop seeding dates	9/24/2008	9/22/2009	10/16/2008	9/25/2009	10/15/2010; 3/9/2011[c]	10/25/2010; 3/15/2011
Cover crop tillage	5/23/2009	5/23/2010	5/15/2009	5/24/2010	5/19/2011	5/25/2011
Dates of Actinovate applications	Foliar 06/03/2009; Soil drench 06/29/2009	Foliar 06/16/2010; Soil drench 06/29/2010	Foliar 06/02/2009; Soil drench 06/29/2009	Foliar 06/08/2010; Soil drench 06/15/2010	Foliar 05/20/2011; Soil drench 06/03/2011	Foliar 05/26/2011; Soil drench 06/07/2010
Transplanting date	06/17/2009	06/18/2010	06/19/2009	06/12/2010	06/03/2011	06/07/2011
Soil dilution date	-	08/23/2010	-	7/20/2010	03/17/2011, 05/20/2011	03/17/2011, 06/22/2011
Soil moisture measurement dates	soil drying[d] 5/18/2009	soil drying 08/23/2010; probe 05/19/2010, 07/08/2010;	soil drying 5/11/2009	soil drying 07/20/2010, 08/31/2010; probe 05/20/2010	soil drying 03/17/2011, 05/20/2011	soil drying 03/17/2011, 06/22/2011

[a]Actinovate AG is a biocontrol product (Natural Industries Inc.) which is labeled for management of Fusarium wilt of watermelon.
[b]UM-LESREC=University of Maryland Lower Eastern Shore Research and Education Center located in Salisbury Maryland. USDA-BARC=United States Department of Agriculture Beltsville Agricultural Research Center located in Beltsville Maryland and UD-REC=University of Delaware's Carvel Research and Education Center located in

Georgetown Delaware.
[c]In 2011 cover crops were overseeded in the early spring to ensure plots had sufficient cover crop biomass
[d]Soil moisture was either measured using a soil moisture probe (FieldScout TDR-300 Spectrum Technologies, East Plainfield, IL, USA) or by weighing and drying soil samples taken by a soil auger.

Table 3.2 Influence of cover crop[a] treatment on micrograms of soil CO_2 flux (µmol m-2 s-1)[b] for five field trials

Location[c], Year	BARC 2009					
Cover crop	**5/18/2009[d]**	5/28/2009	6/9/2009	6/11/2009	7/10/2009	8/3/2009
Vicia villosa	1.06 b[e]	2.40 b	0.71 a	0.88 a	0.85 a	4.72 a
Trifolium incarnatum	1.43 a	4.95 a	1.81 a	0.94 a	0.80 a	3.00 a
Secale cereale	0.89 b	2.91 b	0.89 a	0.88 a	-[f]	2.75 a
Bare ground	0.86 b	2.56 b	1.03 a	1.00 a	0.71 a	3.45 a
P>F	0.0064	0.0062	0.6974	0.2593	0.2056	0.084
Location, Year	BARC 2010				LESREC 2010	
Cover crop	**4/30/2010**	**5/13/2010**	**5/22/2010**	5/24/2010	5/25/2010	
Vicia villosa	0.97 a	0.91 ab	2.22 a	16.02 a	0.97 a	
Trifolium incarnatum	0.69 b	0.99 a	1.37 a	15.77 a	0.69 b	
Secale cereale	0.68 b	0.74 c	1.22 a	14.64 a	0.68 b	
Brassica juncea	0.73 b	0.93 a	1.22 a	11.52 a	0.73 b	
Bare ground	0.41 c	0.76 bc	0.80 a	5.21 b	0.46 c	
P>F	0.0007	0.0109	0.3786	0.0145	<0.0001	
Location, Year	LESREC 2011		UD-REC 2011			
Cover crop	5/20/2011	5/21/2011	5/26/2011	5/27/2011	5/27/2011	
Vicia villosa	1.08 a	1.04 a	0.87 a	0.83 a	0.94 a	
Trifolium incarnatum	0.69 b	0.65 b	0.50 b	0.68 ab	0.65 b	
Secale cereale	0.49 b	0.59 b	0.29 c	0.45 c	0.36 c	
Brassica juncea	0.27 c	0.30 c	0.49 bc	0.64 abc	0.48 b	
Bare ground	0.22 c	0.25 c	0.61 ab	0.62 bc	0.66 b	
P>F	<0.0001	<0.0001	0.0001	0.0121	<0.001	

[a]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[b]Soil respiration measurements were taken using a EGM-4 gas analyzer with a SRC-1 chamber from PP Systems (110 Haverhill Road, Suite 301 Amesbury, MA 01913, USA).
[d]All dates in bold indicate readings taken before cover crop tillage and all dates not bolded were taken following

cover crop tillage.
[e]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the SAS MIXED procedure.
[f]Data not taken.

Table 3.3 Simple effects of cover cropa treatment on the number of Fusarium oxysporum CFUs/g of soil for five field trials

Location[b]**, Year**	BARC, 2010	LESREC, 2010
Cover crop	Sum[c]	Sum
Vicia villosa	713.62 a[d]	228.74 a
Trifolium incarnatum	525.02 a	-
Brassica juncea	459.91 a	102.73 b
Secale cereale	675.05 a	139.74 b
Bare ground	568.29 a	149.29 b
P>F	0.3615	0.0086

Location, Year	LESREC 2011			UD-REC 2011		
Cover crop	Purple	Pink	Sum	Purple	Pink	Sum
Vicia villosa	121.38 a	274.39 a	330.12 a	1093.63 a	-311.91 a	842.57 a
Trifolium incarnatum	169.25 a	134.12 b	305.37 a	475.81 b	246.4 a	727.48 ab
Brassica juncea	216.90 a	121.24 b	279.59 a	526.45 ab	-509.52 a	664.26 ab
Secale cereale	154.68 a	150.80 b	258.92 a	358.77 b	-329.69 a	-17.30 c
Bare ground	149.96 a	154.27 b	242.54 a	70.37 b	-101.94 a	165.75 bc
P>F	0.7148	0.0126	0.3855	0.0318	0.1788	0.0262

[a]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[b]UM-LESREC=University of Maryland Lower Eastern Shore Research and Education Center located in Salisbury. USDA-BARC=United States Department of Agriculture Beltsville Agricultural Research Center located in Beltsville Maryland and UD-REC=University of Delaware's Carvel Research and Education Center located in Georgetown.
[c]Soil from the field was sampled, mixed, and then plated on Komadas media. The number of pink and purple colony forming units (CFUs) that formed on Komadas media after soil plating were counted in 2011 as opposed to just the sum of the two as was done in 2010.
[d]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 3.4 Effects of cover crop[a] and *Fusarium oxysporum* f. sp. *niveum* (FON)[b] inoculation simple effects on the linear rate of watermelon[c] vine growth in two green house experiments

Cover crop	FON Inoculation	Trial 1 (mm/week)[d]	Trial 2 (mm/week)
Trifolium incarnatum	FON	0.49 f	1.15 e
Vicia Villosa x1	FON	0.96 cdef	7.84 d
Vicia villosa x2	FON	0.84 def	6.80 d
Vicia villosa x3	FON	0.67 ef	7.14 d
Nonamended	FON	11.14 cde	17.84 ab
Secale cereale	FON	0.52 ef	6.97 d
Trifolium incarnatum	No FON	2.25 a	17.95 ab
Vicia Villosa x1	No FON	1.83 ab	17.27 ab
Vicia villosa x2	No FON	1.48 bcd	20.26 a
Vicia villosa x3	No FON	1.92 ab	17.88 ab
Nonamended	No FON	1.53 bc	16.26 b
Secale cereale	No FON	0.74 ef	11.71 c
P>F		0.0096	<0.0001

[a]The *V. villosa* green manure amendment was incorporated into the potting soil treatment at three different rates- 2721.55 kg/ha, 3685.43 kg/ha, and 5669.88 kg/ha. *T. incarnatum* was incorporated into the soil at rate of 5102.90 kg/ha and *S. cereale* was incorporated at a rate of 3968.90 kg/ha.

[b]FON inoculum was incorporated into soil at a rate of 25,000 CFUs/g soil.

[c]The watermelon variety 'Sugar Heart' was used in all pots.

[d]Watermelon vine length measured weekly in mm every week for six weeks and the rate of linear vine growth mm/week was calculated.

[e]Means in a column followed by the same letter are not significantly different at $\alpha=0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 3.5 Effects of cover crop[a] and *Fusarium oxysporum* f. sp. *niveum* (FON)[b] inoculation simple effects on watermelon[c] wilt severity ratings in two greenhouse experiments

		Trial 1		Trial 2			
Cover crop	FON Inoculation	Week 1[d]	Week 6	Week 4	Week 5	Week 6	AUDPC
Trifolium incarnatum	FON	0.21ab[f]	2.45 a	2.00 a	2.83 a	2.75 a	7.29 a
Vicia Villosa x1	FON	0.08 b	1.42 bc	0.17 bc	1.29 b	2.00 b	2.60 cd
Vicia villosa x2	FON	0.00 b	1.75 ab	0.17 bc	1.42 b	2.17 ab	2.92 bc
Vicia villosa x3	FON	0.00 b	1.71 ab	0.25 bc	1.92 b	2.75 a	3.83 bc
Nonamended	FON	0.38 a	0.83 cd	0.21 bc	0.13 c	1.00 c	0.88 e
Secale cereale	FON	0.42 a	2.42 a	0.71 b	1.67 b	2.00 b	4.04 b
Trifolium incarnatum	No FON	0.00 b	0.29 de	0.04 c	0.00 c	0.92 cd	0.63 e
Vicia Villosa x1	No FON	0.04 b	0.25 de	0.00 c	0.00 c	0.33 de	0.17 e
Vicia villosa x2	No FON	0.13 b	0.71 cde	0.00 c	0.00 c	0.08 e	0.04 e
Vicia villosa x3	No FON	0.00 b	0.04 e	0.04 c	0.04 c	0.67 cde	0.50 e
Nonamended	No FON	0.00 b	0.92 cd	0.00 c	0.00 c	1.13 c	0.83 e
Secale cereale	No FON	0.00 b	2.46 a	0.13 c	0.38 c	1.25 c	1.29 de
P>F		0.0083	0.0003	<0.0001	<0.0001	<0.0001	<0.0001

[a]*V. villosa* green manure amendment was incorporated into the potting soil treatment at three different rates- 2721.55 kg/ha, 3685.43 kg/ha, and 5669.88 kg/ha. *T. incarnatum* was incorporated into the soil at rate of 5102.90 kg/ha and *S. cereale* was incorporated at a rate of 3968.90 kg/ha.[b]For FON inoculated treatments the fungus was incorporated into soil at a rate of 25,000 CFUs/g soil.

[b]FON inoculum was incorporated into soil at a rate of 25,000 CFUs/g soil.

[c]The watermelon variety ‘Sugar Heart’ was used in all pots.

[d]Watermelon wilt was rated weekly, for six weeks, in both greenhouse trials.

[e]watermelon wilt severity ratings were 0-3 (0=no wilt, 1=1-33% wilted, 2=34-63% wilted 3=64-100% wilted or dead).

[f]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher’s protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

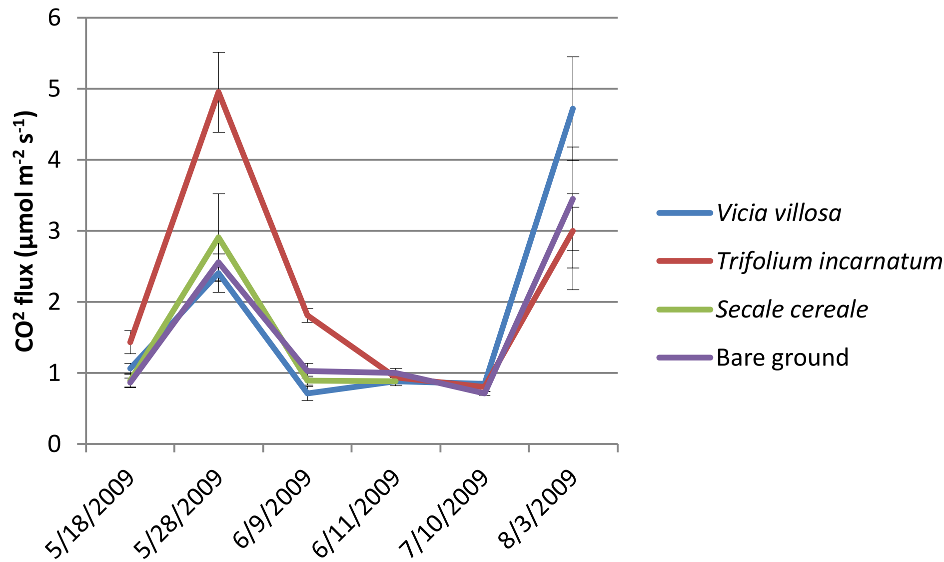

Figure 3.1 Effects over time of green manures planted in the late fall and incorporated in the spring, followed by a spring watermelon crop on soil CO_2 respiration at the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC) in Maryland in 2009. Cover crop tillage was on 5/23/2009 and watermelon fruit were harvested at the end of August

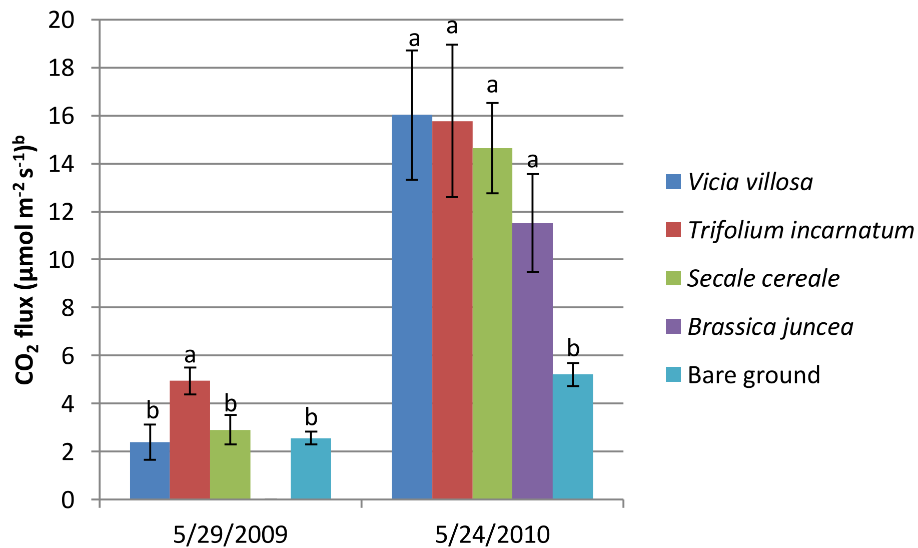

Figure 3.2 Effects of spring incorporated green manures on soil CO_2 respiration measured following cover crop tillage at the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC) in Maryland

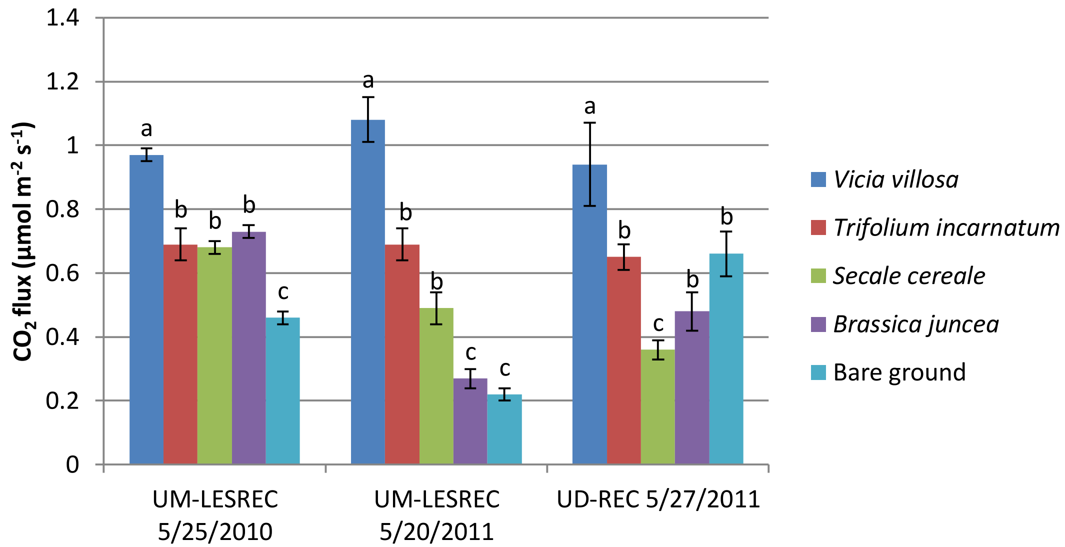

Figure 3.3 Effects of spring incorporated green manures and bare ground on soil CO_2 respiration measured following cover crop tillage at the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) in Salisbury, 2010 and 2011 and the University of Delaware Research and Education Center (UD-REC) in Georgetown, 2011

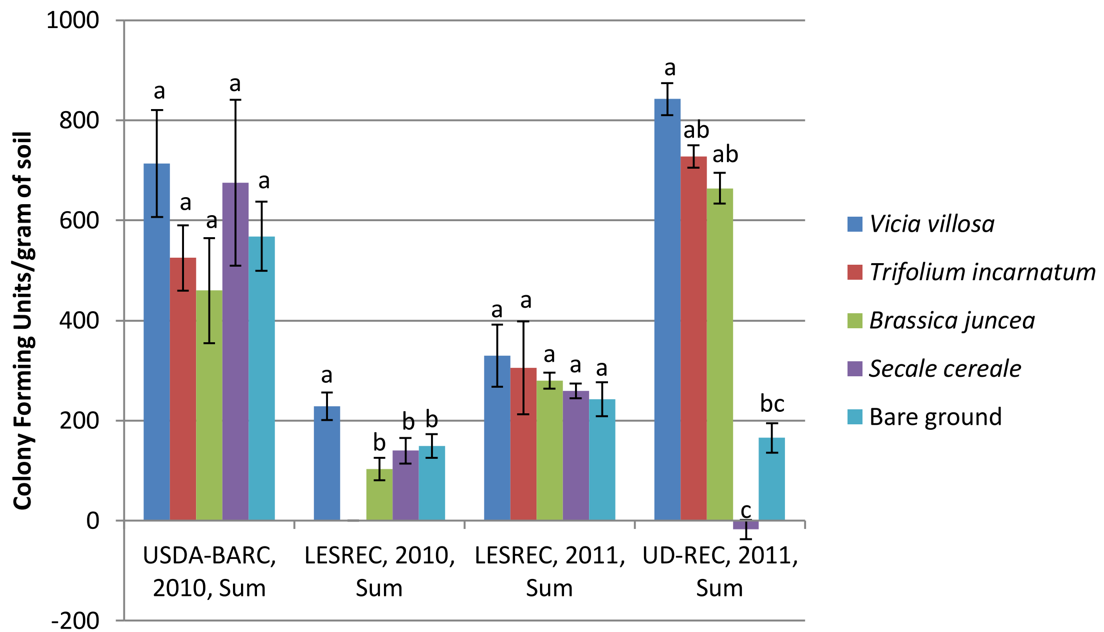

Figure 3.4 Influence of spring incorporated green manures on Colony Forming Units (CFUs) of *Fusarium oxysporum* spp. per a gram of soil of soil at the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC) in Maryland in 2009, the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) in Salisbury, 2010 and 2011 and the University of Delaware Research and Education Center (UD-REC) in Georgetown, 2011

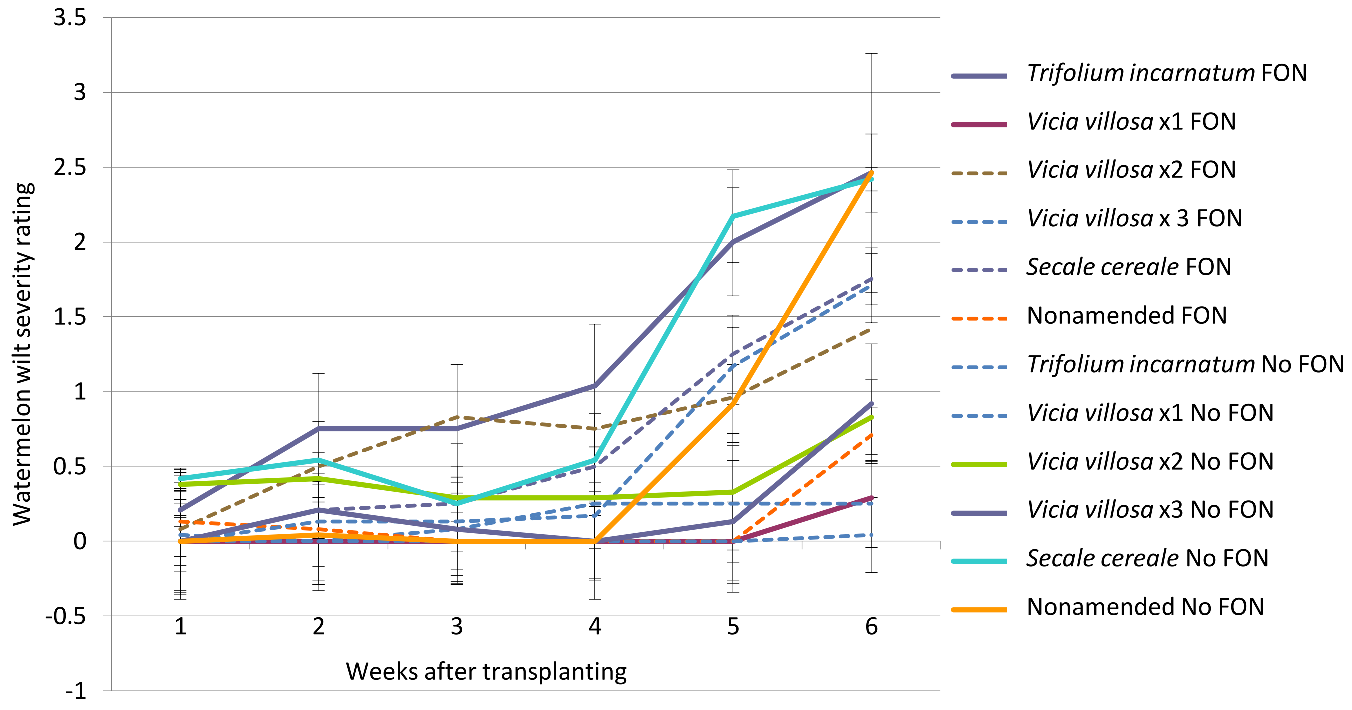

Figure 3.5 Watermelon wilt severity ratings of watermelon plants in pots inoculated or not inoculated with *Fusarium oxysporum* f. sp. *niveum* (FON) and amended with five different cover crop treatments or nonamended in greenhouse experiment 1

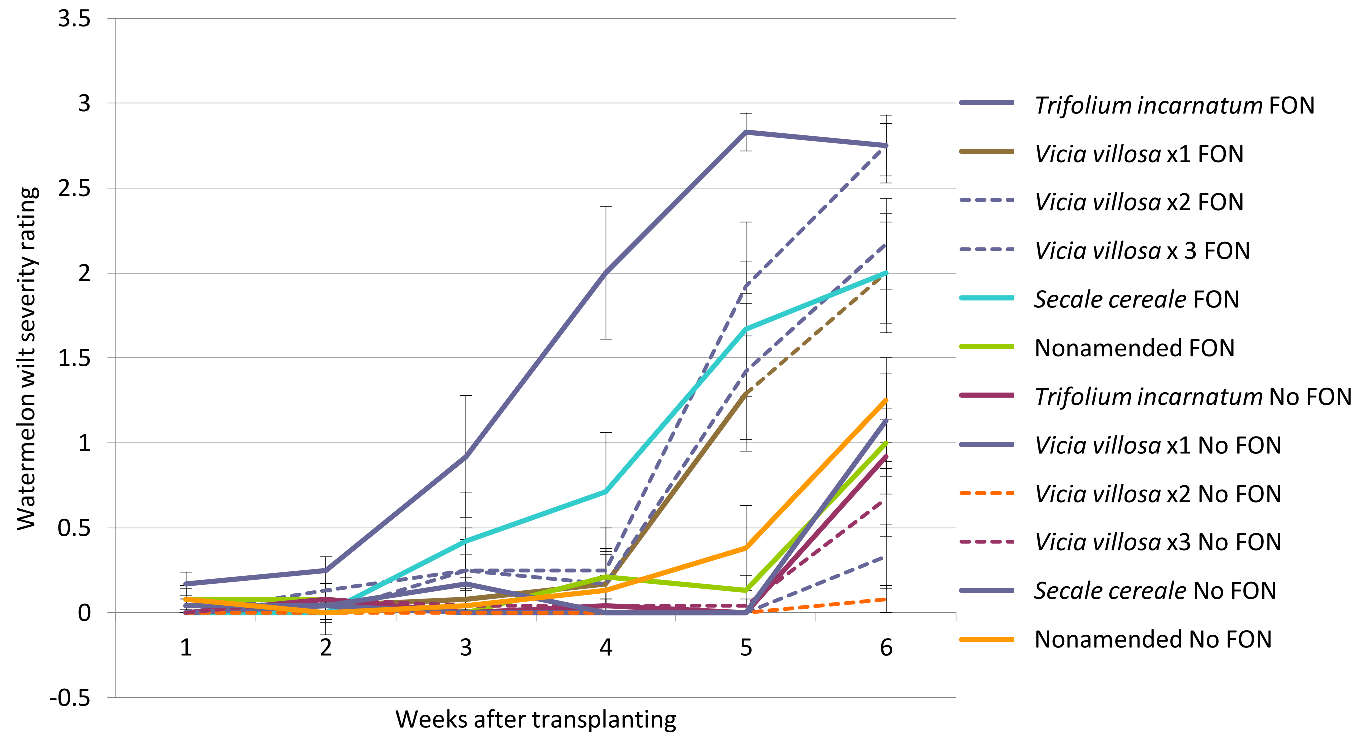

Figure 3.6 Watermelon wilt severity ratings of watermelon plants in pots inoculated or not inoculated with *Fusarium oxysporum* f. sp. *niveum* (FON) and amended with five different cover crop treatments or nonamended in greenhouse experiment 2

Chapter 4: *Vicia villosa* suppression of Fusarium wilt of watermelon via cover crop leachates and augmentation of watermelon mycorrhizal colonization

4.1.Abstract

A *Vicia villosa* green manure incorporated in spring suppresses Fusarium wilt of following watermelon crops, but the means of the disease suppression is unknown. Possible specific mechanisms of disease suppression and the efficacy of an Actinovate biocontrol (*Streptomyces lydicus* WYEC 108) against the causal pathogen, *Fusarium oxysporum* f. sp. *niveum* (FON), were evaluated. Arbuscular mycorrhizal (AM) colonization of watermelon that were grown following a green manure (*V. villosa*, *Trifolium incarnatum*, *Secale cereale*, *Brassica juncea*) or bare ground, and Actinovate were evaluated. *In vitro* experiments were conducted at a pH of 3.5 and 6 to measure the effects of cover crop (*V. villosa*, *T. incarnatum*, *S. cereale*) leachate on the growth rates of FON and *Trichoderma harzianum* and on colony forming units (CFUs) of *S. lydicus*. The percentage of watermelon roots colonized by AM following *V. villosa* and *T. incarnatum* green were significantly higher than in watermelon following bare ground (58% and 44% higher, respectively). Growth rates of FON and *T. harzanium* on *V. villosa* amended media were 66% and 213% faster, respectively, than on nonamended plates. Leachate amendments did not influence *S. lydicus* growth, however, *S. lydicus* significantly inhibited the rate of FON growth, decreasing it as much as 44%.

4.2.Introduction

Watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai) is a major vegetable crop in Maryland and Delaware. In 2011, 2023 hectares of watermelon were planted in these two states (USDA, 2012). Fusarium wilt of watermelon, caused by the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON), is prevalent on the Eastern Shore of Maryland and in Delaware (Zhou et al., 2003b). Increased consumer demand for triploid (seedless) watermelon is problematic as there are currently no commercial triploid watermelon cultivars with resistance to Fusarium wilt (Everts et al., 2010; Everts et al., 2011). Few management options other than growing resistant cultivars exist for Fusarium wilt of watermelon and options that are available vary in cost and effectiveness. One potential control tactic is using Actinovate AG (National Industries Inc., Houston, TX, active ingredient *Streptomyces lydicus* WYEC 108). However, the efficacy of this product has not been evaluated for Fusarium wilt of watermelon.

A spring incorporated *Vicia villosa* Roth (hairy vetch) green manure reduces Fusarium wilt of watermelon (Zhou et al., 2004; Zhou et al., 2006, Zhou et al., 2007). However, the mechanism behind this disease suppression is unknown. One possibility is an increased level of watermelon mycorrhizal root colonization following the soil incorporation of *V. villosa*. Mycorrhizae decrease disease in several host plants, by various means-including improved plant health via enhanced nutrient and water inputs, induced systematic resistance, or changes in host root exudates (Tobar et al., 1994; Cordier et al., 1998; Kaya et al., 2003; Scheffknecht et al. 2006). *Vicia villosa* cover crop amendments increased mycorrhizal populations in the soil rhizosphere of a following tomato cropping system (Buyer et al., 2010), and increased mycorrhizal colonization of

subsequently planted peach seedlings (Rutto et al., 2003; Galvez et al., 1995). Little is known about arbuscular mycorrhizal (AM) watermelon colonization and the role AM play in *V. villosa* disease suppression of Fusarium wilt. The few studies that do examine mycorrhizal associations with watermelon roots focused on the mycorrhizae's ability to increased fruit yield, rather than its effects on disease (Kaya et al., 2003).

Another possible mechanism of this suppression is that plant leachates released by the cover crop influence the population level and virulence of pathogens in the soil. Plant leachates are defined as leachates that occur in the environment naturally, not purposefully induced chemically (Overland, 1966). The fungitoxic effects of *Brassica* spp. leachates on plant pathogens are well known (Angus et al., 1994; Manci et al., 1997; Kirkegaard et al., 1998). Leachates could also impact disease by stimulating antagonists of soil pathogens. *Brassica napus* seed meal suppression of apple root infection (*Pythium* spp.) corresponded with increases in soil populations of actinomycetes and fluorescent psuedomonads (Mazolla et al., 2001).

The objective of this study was to explore the specific mechanism by which *V. villosa* may suppress Fusarium wilt of watermelon and the efficacy of the biocontrol product Actinovate AG in Fusarium wilt suppression. Specific objectives are to 1) examine the percent colonization of watermelon roots by arbuscular mycorrhizae following four fall-planted cover crops and bare ground, with and without an Actinovate biocontrol application; 2) evaluate the *in vitro* effect of *V. villosa, Trifolium incarnatum,* and *Secale cereale* leachate on the mycelial growth of *Fusarium oxysporum* f. sp. *niveum* and *Trichoderma harzianum* and the colony forming units of *S. lydicus*, the active

ingredient of Actinovate AG; and 3) assess the *in vitro* inhibitory effects of *S. lydicus* on *Fusarium oxysporum* f. sp. *niveum*.

4.3.Materials & Methods

4.3.1.Fungal Isolates

Trichoderma harzianum (isolate GJS-00-150) obtained from Beltsville Agricultural Research Center and *Fusarium oxysporum* f. sp. *niveum* race 1 (isolate F-030-1) from the Eastern Shore of Maryland were used in laboratory trials (Kucuk et al., 2003; Zhou et al., 2003b; Wu et al., 2010).

4.3.2.Leachate *in vitro* experiment: FON and *T. harzianum*

Leachate of *V. villosa*, *T. incarnatum* and *S. cereale* were collected by simulating an average rainfall event on the Eastern Shore of Maryland in early spring (approximately 3 mm), as follows. In late spring fresh aboveground biomass of *V. villosa* was collected at the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC), *S. cereale* biomass from the University of Maryland Wye Research and Education Center and *T. incarnatum* biomass from the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC), in Maryland. Cover crop biomass concentrations for leachate production were based on the average above ground cover crop biomass found in previous field trials at UM-LESREC and USDA-BARC. These concentrations were 5101 kg/ha of *T. incarnatum*, 3968 kg/ha of *S. cereale*, and 2834 kg/ ha of *V. villosa*. For leachate collection, biomass concentrations were doubled for *S. cereale* and *T. incarnatum* and quadrupled for *V. villosa* to account for the subsequent dilutions necessary for producing leachate amended

potato dextrose agar (PDA), so final treatment amendments would be representative of field conditions. Cover crops were cut into 5-7 cm length fragments, to simulate rough tillage, and placed on a screen (8788 cm^2) atop a collection bin, and beneath overhead sprinklers. Prior to leachate collection the bin used for the leachate collection was placed below the sprinklers to determine the amount of water that was emitted from the overhead sprinklers every five minutes so that appropriate ratio of cover crop biomass to rain water could be calculated. For each liter of simulated rainwater, 0.53 kg of *S. cereale*, 1.22 kg of *T. incarnatum*, and 2.45 kg of *V. villosa* was placed atop the screen. The water that filtered through the cover crop biomass was the leachate used for the plate assays.

The treatments were PDA plates that were amended with 1) *V. villosa* leachate at x1 and x2 concentration 2) *T. incarnatum* leachate at x1 concentration and 3) *S. cereale* leachate at x1 concentration. Control plates were nonamended PDA plates. All media treatments were adjusted to pH 3.5 and 6 with lactic acid and tested. Each experiment was conducted twice. Ten replicate plates per a treatment were used in the first trial, and then fourteen replicate plates per a treatment in the second.

The leachate was incorporated into the PDA media as follows: commercial PDA was prepared using half the volume of distilled water specified in the recipe and autoclaved. After media had cooled to approximately 50°C, it was brought up to volume with the x2 leachate (or in the case of *V. villosa*, x4), Streptomycin (0.3 g/1000 ml) and oxgall (0.5 g /1000 ml) were added to the media and homogenized. All leachate treatments were produced in this manner except for the *V. villosa* x1 treatment, as the *V. villosa* leachate was collected at x4 the concentration so that x1 and x2 *V. villosa*

treatments could be tested. To produce the *V. villosa* x1 leachate PDA was autoclaved with 3/4th volume of distilled water, brought up to full volume with the *V. villosa* x4 leachate, the same antibiotics were added, and the solution stirred prior to pouring plates.

FON isolates were transferred to PDA plates and grown at 28°C for approximately 10 days and *T. harzianum* for 4 days before transferring 5 mm plugs from the edges of the colony to the center of all leachate amended treatment plates and control plates. Following inoculation, plates were incubated at 25°C.

In the first trial fungal radial growth was measured at 24 hour intervals and in the second trial radial growth was measured every six hours for the initial ten readings and then every 24 hours until the fungus reached the edge of the control plates or significant advances in growth had ceased (Bananomi et al., 2007, Larkin et al., 2007). At this point, 5mm agar plugs were taken from the periphery of fungal growth and placed on nonamended PDA plates to test the viability of the fungi (Yuan et al., 1995; Al-Reza et al., 2010). The morphology of the fungi along the border of the colonies was examined with a dissecting and compound microscope to detect any unusual changes in morphology (abnormal branching, swollen tips of hyphae) or hyphal tip lysis (Yuan et al., 1995).

4.3.3.Leachate *in vitro* Experiment: *Streptomyces lydicus*

Ten replicate leachate plates of the five cover crop treatments (*T. incarnatum* x1, *S. cereale* x1, *V. villosa* x1 and x2, and a nonamended control), at a pH of 3.5 and 6, were prepared using the same techniques as described above. One ml of a 1:10 Actinovate AG product water solution was spread evenly across the leachate amended media plates.

Colony forming units of the *S. lydicus* were counted a week after plates were incubated at 25˚C. The experiment was conducted twice.

4.3.4. Inhibition of FON with *Streptomyces lydicus*

To determine the inhibition of FON mycelial growth in the presence of *S. lydicus,* a Petri dish experiment was conducted. For this purpose, PDA plates were adjusted to a pH of 3.5. The treatments were 1) control (noninoculated) 2) *S. lydicus* isolated directly from the Actinovate AG product 3) *S. lydicus* WYEC 108 isolate obtained directly from Natural Industries. Working cultures from the Actinovate AG product were obtained by pipetting 60 ml of Actinovate at a concentration of 6.1 g/10 L onto sporulation agar (SPA) plates. Plates were incubated at 30˚C for approximately ten days so that the *S. lydicus* sporulation was profuse. The *S. lydicus* isolates were uniformly streaked onto PDA plates from the edge of the working plate to the quarter radius line and grown for five days at 30˚C (Getha et al., 2005; Yuan et al., 1995). A 5 mm diameter agar plug of FON mycelium was then placed on the opposite side of the plate, directly adjacent to the quarter radius line. Nonstreaked SPA plates with FON plugs placed at the quarter radius line served as a control. The plates were incubated at 30˚C and assessed for growth inhibition at 24 hour intervals for twelve days. The experiment was conducted twice. The viability and morphology of the FON was evaluated after mycelia reached the border of the control plates with the same methodology described above.

4.3.5. Arbuscular Mycorrhizae

Cover crops were seeded at USDA-BARC on September 22nd 2009 (*V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, *B. juncea* 6.73 kg/ha.), were killed with a paraquat treatment (Gramoxone Extra 2.5SC, 1.2 kg ai/ha, 1.75 L/ha)

and disked with a tractor-mounted rototiller to a depth of approximately 10 to 20 cm on May 23rd 2010. At UM-LESREC field plots were seeded on September 25th 2009 (*V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 124.54 kg/ha, *B. juncea* 11.21 kg/ha) and incorporated in the same manner on May 24th 2010. Control plots were also cultivated with this method.

In the summer of 2010, watermelon roots were sampled from a field experiment at UM-LESREC and USDA-BARC. The experiment was a completely randomized split-plot design. The main plots were cover crop treatments planted in the fall; *Trifolium incarnatum*, *Secale cereale*, *Brassica juncea*, *Vicia villosa* and no cover. Subplots were Actinovate biocontrol treatments. The field at USDA-BARC was artificially infested with FON. Therefore at this location the subplots were 1) FON inoculation + Actinovate application, 2) FON inoculation, 3) a non-treated and non-inoculated control. In the naturally FON infested field at UM-LESREC the subplots treatments were 1) Actinovate application, or 2) no Actinovate.

An entire watermelon plant, with its root system intact, was gently uprooted from each subplot three weeks after the watermelons were transplanted to the field. Two watermelons were sampled from four blocks at UM-LESREC. One watermelon was sampled from three blocks at USDA-BARC. Roots were scanned using WinRhizo Pro 2004b TM (Regent Instruments Inc.) software to obtain root characteristics-such as total area and diameter.

A protocol described by Morton (2003) was used to evaluate the AM colonization. First, roots were stained with 0.05% trypan blue in lactophenol. Fine roots were washed at least three times, placed in plastic cassettes and cleared in hot 10% KOH

for 10 minutes. The roots were washed again in water, the cassettes immersed in 2% HCL for 20 minutes, rinsed again and then soaked in trypan blue for a minimum of 12 hours before they were stored in a 2:1 water-glycerin mix. Thin feeder roots were examined under a light microscope for mycorrhizal infection (Matsubara et al., 2001). Twenty-five 10 mm root segments from each plant were examined. An infected node is defined as a 10 mm root segment where the presence of an arbuscle has been detected (Newsham et al., 1995; Egerton-Warburton et al. 2000). As only fine roots were examined for AM colonization, the equation for percent AM was adjusted to only include the fine roots, identified by the WinRhizo software as roots with <0.5 cm diameter. Therefore the percent of AM infection was defined by ((number of nodes infected/number of nodes observed)*100) relative to the percentage of roots with <0.5 cm diameter.

4.3.6.Statistical Analysis

Data were analyzed using the MIXED procedure with the Statistical Analysis System (version 9.2; SAS Institute, Cary, NC), which integrates random effects in the statistical model and performs covariance structure modeling (Littell et al., 1998). Treatment means were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. When disease severity ratings were low or fungal growth minimal the data was generally not normally distributed. According to the Central Limit Theorem a sample size (n) over 30 will have a mean which converges to a standard normal deviation (Corbett et al., 2002). As the sample sample size for each trial was larger than 30 the data was considered robust and no transformations were employed (Payton et al., 2006).

4.4. Results

4.4.1. Leachate *in vitro* Experiments

4.4.1.a. FON

FON linear radial growth at a pH of 3.5 was fastest on *V. villosa* x1 amended media, second fastest on *V. villosa* x2 amended media, intermediate on *S. cereale* and *T. incarnatum* plates and slowest on nonamended plates (Table 4.1). In the first trial FON logarithmic radial growth at a pH of 3.5 was significantly faster on all plates amended with a leguminous cover crop leachate, intermediate on *S. cereale* amended media, and slowest on nonamended media (Table 4.1).

In the second trial, at pH 3.5, FON linear growth on *V. villosa* x2 amended agar plates was faster than on nonamended or *S. cereale* amended media (Table 4.1). Almost parallel to these results, FON logarithmic growth in the second trial at a pH of 3.5 was faster on *V. villosa* x1 amended plates compared to on nonamended and *S. cereale* amended media (Table 4.1).

There were no significant treatment differences in FON linear or logarithmic growth at a pH of 6 in the first trial. However, FON linear growth in the second trial at pH 6 was faster on *V. villosa* x1 amended plates compared to on all other treatments except for that of *V. villosa* x2. FON linear growth on *V. villosa* x2 also was only significantly faster than growth on nonamended and *T. incarnatum* amended plates (Table 4.1). Similarly, for the second trial at a pH of 6 FON logarithmic growth was fastest on *V. villosa* x1 amended plates compared to all other plate treatments except for that of *V. villosa* x2 amended media (Table 4.1).

4.4.1.b.Trichoderma

Linear and logarithmic growth rates of *T. harzianum* at a pH of 3.5 were significantly faster on *V. villosa* x1 and *V. villosa* x2 plates in the first trial compared to on all other treatments. The logarithmic growth of *V. villosa* x1 at a pH of 3.5 in the second trial also was also greater than all other treatments (Table 4.2). In the second trial *T. harzianum* linear growth at a pH of 3.5 was significantly faster on *V. villosa* x1 and *S. cereale* amended plates compared to all other leachate amended and nonamended treatments (Table 4.2).

The linear rate of growth of *T. harzianum* at a pH of 6, in the first trial was significantly faster on *V. villosa* x1 and *V. villosa* x2 amended plates than on *T. incarnatum* leachate amended plates (Table 4.2). The logarithmic rate of growth of *T. harzianum* at a pH of 6 in the first trial was significantly faster on *V. villosa* x1 and *V. villosa* x2 (66.91 mm/24 hours) amended plates than on *T. incarnatum* or nonamended plates. Likewise, the linear and logarithmic growth rates of *T. harzianum* in the second trial done at a pH of 6 were faster on *V. villosa* x1 compared to all other leachate amended and nonamended media except for that of *V. villosa* x2. Additionally, in the second trial at a pH of 6, *T. harzianum* linear growth on *V. villosa* x2 amended plates was significantly faster than on *S. cereale* and nonamended plates (Table 4.2).

All FON and *T. harzianum* fungi were found to be viable after growth had reached the edge of the plate or had slowed. No abnormal mycelia growth was observed.

4.4.1.c.*Streptomyces lydicus*

Leachate plate amendments had no significant effect on the number of *S. lydicus* CFUs formed for either plate experiment (data not shown).

4.4.2.Inhibition of FON with *Streptomyces lydicus*

FON linear growth rate was significantly inhibited on plates with *S. lydicus* compared to the linear growth rate on control plates where no *S. lydicus* was streaked for both plate experiments (Table 4.3). In the first trial FON linear growth was significantly slower on plates streaked with the *S. lydicus* WYEC 108 pure isolate compared to plates streaked with *S. lydicus* isolated from the commercial Actinovate product, and the opposite relationship occurred in the second trial (Table 4.3). Abnormal mycelia growth was observed at the line of inhibition, where FON approached the *S. lydicus* streaking. Here the the FON mycelia formed a mound of mycelia, clearly being influenced by something the *S. lydicus* was producing *in vitro*. However, FON growth was normal when the mycelia were transferred to nonamended PDA plates.

4.4.3.Arbuscular Mycorrhizae

The roots of watermelon plants grown following a *V. villosa* and *T. incarnatum* cover crop had a significantly higher percent of arbuscular mycorrhizal colonization than watermelon roots following *S. cereal*e, or *B. juncea*, cover crops or in nonamended plots at UM-LECREC in 2010 ($P < 0.0001$) (Table 4.4). Arbuscular mycorrhizal root colonization of watermelon was also significantly higher following *S. cereale* than in watermelon following *B. juncea* or nonamended plots (Table 4.4).

The percentage of mycorrhizal arbuscules was significantly higher in watermelon roots grown following *V. villosa* cover crop amended soil compared to all other amended and nonamended plots at USDA-BARC in 2010 (*P* <0.0001) (Table 4.4). Arbuscular mycorrhizal root colonization of watermelon in *T. incarnatum* amended plots was significantly intermediate and higher compared to plants in *S. cereale*, *B. juncea*, and nonamended plots (Table 4.4).

The Actinovate biocontrol treatment had no significant effect on the percentage of watermelon roots colonized by arbuscular mycorrhizae at either location (data not shown).

4.5.Discussion

The leachate used in our study was designed to represent a single rainfall event on the Eastern Shore of Maryland. Similar protocols for leachate or plant extract experiments were used in only a few studies (Chou et al., 1973; Hussain et al., 2011) whereas the majority of studies did not employ field based estimation (Cote, et al., 1988; Conway et al., 2002; Suman et al., 2002; Orr et al., 2005; Chou et al., 1972; Hanson et al., 1987).

Plant extracts, essential oils and various phenolics can reduce *F. oxysporum* spp. *in vitro* growth (Bowers et al., 2000; Wu et al., 2008; Wu et al., 2009a; Al-Reza et al., 2010; Linde et al., 2010; Osorio et al., 2010; Vaz et al., 2010; Wu et al., 2010). We therefore expected that *V. villosa* leachate would inhibit FON growth. However, in our study, the *V. villosa* leachate amended plates significantly stimulated FON growth, especially at a pH of 3.5. It is notable that *V. villosa* leachate was effective at enhancing FON growth at the lower pH, because Fusarium wilt of watermelon is most prevalent in

slightly acidic soils (pH 5-6), and less prevalent in more neutral or higher pH soil environments (Zitter et al., 1996). As nonpathogenic *F. oxysporum* and FON occupy a similar saprophytic niche in absence of a host plant they have similar nutrient requirements (Lamanceau et al., 1993). The competition among the species often results in the reduction of FON growth and virulence (Zhou et al., 2003b; Alabouvette et al., 1993). Therefore, it is possible that *V. villosa* leachate may also stimulate nonpathogenic *F. oxysporum* spp.. A recent field study which observed *V. villosa* green manure disease suppression of Fusarium wilt of watermelon found a parallel increase in *F. oxysporum* spp. in watermelon plots amended with *V. villosa* (unpublished data). Increased growth and activity of nonpathogenic *F. oxysporum* spp. and other commonly antagonistic microorganisms (i.e. fluorescent *Psuedomonas* spp.) are cited as the mechanisms of disease suppression in soils naturally suppressive to FON and with induced FON suppression via a monoculture of 'Crimson Sweet' (Alabouvette et al., 1993; Larkin et al., 1993a; Larkin et al., 1993b; Larkin et al., 1996). The ability of nonpathogenic *F. oxysporum* spp. to protect plants from pathogenic FON through direct competition or induced systemic resistance has been demonstrated in numerous experiments. For example, preinoculation of watermelon plants with nonpathogenic *F. oxysporum* spp. conferred 'cross protection' against FON (Biles et al., 1989; Freeman et al., 2002).

In this experiment, *T. harzianum* growth was also stimulated on *V. villosa* leachate amended plates, at a pH of 3.5 and 6. *Trichoderma* species are widespread soil fungi, fast growing opportunists and occasional mycoparasites (Samuels et al., 2006). The antagonistic role *Trichoderma* spp. play in disease suppression is well known. They are often used as a form of biocontrol or as indicators of elevated antagonistic soil

microbial populations (Bailey et al., 2008; Busko et al., 2008; Bonanomi et al., 2010). A bio-organic fertilizer containing a combination of *T. harzianum* and *Paenibacillus polymyxa* successfully reduced Fusarium wilt of watermelon by inducing systemic acquired resistance (Wu et al., 2009b). *Trichoderma* spp. employed as a biocontrol for *Fusarium* spp. pathogens of other plants have also demonstrated successful disease reductions, often through direct antagonism (Rojo et al., 2007; Yang et al., 2011). *Trichoderma* spp. stimulated by green manure leachates could induce systemic acquired resistance in watermelon or function as direct antagonists of the FON pathogen, thereby playing a role in *V. villosa* disease management of Fusarium wilt. However, it is also possible that the leachates stimulation of *T. harzanium* is merely representative of the cover crops ability to cause an overall elevation in soil microbial activity, rather than its capacity to promote this specific antagonist or other definite antagonistic microbial groups. A detailed molecular analysis of the specific microbial changes that result from *V. villosa* cover crop planting and incorporation would help elucidate the role *Trichoderma* spp. and nonpathogenic *F. oxysporum* play in this disease suppression.

In the *in vitro* study, cover crop leachates had no significant effect on the number of *S. lydicus* CFUs. The Actinovate biocontrol was not consistently effective as a management tool for Fusarium wilt of watermelon in previous field and greenhouse trials in central Maryland and on the Eastern Shore of Maryland and Delaware (unpublished data). We were unable to isolate the active ingredient *S. lydicus* from watermelon roots in field and greenhouse studies indicating that it may not have successfully colonized the rhizosphere (unpublished). However, during the in vitro Petri dish experiments both *S. lydicus* treatments significantly inhibited FON growth. An *in vitro* experiment evaluating

the antagonism of 82 actinomycete isolates against *Pythium ultimum* found that *S. lydicus* WYEC 108 was one of the four isolates that showed a very strong antagonism to the pathogen (Crawford et al., 1993). *Streptomyces lydicus* can clearly be a very competent and highly competitive antagonist in *in vitro* conditions. However, it is unlikely to be effective if used in soil conditions that are not amenable to its growth and establishment. The soil on the Eastern Shore of Maryland and in Delaware, low in pH and organic matter may not be conducive to the growth of *S. lydicus,* which grows best in high soil pH conditions with complex organic matter (Hiltunen et al., 2008). Although the pH's 6.5 to 8.0 were suitable for the *in vitro* growth of the majority of 82 actinomycete strains tested in a study, some could not grow at pH 6.0 and a significant amount were unable to grow at pH 5.5 (Crawford et al., 2003). It is interesting to note that the efficacy of Actinovate has been demonstrated for many foliar pathogens, such as powdery mildew (*Podosphaera xanthii*) of summer squash (*Cucurbita pepo*) and cantaloupe (*Cucumis melo*) and downy mildew (*Peronospora belbahrii*) of basil (*Osicmum basilicum* 'Genovese'), perhaps indicating that *S. lydicus* is more competent in the phyllosphere, an environment which could provide less intense competition from saprophytes (Zhang et al., 2011; Mersha et al., 2010).

Vicia villosa and *T. incarnatum* green manure amendments increased the percentage of watermelon root colonization by arbuscular mycorrhizae compared to watermelon roots in all other amended and nonamended plots, for both field locations. This is consistent with the findings of Kipkoriony et al., (2003) and Galvez et al., (1995) who found a *V. villosa* cover crop significantly increased soil inoculum levels of arbuscular mychorrhizae for potential colonization of subsequent crop roots. These

results are also of interest as an unpublished study recently demonstrated that both *V. villosa* and *T. incarnatum* green manures can reduce Fusarium wilt of watermelon to a similar extent. Additionally, in one of the few studies done on mycorrhizal colonization of watermelon Kaya et al. (2003) found that mycorrhizal watermelon root inoculation increased fruit yield, water use efficiency and early plant growth.

Watermelon roots in *S. cereale* amended plots were colonized by a significantly higher percentage of arbuscular mycorrhizae than in *B. juncea* amended and nonamended plots at USDA-BARC in 2010. This is not surprising as the presence of compatible host plant roots can help sustain and increase soil arbuscular mycorrhizae populations (Kabir et al., 2000). However, plants like those from the Brassicaceae family can decrease soil mycorrhizae populations (Schreiner et al., 1993; Roberts et al., 2001), resulting in reduced root mycorrhizal colonization of future field crops. In our experiment the arbuscular mycorrhizal root colonization of watermelon in *B. juncea* plots was not significantly different than that of watermelon roots in nonamended plots.

4.6.Conclusion

Bradow et al. (1990) analyzed volatiles derived from *V. villosa* and *T. incarnatum* leachates and found that many of the compounds they characterized stimulate the germination of some fungal spp.. Results of our study support the theory that the stimulatory effects of *V. villosa* leachate on antagonistic microorganisms, like *Trichoderma spp*. and possibly nonpathogenic *Fusarium oxysporum* spp., could contribute to *V. villosa* disease suppression of Fusarium wilt of watermelon. Aditionally, this study establishes that *V. villosa* and *T. incarnatum*, both of which have been proven

to suppress Fusarium wilt of watermelon, increase arbuscular mycorrhizal root colonization of following watermelon crops.

There are many variables in the environment and more than one mechanism may be required to achieve disease suppression. Leachates of cover crops have been shown (El-Atrach et al., 1989; Roberts et al., 2001) to decrease mycorrhizal soil populations but it is also probable that they could increase it (Siqueira et al., 1991). Buyer et al. (2010) observed a green manure amendment of *V. villosa* shoots increased overall microbial biomass and mycorrhizal soil populations in a following tomato cropping system, a mechanism that cannot be attributed to colonization of *V. villosa* roots. Several papers look at the additive effects of *Trichoderma* spp., *Streptomyces* spp. and arbuscular mycorrhizae on disease suppression (Datnoff et al., 1995; Martínez-Medina et al., 2010; Srivastava et al., 2010). Co-inoculation of melon plants with *T. harzianum* and arbuscular mycorrhizae (*Glomus intraradices*) synergistically decreased Fusarium wilt of melon, caused by the pathogen *Fusarium oxysporum* f. sp. *melonis* (Martínez-Medina et al., 2009). Certain strains of *Streptomyces* spp. can stimulate the development of arbuscular mycorrhizae (Abdel-Fattah et al., 2000).

Further exploration of the role of green manure leachates, *T. harzianum* and nonpathogenic *F. oxysporum* spp. play in *V. villosa* disease suppression of Fusarium wilt is necessary for optimal utilization of this disease management method. For instance, examination of a correlation between cover crop enhancement of arbuscular mycorrhizal colonization of watermelon roots and disease suppression of Fusarium wilt could help elucidate the disease suppressive process. A detailed molecular analysis of *V. villosa*

effects on soil microorganisms antagonistic to FON would also help elucidate the different elements of this disease suppression.

Table 4.1 Linear and logarithmic rate of radial growth (mm)[a] of *Fusarium oxysporum* f. sp. *niveum* on Potato Dextrose Agar plates amended with cover crop leachate

	pH 3.5		pH 6	
Cover crop[b] leachate amendment				
Linear growth rate	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Nonamended[c]	2.70 d[d]	2.54 b	3.70 a	2.61 c
Trifolium Incarnatum	3.33 c	2.70 ab	3.73 a	2.76 c
Vicia villosa x1	4.49 a	2.75 ab	3.74 a	3.24 a
Vicia villosa x2	3.67 b	2.84 a	4.17 a	3.07 ab
Secale cereale	3.40 c	2.55 b	3.79 a	2.81 bc
P>F	<0.0001	0.0388	0.4808	0.0005
Logarithmic growth rate				
Nonamended	22.83 c	23.51 b	24.53 a	24.57 b
Trifolium incarnatum	26.45 a	24.49 ab	27.22 a	25.25 b
Vicia villosa x1	26.34 a	26.19 a	25.07 a	27.72 a
Vicia villosa x2	27.29 a	24.89 ab	24.77 a	26.43 ab
Secale cereale	24.46 b	23.38 b	25.00 a	24.03 b
P>F	<0.0001	0.0230	0.6256	0.0206

[a]For Experiment 1 radial growth measurements were taken every 24 hours for 24 days at a pH of 3.5 and every 24 hours for 16 days for pH 6. For Experiment 2 measurements were taken every 6 hours ten times, again after 12 hours, and then every 24 hours for nineteen days.
[b]Amounts of cover crop biomass used to simulate an early spring rainfall was representative of field conditions (*T. incarnatum*: 5101.88 kg/ha, *S. cereale*: 3968.13 kg/ha, *V. villosa* x1: 2834.38 kg/ha and for *V. villosa* x2: 5668.75 kg/ha). Leachate was collected at twice the concentration of these field conditions for *S. cereale* and *T. incarnatum* and four times the concentration for *V. villosa* so that after PDA was made it was brought up to volume with the leachate to achieve field leachate concentrations.
[c]Control plates were nonamended Potato Dextrose Agar.
[d]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 4.2 Linear and logarithmic rate of radial growth (mm)[a] of *Trichoderma harzianum* on Potato Dextrose Agar plates amended with cover crop leachate

Cover crop[b] amendment	pH 3.5		pH 6	
Linear growth rate	Trial 1	Trial 2	Trial 1	Trial 2
Nonamended[c]	7.81 b[d]	7.18 b	44.88 ab	43.00 c
Trifolium incarnatum	7.88 b	5.76 c	43.60 b	43.48 bc
Vicia villosa x1	24.47 a	10.88 a	45.40 a	45.04 ab
Vicia villosa x2	23.66 a	6.87 b	45.99 a	45.88 a
Secale cereale	9.71 b	11.23 a	44.70 ab	42.24 c
P>F	<0.0001	<0.0001	0.0002	0.0007
Logarithmic growth rate				
Nonamended[c]	29.49 b	27.68 b	61.44 c	31.43 b
Trifolium incarnatum	28.19 b	26.57 b	63.45 bc	31.58 b
Vicia villosa x1	44.88 a	31.43 a	66.05 a	32.96 a
Vicia villosa x2	45.18 a	26.71 b	66.91 a	32.07 ab
Secale cereale	28.18 b	26.61 b	65.05 ab	31.48 b
P>F	<0.0001	<0.0001	0.0174	0.0070

[a]For Experiment 1 radial growth measurements were taken every 24 hours for 24 days at a pH of 3.5 and every 24 hours for 16 days for pH 6 plates. For Experiment 2 measurements were taken every 6 hours ten times, again after 12 hours, and then every 24 hours for nineteen days
[b]Amounts of cover crop biomass used to simulate an early spring rainfall was representative of field conditions (*T. incarnatum*: 5101.88 kg/ha, *S. cereale*: 3968.13 kg/ha, *V. villosa* x1: 2834.38 kg/ha and for *V. villosa* x2: 5668.75 kg/ha). Leachate was collected at twice the concentration of these field conditions for *S. cereale* and *T. incarnatum* and four times the concentration for *V. villosa* so that after PDA was made it was brought up to volume with the leachate to achieve field leachate concentrations.
[c]Control plates were nonamended Potato Dextrose Agar.
[d]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 4.3 Effects of two isolates of *Streptomyces lydicus*[a] on in vitro linear rate of growth (mm) of *Fusarium oxysporum* f. sp. *niveum* (FON) for trial 1 and 2

Trial 1	Week												
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	Rate of Growth (Linear)
Control[b]	5.91 a[c]	10.47 a	15.28 a	20.25 a	24.94 a	29.60 a	35.05 a	38.77 a	42.93 a	46.95 a	52.37 a	57.00 a	4.60 a
S. lydicus (Actinovate)	5.16 b	9.91 ab	15.13 a	20.06 a	24.94 a	29.41 a	34.98 a	35.69 b	36.63 b	38.08 b	38.23 b	37.34 b	3.10 b
S. lydicus WYEC 108	5.16 b	9.28 b	13.19 b	15.66 b	18.34 b	20.38 b	24.41 b	26.88 c	29.16 c	32.46 c	35.85 c	36.88 b	2.86 c
P>F	0.0267	0.0089	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Trial 2													
Control	5.16 a	10.19 a	14.84 a	19.72 a	24.78 a	29.50 a	34.50 a	39.31 a	44.00 a	49.06 a	53.69 a	60.00 a	4.89 a
S. lydicus (Actinovate)	4.75 b	9.56 b	13.78 b	17.38 b	20.84 b	23.66 b	27.03 b	29.56 b	31.38 b	33.80 b	35.50 b	39.06 b	2.73 c
S. lydicus WYEC 108	4.56 b	9.19 b	12.93 c	16.09 c	19.41 c	22.59 c	25.41 c	28.06 c	30.84 b	33.94 b	36.91 b	37.67 c	2.93 b
P>F	0.0060	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]*Streptomyces lydicus* WYEC 108 is the active in gradient in Actinovate AG, a biocontrol product made by Natural Industries. *Streptomyces* isolated from the biocontrol product and a pure culture of *S. lydicus* WYEC 108 were streaked onto a quarter section of Potato Dextrose Agar plates. A 5 mm plug of FON was placed 40 mm away on the opposite quarter line. The growth of the FON towards the two Streptomyces treatments was measured over time.
[b]Control plates were Potato Dextrose Agar plates with a 5 mm FON plug placed at a line 20 mm from the edge of the plate (the quarter line).
[c]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table 4.4 Percentage of watermelon roots colonized by arbuscular mycorrhizae in plots amended with four cover crops[a] and bare ground at the United States Department of Agriculture Beltsville Agricultural Reseach Center (USDA-BARC) and the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) in 2010

Cover crop	UM-LESREC	USDA-BARC
Vicia villosa	63.83 a[b]	84.12 a
Trifolium incarnatum	58.67 a	56.06 b
Secale cereale	24.04 b	13.48 c
Brassica juncea	14.73 c	18.84 c
Bare ground	14.30 c	25.65 c
P>F	<0.0001	<0.0001

[a]Seeding rate at UM-LESREC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[b]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

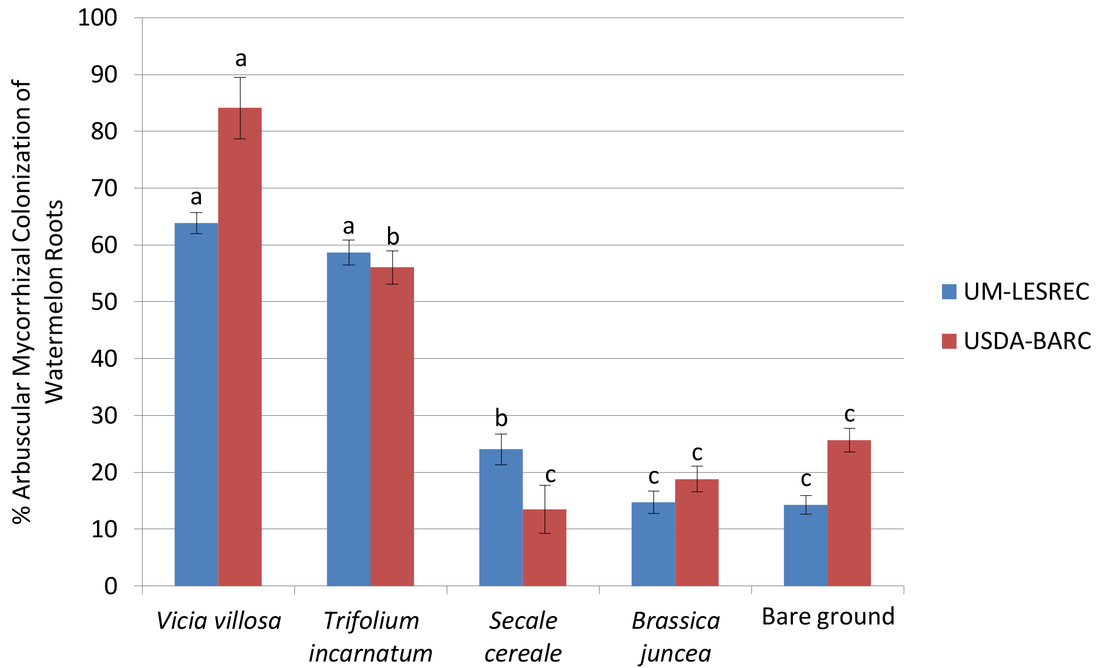

Figure 4.1 Percent arbuscular mycorrhizal root colonization of watermelon plants following four different spring incorporated green manures and bare ground at the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) and the United States Department of Agriculture Beltsville Agriculture Research Center in Maryland, 2010

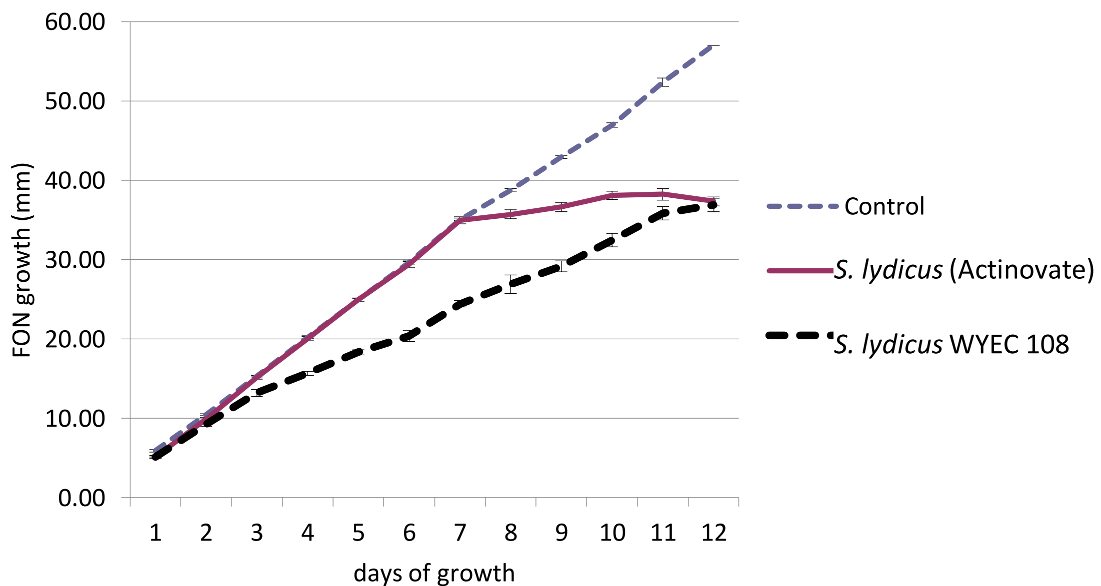

Figure 4.2 Experiment 1 in vitro growth inhibition of a pure isolate of Streptomyces lydicus WYEC 108 and S. lydicus WYEC 108 isolated from Actinovate AG biocontrol product on the pathogen Fusarium oxysporum f. sp. niveum (FON)

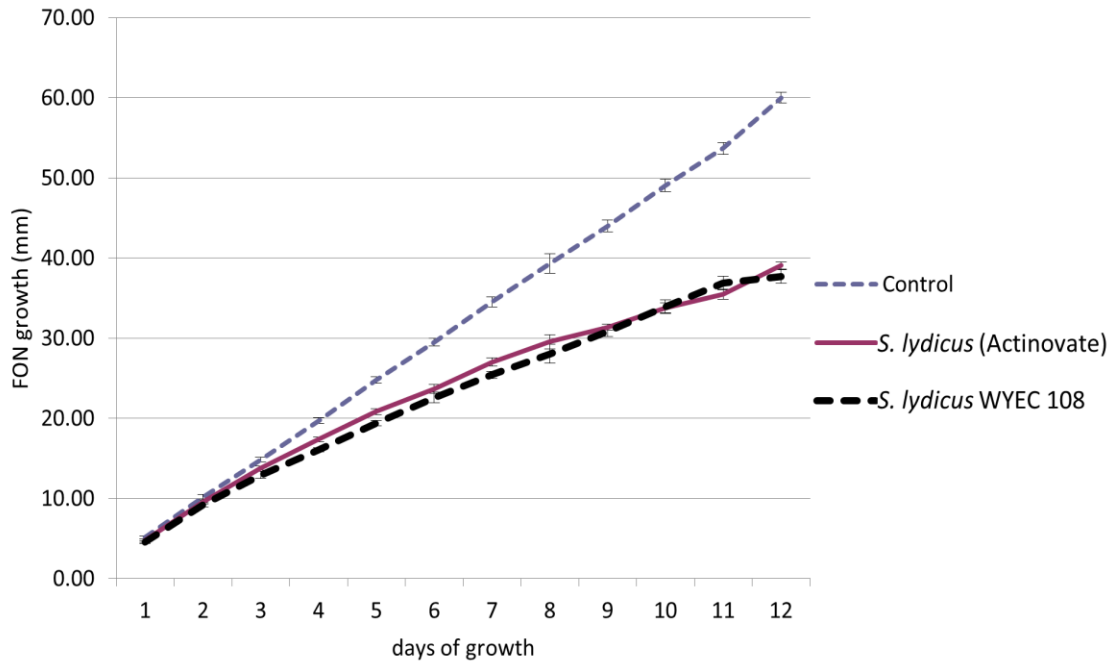

Figure 4.3 Experiment 2 *in vitro* growth inhibition of a pure isolate of *Streptomyces lydicus* WYEC 108 and *S. lydicus* WYEC 108 isolated from Actinovate AG biocontrol product on the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON)

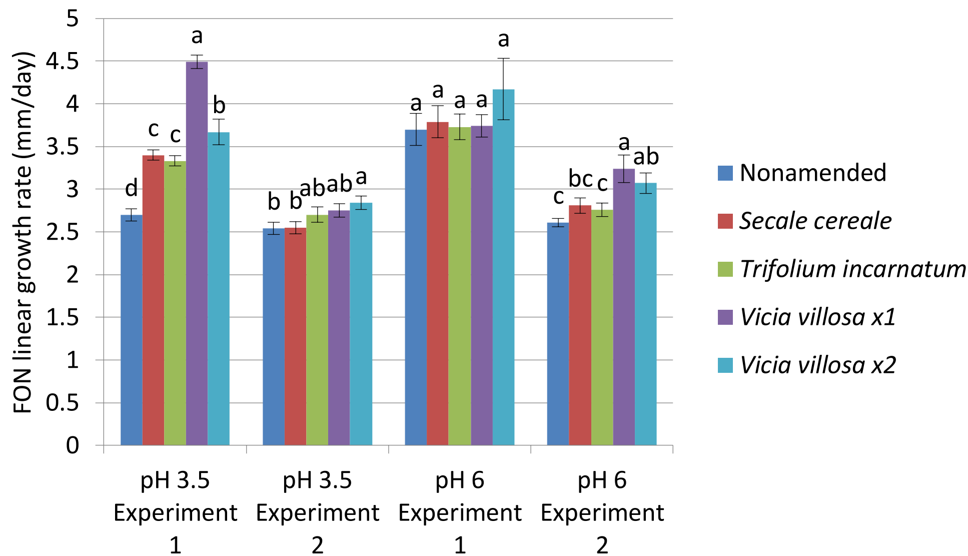

Figure 4.4 *Fusarium oxysporum* f. sp. *niveum* (FON) linear growth rate on PDA plates amended with four different cover crop leachate treatments and nonamended PDA at a pH of 3.5 and 6 for two *in vitro* experiments

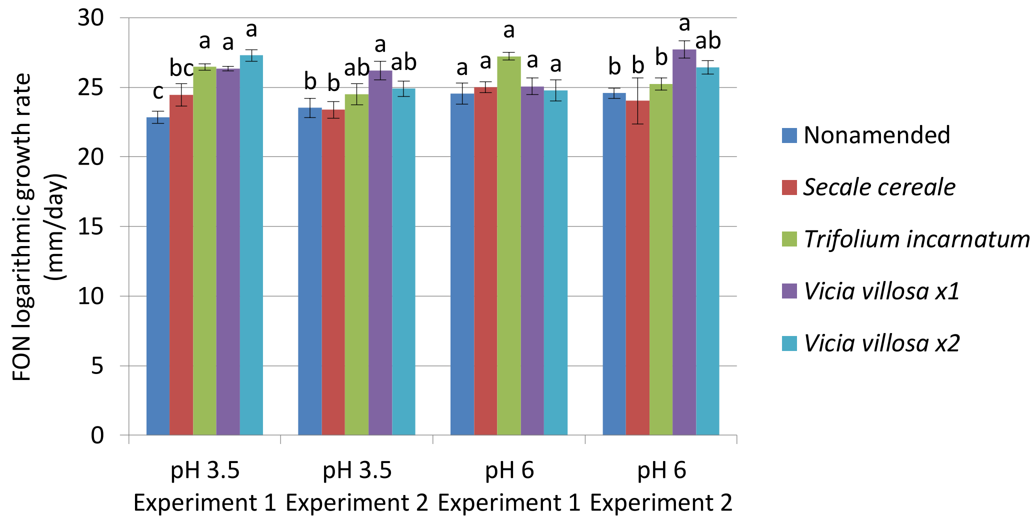

Figure 4.5 *Fusarium oxysporum* f. sp. *niveum* (FON) logarithmic growth rate on PDA plates amended with four different cover crop leachate treatments and nonamended PDA at a pH of 3.5 and 6 for two *in vitro* experiments

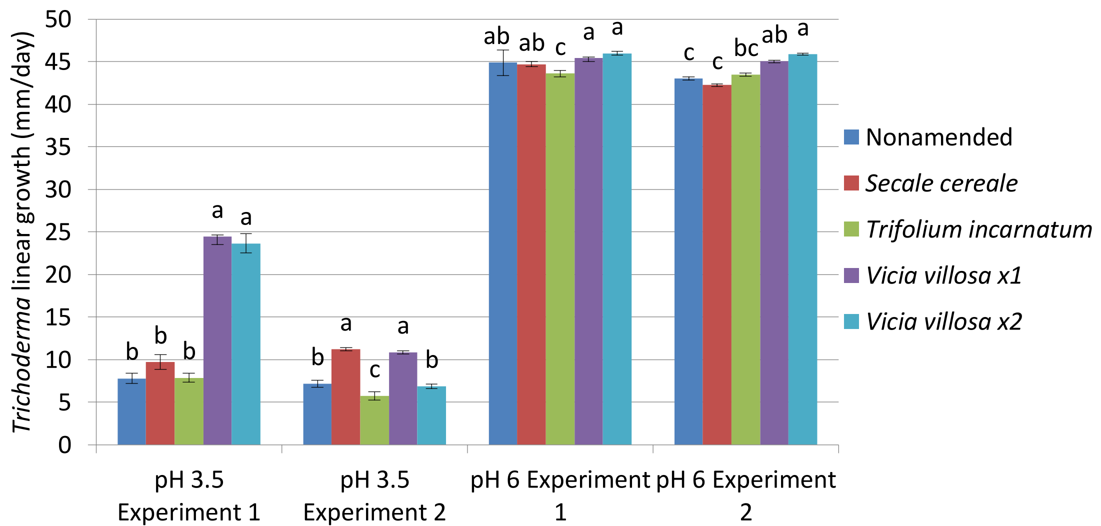

Figure 4.6 *Trichoderma harzianum* linear growth rate on PDA plates amended with four different cover crop leachate treatments and nonamended PDA at a pH of 3.5 and 6 for two *in vitro* experiments.

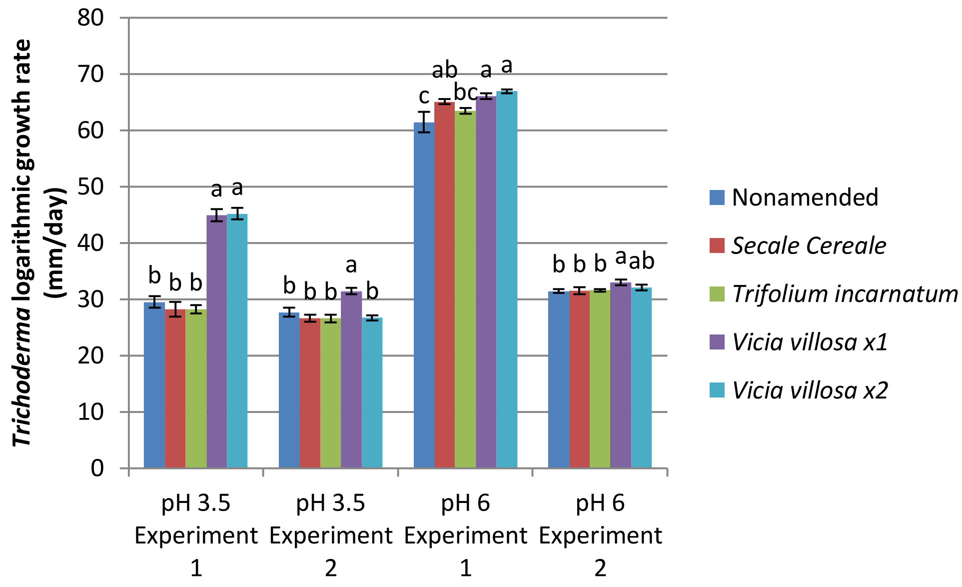

Figure 4.7 *Trichoderma harzianum* logarithmic growth rate on PDA plates amended with four different cover crop leachate treatments and nonamended PDA at a pH of 3.5 and 6 for two *in vitro* experiments

Appendices

Table A1.1 Differences in nutrient content of aboveground cover crop[a] foliar biomass sampled from the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) and the University of Deleware Research and Education Center (UD-REC) in 2011

UM-LESREC	Al (ppm)	Bo (ppm)	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	Mg (ppm)	Mn (ppm)	N (%)	P (%)	S (%)	Zn (ppm)
Vicia villosa	304.50 bc[b]	22.80 a	0.81 a	7.85 b	173.25 b	1.89 a	80.20 b	0.25 b	3.20 a	0.35 a	0.23 a	34.28 a
Trifolium incarnatum	544.00 bc	25.67 a	0.64 a	6.63 bc	254.50 b	2.13 a	96.60 ab	0.37 a	2.29 a	0.28 b	0.22 a	34.52 a
Secale cereale	213.75 c	9.05 a	0.29 a	4.83 c	129.60 b	2.13 a	60.30 b	0.13 c	1.56 a	0.27 b	0.13 a	27.87 a
Brassica juncea	1630.00 a	24.50 a	0.48 a	10.85 a	1131.33 a	2.15 a	142.63 a	0.38 a	3.20 a	0.26 b	0.17 a	21.47 a
Bare ground[c]	535.67 b	25.43 a	0.43 a	4.79 c	257.33 b	2.15 a	161.33 a	0.31 ab	2.44 a	0.31 ab	0.16 a	23.60 a
P>F	<0.0001	0.0532	0.2466	0.0030	0.0007	0.2041	0.0078	0.0020	0.1274	0.0265	0.1382	0.1678
CARVEL-REC												
Vicia villosa	745.67 a	18.25 a	1.07 a	7.62 a	288.33 a	2.82 a	33.57 a	0.30 a	2.59 a	0.33 ab	0.13 a	53.38 a
Trifolium incarnatum	798.17 a	16.62 a	0.94 a	6.90 a	309.83 a	2.64 ab	38.28 a	0.28 a	2.44 a	0.38 a	0.17 a	39.85 b
Secale cereale	796.33 a	15.10 a	0.86 a	6.33 a	300.22 a	2.60 ab	41.12 a	0.32 a	1.75 ab	0.35 a	0.16 a	25.55 c
Brassica juncea	339.17 a	12.22 a	0.77 a	5.67 a	163.70 a	2.00 b	22.37 a	0.22 a	1.40 b	0.30 a	0.11 a	53.77 a
Bare ground	488.00 ac	9.88 a	0.60 a	5.37 a	224.37 a	2.00 b	29.78 a	0.17 a	1.29 b	0.24 b	0.16 a	56.20 a
P>F	0.2677	0.0710	0.2466	0.1240	0.3146	0.0488	0.4781	0.1096	0.0118	0.0250	0.1653	<0.0001

[a]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, and *B. juncea* 11.21 kg/ha.
[b]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.
[c]All aboveground vegetation found within the randomly selected m^2 area of the bare ground plots was sampled for treatment analysis.

Table A1.2 Simple effects of cover crop and *Fusarium oxysporum* f. sp. *niveum* (FON) inoculation and Actinovate application on watermelon market yield (kg/ha)[a] at the United States Department of Agriculture Beltsville Agriculture Research Center in 2009

Cover Crop[b]	Treatment[c]	Market Yield
Vicia villosa	none	2,727 ab[e]
Vicia villosa	FON[d]	2,530 abc
Vicia villosa	FON + Actinovate[d]	2,709 ab
Trifolium incarnatum	Control	2,547 abc
Trifolium incarnatum	FON	2,619 ab
Trifolium incarnatum	FON + Actinovate	2,727 ab
Secale cereale	none	2,189 cd
Secale cereale	FON	2,350 bcd
Secale cereale	FON + Actinovate	2,834 a
Bare ground	none	2,655 ab
Bare ground	FON	2,099 d
Bare ground	FON + Actinovate	2,422 bcd
P<F		0.0109

[a] Marketable watermelon yield is qualified as the mean number of watermelon fruit per hectare that weighed more than 3.18 kg. The watermelon variety Sugar heart was used for all field trials.
[b]Seeding rate at USDA-BARC was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[c]In 2009 at USDA-BARC 6 ml of FON inoculum at a concentration of 2.45×10^6 CFU/ml was pippeted into a hole (approximately 3 cm in diameter and 8 cm deep) 7 cm away from the crown of each watermelon in the bed immediately after watermelon transplanting. Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at a rate of 0.011 g/12.2 ml H_2O per 0.156 m^2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H2O/1.89 m^2 per a plant.
[d]FON=*Fusarium oxysporum* f. sp. *niveum.*
[e]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table A1.3 Impact of bare ground and cover crop treatments on main effect means of watermelon sugars content (% Soluble Solids)[a] at the University of Maryland Lower Eastern Shore and Education Center (UM-LESREC), University of Delaware Research and Education Center (UD-REC), and United States of Department of Agriculture Beltsville Agriculture Research Center (USDA-BARC) in 2009, 2010 and 2011

Cover Crop[b]	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	USDA-BARC 2009	USDA-BARC 2010	UD-REC 2011
Vicia villosa	10.54 b[c]	10.83 a	10.43 a	12.77 a	10.81 a	9.96 a
Trifolium incarnatum	-[d]	-	10.69 a	12.87 a	10.55 a	10.64 a
Secale cereale	11.34 a	11.21 a	10.10 a	12.66 a	10.95 a	10.39 a
Brassica juncea	-	11.12 a	10.07 a	-	11.18 a	10.31 a
Bare ground	10.81 b	10.98 a	10.15 a	12.77 a	11.21 a	10.26 a
P<F	0.0064	0.7796	0.0921	0.9015 a	0.0845	0.3764*[e]

[a]Percent soluble solids was measured using a hand held refractometer.

[b]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.

[c]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

[d]The symbol – indicates the absence of the specific cover crop treatment for the given time and location.

[e]* signifies the presence of a significant interaction of cover crop and inoculation treatment factors so Table A1.4 must be referred to for the simple effects and main effect means must be discounted.

Table A1.4 Simple effects of cover crop and inoculation treatments on sugar content (% Soluble Solids)[a] in a field infested with *Fusarium oxysporum* f. sp. *niveum* (FON) at the University of Delaware Research and Education Center (UD-REC) in 2011

Cover Crop[b]	Treatment[c]	Fruit Sugar Content
Vicia villosa	-	9.98 bc[d]
Vicia villosa	Actinovate	9.68 c
Trifolium incarnatum	-	10.39 abc
Trifolium incarnatum	Actinovate	10.73 ab
Secale cereale	-	10.70 ab
Secale cereale	Actinovate	9.75 c
Brassica juncea	-	10.31 abc
Brassica juncea	Actinovate	10.92 a
Bare ground	-	10.60 abc
Bare ground	Actinovate	10.12 abc
P<F		0.0127

[a]Percent soluble solids was measured using a hand held refractometer.
[b]Seeding rate at UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, and *B. juncea* 11.21 kg/ha.
[c]Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at a rate of 0.011 g/12.2 ml H2O per 0.156 m2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H2O/1.89 m^2 per a plant.
[d]Means in a column followed by the same letter are not significantly different at $\alpha=0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table A1.5 Impact of Actinovate biocontrol treatment on watermelon vine length (cm) at the University of Maryland Lower Eastern Shore Research and Education Center in Salisbury 2011

Treatment[a]	6/13/2011	6/20/2011
Actinovate	117.70 a[b]	216.36 b
None	131.60 b	237.16 a
P>F	0.0002	0.0097

[a]Fields at UM-LESREC in 2011 were naturally infested with *Fusarium oxysporum* f. sp. *niveum* so there were no beds that did not contain the FON treatment. Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants two weeks after seeding at a rate of 0.011 g/12.2 ml H2O per 0.156 m^2 transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g/172 ml H2O/1.89 m^2 per a plant.

[b]Numbers in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test using the Statistical Analysis System MIXED procedure.

Table A1.6 Impact of bare ground and cover crop treatments on main effect means of the number of sunburned watermelon per hectare at the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC), the University of Delaware's Carvel Research and Education Center (UD-REC) and the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC) in 2009, 2010, and 2011.

Cover Crop[a]	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	USDA-BARC 2009	USDA-BARC 2010	UD-REC 2011
Vicia villosa	2870 a[b]	3839 b	2172 a	1134 a	188 a	260 a
Trifolium incarnatum	-[c]		2936 a	1134 a	148 a	260 a
Secale cereale	2713 a	3092 b	1957 a	775 a	224 a	76 a
Brassica juncea	-	3636 b	2499 a	-	260 a	148 a
Bare ground	3746 a	5430 a	2720 a	696 a	372 a	336 a
$P<F$	0.0939	0.0191	0.5147	0.4554	0.7197	0.5285

[a]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54-134.50 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[b]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.
[c]The symbol – indicates the absence of the specific cover crop treatment for the given time and location.

Table A1.7 Main effects of green manures on foliar nutrient content of watermelon[a] cultivar 'Sugar Heart' at the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) and the University of Delaware Research and Education Center (UD-REC) in 2011

Location, Cover crop[b]	Al (ppm)	Bo (ppm)	Ca (%)	Cu (ppm)	Fe (ppm)	K (%)	Mg (ppm)	Mn (ppm)	N (%)	P(%)	S (%)	Zn (ppm)
UM-LESREC												
Vicia villosa	45.70 a[c]	230.25 c	6.61 a	8.02 a	220.72 a	1.27 a	1.49 ab	865.42 bc	2.84 a	0.18 a	0.21 a	45.70 bc
Trifolium incarnatum	32.88 a	247.75 c	5.91 b	5.92 a	237.97 a	1.13 a	1.65 a	544.50 c	2.67 a	0.18 a	0.20 a	32.88 c
Brassica juncea	64.58 a	340.50 a	5.83 b	6.53 a	228.47 a	1.38 a	1.33 b	1298.83 a	2.82 a	0.19 a	0.22 a	64.58 a
Secale cereale	56.41 a	295.83 b	5.62 b	7.03 a	255.14 a	1.47 a	1.28 c	945.25 ab	2.73 a	0.19 a	0.22 a	56.41 ab
Bare ground	66.59 a	332.00 ab	6.00 b	6.61 a	286.97 a	1.38 a	1.36 b	1289.17 a	2.38 a	0.16 a	0.21 a	66.59 a
P>F	0.2968	<0.0001	0.0069	0.0744	0.2651	0.2218	0.0020	0.0003	0.0857	0.1978	0.5006	0.0046
UD-REC												
Vicia villosa	902.47 a	36.83 a	2.38 a	11.79 a	324.34 a	2.87 a	52.45 a	0.51 a	4.03 a	0.36 a	0.29 a	30.94 a
Trifolium incarnatum	1156.43 a	34.79 a	2.65 a	11.47 a	380.66 a	2.60 a	56.19 a	0.54 a	3.95 a	0.33 a	0.27 b	29.72 a
Secale cereale	881.87 a	48.91 a	2.39 a	11.67 a	329.10 a	2.70 a	57.40 a	0.48 a	4.10 a	0.34 a	0.28 ab	30.94 a
Brassica juncea	1313.22 a	49.85 a	2.54 a	11.92 a	417.25 a	2.62 a	61.34 a	0.53 a	4.27 a	0.34 a	0.28 ab	32.56 a
Bare ground	1025.50 a	43.97 a	2.34 a	12.02 a	361.20 a	2.74 a	77.08 a	0.54 a	4.06 a	0.35 a	0.30 a	33.50 a
P>F	0.6991	0.1274	0.994	0.6	0.8879	0.605	0.408	0.8843	0.193	0.8609	0.0423	0.2991

[a]Directly before harvest five watermelon leaves that were the second or third leaf from vines' terminal point were randomly sampled from every bed, dried, and sent for nutrient analysis to Brookside Laboratories Inc.

[b]Seeding rate at UM-LESREC and UD-REC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, and *B. juncea* 11.21 kg/ha.

[c]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.

Table A3.1 Effects of cover crop green manure on soil temperature (C°)[a] at the United States Department of Agriculture Beltsville Education and Research Center (USDA-BARC) in 2009 and 2010 and the Lower Eastern Shore Research and Education Center (UM-LESREC) in 2010

Location, Year						
USDA-BARC, 2009						
	5/18[c]	5/28	6/9	6/11	7/10	8/3
Vicia villosa[b]	17.72 a[d]	21.36 a	23.69 b	16.62 a	17.15 a	24.57 a
Trifolium incarnatum	17.66 a	21.17 a	23.88 b	15.51 b	15.54 b	23.35 a
Secale cereale	17.93 a	21.49 a	24.43 a	16.64 a	-[e]	24.85 a
Bare ground	17.80 a	21.75 a	23.98 b	17.29 a	17.79 a	24.83 a
P>F	0.5086	0.5869	0.0012	0.0167	<0.0001	0.6500
USDA-BARC, 2010						
	4/30	**5/13**	**5/24**	5/22		
Trifolium incarnatum	15.02 a	16.12 ab	21.35 a	22.88 a		
Vicia villosa	14.32 a	15.34 b	19.73 a	22.32 a		
Brassica juncea	16.10 a	16.94 a	21.13 a	22.07 a		
Secale cereale	16.20 a	17.17 a	20.60 a	21.95 a		
Bare ground	15.51 a	16.92 a	21.54 a	22.07 a		
P>F	0.875	0.0147	0.1341	0.8066		
UM-LESREC, 2010						
	5/25					
Trifolium incarnation	26.77 ab					
Vicia villosa	27.10 ab					
Secale cereale	27.72 a					
Brassica juncea	26.51 b					
Bare ground	26.00 b					
P>F	0.0408					

[a]Soil temperature was taken along with soil respiration measurements using a EGM-4 gas analyzer with a SRC-1 chamber from PP Systems (110 Haverhill Road, Suite 301 Amesbury, MA 01913, USA).
[b]Seeding rate at UM-LESREC was *V. villosa* 50.44 kg/ha, *T. incarnatum* 11.21 kg/ha, *S. cereale* 125.54 kg/ha, *B. juncea* 11.21 kg/ha and at USDA-BARC the seeding rate was *V. villosa* 44.83 kg/ha, *T. incarnatum* 28.02 kg/ha, *S. cereale* 134.50 kg/ha, and *B. juncea* 6.73 kg/ha.
[c]All dates in bold indicate readings taken before cover crop tillage and all dates not bolded were taken following cover crop tillage.
[d]Means in a column followed by the same letter are not significantly different at α=0.05 according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure.
[e]Data not taken.

Bibliography

1. Abawi, G. S., and Widmer, T. L. 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology 15(1):37-47.

2. Abdel-Fattah, G. M., and Mohamedin, A. H. 2000. Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biology and Fertility of Soils 32(5):401-409.

3. Alabouvette, C. 1999. Fusarium wilt suppressive soils: an example of disease-suppressive soils. Australasian Plant Pathology 28(1):57-64.

4. Alabouvette, C., Louvet, J., and Couteaudier, Y. 1985. Disease suppressiveness of soils. XII. Respiratory activity after addition of glucose to a wilt-suppressive and a wilt-conducive soil. Agronomie 5(1):69-72.

5. Alabouvette, C., Steinberg, C., and Lemanceau, P. 1993. Recent advances in the biological control of Fusarium wilts. Pesticide Science 37(4):365-373.

6. Al-Reza, S. M., Rahman, A., Ahmed, Y., and Kang, S. C. 2010. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L.. Pesticide Biochemistry and Physiology 96(2):86-92.

7. Amir, H., and Alabouvette, C. 1993. Involvement of soil abiotic factors in the mechanisms of soil suppressiveness to Fusarium wilts. Soil Biology and Biochemistry 25(2):157-164.

8. Andrade, G., Mihara, K. L., Linderman, R. G., and Bethlenfalvay, G. J. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and Soil 192:71-79.

9. Angus, J. F., Gardner, P. A., Kirkegaard, J. A., and Desmarchelier, J. M. 1994. Biofumigation: Isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant and Soil 162(1):107-112.

10. Antherton Lee, H., and Martin, J. P. 1928. Effect of fertilizer constituents on the eye-spot disease of sugar cane. Industrial and Engineering Chemistry 20(2):220-224.

11. Augé, R. M. 2001.Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11(1):3-42.

12. Azcón-Aguilar, C., and Barea, J. M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. Mycorrhiza 6(6):457-464.

13. Bailey, B. A., Bae, H., Strem, M. D., Crozier, J., Thomas, S. E., Samuels, G. J., Vinyard, B. T., and Holmes, K. A. 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Biological Control 46:24-35.

14. Baker, K. F., and Cook, R. J. 1974. Biological control of plant pathogens. W. H. Freeman and Company, San Francisco, CA. 433 pp. (Book reprinted in 1982, Am. Phytopathol. Soc., St. Paul, MN).

15. Biles, C. L., and Martyn, R. D. 1989. Local and systemic resistance induced in watermelons by formae speciales of *Fusarium oxysporum*. Phytopathology 79(8):856-860.

16. Bolan, N. S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134(2):189-207.

17. Bonanomi, G., Antignani, V., Capodilupo, M., and Scala, F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology & Biochemistry 42:136-144.

18. Bonanomi, G., Antignani, V., Pane, C., and Scala, F. 2007a. Invited review: Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology 89(3):311-324.

19. Bonanomi, G., Del Sorbo, G., Mazzoleni, S., and Scala, F. 2007b. Autotoxicity of decaying tomato residues affects susceptibility of tomato to *Fusarium* wilt. Journal of Plant Pathology 89(2):219-226.

20. Bowers, J. H., and Locke, J. C. 2000. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusarium wilt in the greenhouse. Plant Disease 84(3):300-305.

21. Bradow, J. M., and Connick, Jr., W. J. 1990. Volatile seed germination inhibitors from plant residues. Journal of Chemical Ecology 16(3):645-666.

22. Buśko, M., Chellkowsi, J., Popiel, D., and Perkowski, J. 2008. Solid substrate bioassay to evaluate impact of Trichoderma on trichothecene mycotoxin production by Fusarium species. Journal of the Science of Food and Agriculture 88:536-541.

23. Buyer, J. S., Roberts, D. P., Millner, P., and Russek-Cohen, E. 2001. Analysis of fungal communities by sole carbon source utilization profiles. Journal of Microbiological Methods 45:53-60.

24. Buyer, J. S., Teasdale, J. R., Roberts, D. P., Zasada, I. A., and Maul, J. E. 2010. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biology & Biochemistry 42:831-841.

25. Campiglia, E., Caporali, F., Radicetti, E., and Mancinelli, R. 2010a. Hairy vetch (*Vicia villosa* Roth.) cover crop residue management for improving weed control and yield in no-tillage tomato (*Lycopersicon esculentum* Mill.) production. European Journal of Agronomy 33(2):94-102.

26. Campiglia, E., Mancinelli, R., Radicetti, E., and Caporali, F. 2010b. Effect of cover crops and mulches on weed control and nitrogen fertilization in tomato (*Lycopersicon esculentum* Mill.). Crop Protection 29(4):354-363.

27. Candole, B. L., and Rothrock, C. S. 1998. Using marked strains to assess the effect of hairy vetch amendment on the inoculum densities of *Thielaviopsis basicola*, *Pythium ultimum* and *Rhizoctonia solani*. Soil Biology and Biochemistry 30(4):443-448.

28. Carlile, M. J. 1956. A study of the factors influencing non-genetic variation in a strain of *Fusarium oxysporum.* J. gen. Microbiol. 14:643-654.

29. Carrera, L. M., Buyer, J. S., Vinyard, B., Abdul-Baki, A. A., Sikora, L. J., and Teasdale, J. R. 2007. Effects of cover crops, compost, and manure amendments

on soil microbial community structure in tomato production systems. Applied Soil Ecology 37:247-255.

30. Cavigelli, M. A., and Thien, S. J. 2003. Phosphorus bioavailability following incorporation of green manure crops. Soil Science Society of America Journal 67:1186-1194.

31. Chou, C.-H., and Muller, C. H. 1972. Allelopathic mechanisms of *Arctostaphylos glandulosa* var. *zacaensis*. American Midland Naturalist 88(2):324-347.

32. Clark, Andy (ed.). 2001. Managing cover crops profitably. 3rd ed. Handbook Series Book 9. Sustainable Agriculture Network, Beltsville, MD.

33. Cohen, M. F., Yamasaki, H., and Mazzola, M. 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. Soil Biology and Biochemistry 37(7):1215-1227.

34. Conway, W. C., Smith, L. M., and Bergan, J. F. 2002. Potential allelopathic interference by the exotic Chinese tallow tree (*Sapium sebiferum*) Am. Midl. Nat. 148:43-53.

35. Corbett, C. J., and Pan, J. N. 2002. Evaluating environmental performance using statistical process control techniques. Euuropean Journal of Operational Research 139:68-83.

36. Cordier, C., Pozo, M. J., Barea, J. M., Gianinazzi, S., and Gianinazzi-Pearson, V. 1998. Cell defense responses associated with localized and systemic resistance to *Phytophthora* parasitic induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Interactions 11(10):1017-1028.

37. Cote, J. F., and Thibault, J. R. 1988. Allelopathic potential of raspberry foliar leachates on growth of ectomycorrhizal fungi associated with black spruce. American Journal of Botany 75(7):966-970.

38. Coumans, J. V. F., Moens, P. D. J., Poljak, A., Al-Jaaidi, S., Pereg, L., and Raftery, M. J. 2010. Plant-extract-induced changes in the proteome of the soil-borne pathogenic fungus *Thielaviopsis basicola*. Proteomics 10(8):1573-91.

39. Crawford, D. L., Lynch, J. M., Whipps, J. M., and Ousley, M. A. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology 59(11):3899-3905.

40. Cummings, J. A., du Toit, L. J., and Miles, C. A. 2008. Evaluation of seed and drench treatments for organic management of soilborne diseases of spinach in Sequim, WA, 2007. Plant Disease Management Reports 2:V133. Online publication. doi:10.1094/PDMR02.

41. Damicone, J. P., and Pierce, L. R. 2006. Evaluation of fungicides for control of watermelon anthracnose, 2006. 1:V036. Plant Disease Management Reports Online publication. doi:10.1094/PDMR01.

42. Datnoff, L. E., Nemec, S., and Pernezny, K. 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biological Control 5(3):427-431.

43. Davis, A. R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J. V., Lee, S. G., Huh, Y. C., Sun, Z., Miguel, A., King, S. R., Cohen, R., and Lee, J. M. 2008. Cucurbit grafting. Critical Reviews in Plant Sciences 27(1):50-74.

44. de Capdeville, G., Wilson, C. L., Beer, S. V., and Aist, J. R. 2002. Alternative disease control agents induce resistance to blue mold in harvested ‘red delicious’ apple fruit. Phytopathology 92(8):900-908.

45. De Marco, J. L., Lima, L. H. C., Valle de Sousa, M., and Felix, C. R. 2000. A *Trichoderma harzianum* chitinase destroys the cell wall of the phytopathogen *Crinipellis perniciosa*, the causal agent of witches’ broom disease of cocoa. World Journal of Microbiology & Biotechnology 16:383-386.

46. De Cal, A., Sztejnberg, A., Sabuquillo, P., and Melgarejo, P. 2009. Management Fusarium wilt on melon and watermelon by *Penicillium oxalicum*. Biological Control 51(3):480-486.

47. De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93(4):428-35.

48. Dhananjeyan, V., Selvan, N., and Dhanapal, K. 2010. Isolation, characterization, screening and antibiotic sensitivity of *Actinomycetes* from locally (near MCAS) collected soil samples. Journal of Biological Sciences 10(6):514-519.

49. Diab, H. G., Hu, S., and Benson, D. M. 2003. Suppression of *Rhizoctonia solani* on impatiens by enhanced microbial activity in composted swine waste-amended potting mixes. Phytopathology 93(9):1115-1123.

50. Doumbou, C. L., Hamby Salove, M. K., Crawford, D. L., and Beaulieu, C. 2010. Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection 82(3):85-102.

51. Drinkwater, L. E., Letourneau, D. K., Workneh, F., van Bruggen, A. H. C., and Shennan, C. 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecological Applications 5(4):1098-1112.

52. Dubey, S. C., Suresh, M., and Singh, B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. Biological Control 40(1):118-127.

53. Duffy, B. K., and Défago, G. 1997. Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Psuedomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology 87(12):1250-1257.

54. Egel, D. S., and Martyn, R. D. 2007. Fusarium wilt of watermelon and other cucurbits. Online. The Plant Health Instructor. doi:10.1094/PHI-I-2007-0122-01. Available at: http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/FusariumWatermelon.aspx

55. Egerton-Warburton L. M., and Allen, E. B. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10(2):484- 496.

56. El-Atrach, F., Vierheilig, H., and Ocampo, J. A. 1989. Influence of non-host plants on vesicular-arbuscular mycorrhizal infection of host plants and on spore germination. Soil Biology and Biochemistry 21(1):161-163.

57. El-Khallal, S. M. 2007. Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (Jasmonic acid & Salicylic acid): 1- Changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. Australian Journal of Basic and Applied Sciences 1(4):691-705.

58. El-Tarabily, K. A. 2006. Rhizosphere-competent isolates of streptomycete and non-streptomycete actinomycetes capable of producing cell-wall-degrading enzymes to control *Pythium aphanidermatum* damping-off disease of cucumber. Canadian Journal of Botany 84(2):211-222.

59. Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70(2):119-121.

60. Elmer, W. H., and Elliott, G. 2000. The influence of microbial inoculants on Fusarium corm rot of gladiolus, 2000. Biol. Cult. Tests Control Plant Dis. Rep. 16:O42. www.apstnet.org/online/BCTests/Vol16/sections/ornamentals_trees.htm.

61. 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(10):909-914.

62. Entry, J. A., Strasbaugh, C. A., and Sojka, R. E. 2000. Wood chip-polyacrylamide medium for biocontrol bacteria decreases *Verticillium dahliae* infection on potato. Biocontrol Science and Technology 10(5):677-686.

63. Eshel, D., Gamliel, A., Grinstein, A., Di Primo, P., and Katan, J. 2000. Combined soil treatments and sequence of application in improving the control of soilborne pathogens. Phytopathology 90(7):751-757.

64. Everts, K. L., and Hochmuth, M., 2011. Field evaluation of triploid cultivars for resistance to Fusarium wilt of watermelon in Delaware, 2010. Report 5:V175. doi: 10.1094/PDMR05.

65. Everts, K. L. and Zhou, X. G. 2010. Field evaluation of triploid cultivars for resistance to Fusarium wilt of watermelon in Delaware, 2009. Report 4:V066. doi: 10.1094/PDMR04.

66. Ferguson, W., and Yee, J. 1997. Phasing out registered pesticide uses as an alternative to total bans: A case study of methyl bromide. Journal of Agribusiness 15(1):69-84.

67. Förster, H., Adaskaveg, J. E., Kim, D. H., and Stanghellini, M. E. 1998. Effect of phosphite on tomato and pepper plants and on susceptibility of pepper to

Phytophthora root and crown rot in hydroponic culture. Plant Disease 82(10):1165-1170.

68. Freeman, S., Zveibil, A., Vintal, H., and Maymon, M. 2002. Isolation of nonpathogenic mutants of *Fusarium oxysporum* f. sp. *melonis* for biological control of Fusarium wilt in cucurbits. Phytopathology 92(2):164-168.

69. Fujii, Y. 2001. Screening and future exploitation of allelopathic plants as alternative herbicides with special reference to hairy vetch. Journal of Crop Production 4(2):257-275.

70. Galvez, L., Douds, D. D., Drinkwater, L. E., and Wagoner, P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant and Soil 228:299-308.

71. Galvez, L., Douds, D. D., Wagoner, P., Longnecker, L. R., Drinkwater, L. E., and Janke, R. R. 1995. An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. American Journal of Alternative Agriculture 10(4):152-156.

72. Gareau, B. J. 2010. A critical review of the successful CFC phase-out versus the delayed methyl bromide phase-out in the Montreal Protocol. International Environmental Agreements: Politics, Law and Economics 10(3):209-231.

73. Getha, K., Vikineswary, S., Wong, W. H., Seki, T., Ward, A., Goodfellow, M. 2005. Evaluation of *Streptomyces spp*. strain g10 for suppression of Fusarium wilt and rhizosphere colonization in pot-grown banana plantlets. Journal of Industrial Microbiology & Biotechnology 32(1):24-32.

74. Ghorbani, R., Wilcockson, S., Koocheki, A., and Leifert, C. 2008. Soil management for sustainable crop disease control: a review. Environmental Chemistry Letters 6(3):149-162.

75. Gonzalez-Franco, A. C., and Hernandez, L. R. 2009. Actinomycetes as biological control agents of phytopathogenic fungi. Tecnociencia Chihauhau 3(2):64-73.

76. Graham, J. H., and Menge, J. A. 1982. Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat. Phytopathology 72(1):95-98.

77. Gullino, M. L., Camponogara, A., Gasparrini, G., Rizzo, V., Clini, C., and Garibaldi, A. 2003. Replacing methyl bromide for soil disinfestation: The Italian experience and implications for other countries. Plant Disease 87(9):1012-1021.

78. Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69(1):64-68.

79. Haramoto, E. R., and Gallandt, E. R. 2004. *Brassica* cover cropping for weed management: A review. Renewable Agriculture and Food Systems 19(4):187-198.

80. Haran, S., Schickler, H., and Chet, I. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology 142:2321-2331.

81. Hartwig, N. L., and Ammom, H. U. 2002. Cover crops and living mulches. Weed Science 50:688-699.

82. Henn, A., and Burdine, A., 2010. Management of *Fusarium* surface rot of sweetpotatoes after packing in Mississippi, 2009. Plant Disease Management Reports 4:V109 Online publication. doi:10.1094/PDMR04.

83. Hiltunen, L .H., Ojanperä, T., Kortemaa, H., Richter, E. Lehtonen, M. J., and Valkonen, J. P. T. 2009. Interactions and biocontrol of pathogenic *Streptomyces* strains co-occurring in potato scab lesions. Journal of Applied Microbiology 106 (1):199-212.

84. Höper, H., Steinberg, C., and Alabouvette, C. 1995. Involvement of clay type and pH in the mechanisms of soil suppressiveness to *Fusarium* wilt of flax. Soil Biology and Biochemistry 27(7):955-967.

85. Howell, C. R. 2002. Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and it biological control with *Trichoderma* spp. Phytopathology 92(2):177-180.

86. Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease 87(1):4-10.

87. Howell, C. R. 2005. The nature and application of biocontrol microbes II : *Trichoderma* spp. understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology (14):178-180.

88. Howell, C. R., Hanson, L. E., Stipanovic, R. D., and Puckhaber, L. S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology 90(3):248-252.

89. Hussain, F., Saeed, I. I., Malik, S. A., Dasti, A. A., and Ahmad, B. 2011. Allelopathic effects of rain leachates and root exudates of *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) A. Camus. Pak. J. Bot. 43(1):341-350.

90. Hwang, S. F. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of *Verticillium* and Fusarium wilts of alfalfa. Plant Disease 76:239-243.

91. Isik, D., Kaya, E., Ngouajio, M., and Mennan, H. 2009. Weed suppression in organic pepper (*Capsicum annuum* L.) with winter cover crops. Crop Protection 28(4):356-363.

92. Jacobsen, K. L., Gallagher, R. S., Burnham, M., Bradley, B. B., Larson, Z. M., Walker, C. W., and Watson, J. E. 2010. Mitigation of seed germination impediments in hairy vetch. Agronomy Journal. 102:1346-1351 doi: 10.2134/agronj2010.0002n.

93. Kabir, Z., and Koide, R. T. 2000. The effect of dandelion or a cover crop on mycorrhizal inoculum potential, soil aggregation and yield of maize. Agriculture, Ecosystems & Environment 78(2):167-174.

94. Kawaide, T. 1985. Utilization of rootstocks in cucurbits production in Japan. Japanese Agricultural Research Quarterly 18(4):284-289.

95. Kaya, C., Higgs, D., Kirnak, H., and Tas, I. 2003. Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. Plant and Soil 253:287-292.

96. Keinath, A. P., Hassell, R. L., Everts, K. L., and Zhou, X.-G. 2010. Cover crops of hybrid common vetch reduce Fusarium wilt of seedless watermelon in the

eastern United States. Online. Plant Health Progress doi:110.1094/PHP-2010-0914-01-RS.

97. King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for disease resistance. HortScience 43(6):1673-1676.

98. Kirkegaard, J. A., and Sarwar, M. 1998. Biofumigation potential of brassicas I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant and Soil 201:71-89.

99. Koger, C. H. and Reddy, K. N. 2005. Effects of hairy vetch (*Vicia villosa*) cover crop and banded herbicides on weeds, grain yield, and economic returns in corn (*Zea mays*). Journal of Sustainable Agriculture 26(3):107-124.

100. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:115-125.

101. Kort, J., Collins, M., and Ditsch, D. 1998. A review of soil erosion potential associated with biomass crops. Biomass and Bioenergy 14(4):351-359.

102. Krause, M. S., Madden, L. V., and Hoitink, H. A. J. 2001. Effect of potting mix microbial carrying capacity on biological control of *Rhizoctonia* damping-off of radish and *Rhizoctonia* crown and root rot of poinsettia. Phytopathology 91(11):1116-1123.

103. Kucuk, C., and Kivanc, M. 2003. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turkish Journal of Biology 27:247-253.

104. Lamanceau, P., Bakker, P. A. H. M., Jan de Kogel, W., Alabouvette, C., and Schippers, B. 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and *Psuedobactin* 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. Applied and Environmental Microbiology 59(1):74-82.

105. Larkin, R. P., and Fravel, D. B. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Disease 82(9):1022-1028.

106. Larkin, R. P., and Fravel, D. R. 2002. Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92(11):1160-1166.

107. Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1993a. Ecology of *Fusarium oxysporum* f. sp. *niveum* in soils suppressive and conducive to Fusarium wilt of watermelon. Ecology and Epidemiology 83(10):1105-1116.

108. Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1993b. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Ecology and Epidemiology 83(10):1097-1105.

109. Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. Phytopathology 86(8):812-819.

110. Larkin, R. P., and Griffin, T. S. 2007. Control of soilborne potato diseases using *Brassica* green manures. Crop Protection 26(7):1067-1077.

111. Leslie, J. F., and Summerell, B. A. 2006. The Fusarium Laboratory Manual. Blackwell Professional, Ames, Iowa.

112. Lewis, J. A., and Papavizas, G. C. 1977. Effect of plant residues on chlamydospore germination of *Fusarium solani* f. sp. *phaseoli* and on *Fusarium* root rot of beans. Phytopathology 67:925-929.

113. Lewis, J. A., and Papavizas, G. C. 1984. A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. Phytopathology 74(10):1240-1244.

114. Linde, J. H., Combrinck, S., Regnier, T. J. C., and Virijevic, S. 2010. Chemical composition and antifungal activity of the essential oils of *Lippia rehmannii* from South Africa. South African Journal of Botany 76:37-42.

115. Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology 78(3):366-371.

116. Linderman, R. G., and Gilbert, R. G. 1973. Influence of volatile compounds from alfalfa hay on microbial activity in soil in relation to growth of *Sclerotium rolfsii*. Phytopathology 63:359-362.

117. Ling, G., Huang, Q., Guo, S., and Shen, Q. 2011. *Paenibacillus polymyxa* SQR-21 systematically affects root exudates of watermelon to decrease the conidial germination of *Fusarium oxysporum* f. sp. *niveum*. Plant and Soil 341:1485-493.

118. Ling, N., Xue, C., Huang, Q., Yang, X., Xu, Y., and Shen, Q. 2010. Development of a mode of application of bioorganic fertilizer for improving the biocontrol efficacy to Fusarium wilt. BioControl 55(5):673-683.

119. Littell, R. C., Henry, P. R., and Ammerman, C. B. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science 76:1216-1231.

120. Lucier, G., and Biing-Hwan, L. 2001. Factors affecting watermelon consumption in the United States. Vegetables and Specialties Situation and Outlook 287:23-29.

121. Mandeel, Q., and Baker, R. 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81(4):462-469.

122. Manici, L. M., Lazzeri, L., and Palmieri, S. 1997. *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. J. Agric. Food Chem. 45(7):2768-2773.

123. Martínez-Medina, A., Pascual, J. A., Lloret, E., Roldán, A. 2009. Interactions between arbuscular mycorrhizal fungi and *Trichoderma harzianum* and their effects on *Fusarium* wilt in melon plants grown in seedling nurseries. Journal of the Science of Food and Agriculture 89(11):1843-1850.

124. Matsubara, Y., Ohba, N., and Fukui, H. 2001. Effect of arbuscular mycorrhizal fungus infection on the incidence of Fusarium root rot in asparagus seedlings. Japanese Society of Horticultural Science 70(2):202-206.

125. Matsubara, Y., Tamura, H., and Harada, T. 1995. Growth enhancement and *Verticillium* wilt control by vesicular-arbuscular mycorrhizal fungus inoculation in eggplant. Journal of the Japanese Society for Horticulture Science 64(3):555-561.

126. Mazzola, M., Granatstein, D. M., Elfving, D. C., and Mullinix, M. K. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. Phytopathology 91(7):673-679.

127. McCann, I., Kee, E., Adkins, J., Ernest, E., and Ernest, J. 2007. Effect of irrigation rate on yield of drip-irrigated seedless watermelon in a humid region. Scientia Horticulturae 113(2):155-161.

128. McGonigle T. P. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 33(3):261-501.

129. McGonigle, T. P., Yano, K., and Shinhama, T. 2003. Mycorrhizal phosphorus enhancement of plants in undisturbed soil differs from phosphorus uptake stimulation by arbuscular mycorrhizae over non-mycorrhizal controls. Biol Fertil Soils 37:268-273.

130. Mersha, Z., Zhang, S., and Mo, X. 2010. Evaluation of application timing of biological and biofungicides for control of downy mildew on basil, 2010. Plant Disease Management Reports. 06:V060. Online publication. doi: 10.11094/PDMR04.

131. Morton, J., 2003.Staining of mycorrhizal roots, International Culture Collection of (Vesicular) Arbuscular Mycorrhizae. Retrieved November 5, 2010 from: http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm.

132. Mucharromah, E., and Kuc, J. 1991. Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Protection 10(4):265-270.

133. Mwaja, V. N., Masiunas, J. B., and Weston, L. A. 1995. Effects of fertility on biomass, phytotoxicity, and allelochemical content of cereal rye. Journal of Chemical Ecology 21(1):81-96.

134. Netzer, D. 1976. Physiological races and soil population level of Fusarium wilt of watermelon. Phytoparasitica 4(2):131-136.

135. Newsham, K. K., Fitter, A. H., and Watkinson, A. R. 1995.Arbusuclar mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* 83:991-1000.

136. Nga, N. T. T., Giau, N. T., Long, N. T., Lübeck, M., Shetty, N. P., De Neergaard, E., Thuy, T. T. T., Kim, P. V., and Jørgensen, H. J. L. 2010. Rhizobacterially induced protection of watermelon against *Didymella bryoniae*. Journal of Applied Microbiology 109(2):567-582.

137. Njoroge, S. M. C., Riley, M. B., and Keinath, A. P. 2008. Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on damping-off and *Fusarium* wilt of watermelon. Plant Disease 92(2):287-294.

138. Ochiai, N., Powelson, M. L., Crowe, F. J., and Dick, R. P. 2008. Green manure effects on soil quality in relation to suppression of *Verticillium* wilt of potatoes. Biol Fert Soils 44:1013-1023.

139. Orr., S. P., Rudgers, J. A., and Clay, K. 2005. Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecology 181:153-165.

140. Osorio, E., Flores, M., Hernández, D., Ventura, J., Rodríguez, R., and Aguilar, C. N. 2010. Biological efficiency of polyphenolic extracts from pecan nuts shell (*Carya illinoensis*), pomegranate husk (*Punica granatum*) and creosote bush leaves (*Larrea tridentata* Cov.) against plant pathogenic fungi. Industrial Crops and Products 31(1):153-157.

141. Overland, L. 1966. The role of allelopathic substances in the “smother crop” barley. American Journal of Botany 53(5):423-432.

142. Payton, M. E., Richeter, S. J., Giles, K. L., Royer, T. A. 2006. Transformations of count data for tests of interaction in factorial and split-plot experiments. BioOne 99(3):1002-1006.

143. Pozo, M. J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J. M., and Azcon-Aguilar, C. 2002. Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. Journal of Experimental Botany 53(368):525-534.

144. Pratt, R. G. 1982. A new vascular wilt disease caused in crimson clover by *Fusarium oxysporum.* Phytopathology 72:622-627.

145. Rangappa, M., Hamama, A. A., Bhardwai, H. L. 2002. Legume and grass cover crops for seedless watermelon production. HortTechnology 12(2):245-249.

146. Reid, L. M., Zhu, X., and Ma, B. L. 2001. Crop rotation and nitrogen effects on maize susceptibility to Gibberella (*Fusarium graminearum)* ear rot. Plant and Soil 237:1-14.

147. Ren, L., Su, S., Yang X., Xu, Y., Huang, Q., and Shen, Q. 2008. Intercropping with aerobic rice suppressed *Fusarium* wilt in watermelon. Soil Biology and Biochemistry 40(3):834-844.

148. Ristaino, J. B. 1997. Suppression of *Phytophthora* blight in bell pepper by a no-till wheat cover crop. Disease Control and Pest Management 87(3):242-249.

149. Roberts, K. J., and Anderson, R. C. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. The American Midland Naturalist 146(1):146.

150. Rojo, F. G., Reynoso, M. M., Ferez, M., Chulze, S. N., and Torres, A. M. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop protection 26:549-555.

151. Rosecrance, R. C., McCarty, G. W., Shelton, D. R., and Teasdale, J. R. 2000. Denitrification and N mineralization from hairy vetch (*Vicia villosa* Roth) and rye (*Secale cereale* L .) cover crop monocultures and bicultures. Plant and Soil 227:283-290.

152. Rothrock, C. S., Kirkpatrick, T. L., Frans, R. E., and Scott, H. D. 1995. The influence of winter legume cover crops on soilborne plant pathogens and cotton seedling diseases. Plant Disease 79(2):167-171.

153. Rovira, A. D., and Harris, J. R. 1961. Plant root excretions in relation to the rhizosphere effect V. The exudation of B-group vitamins. Plant and Soil 14(3):199-214.

154. Rutto, K. L., Mizutani, F., Moon, D. G., Cho, Y. S., and Kadoya, K. 2003. Seasonal fluctuations in mycorrhizal spore populations and infection rates of vineyard soils planted with five legume cover crops. Journal for Japanese Society for Horticultural Science 72(4):262-267.

155. Samuels, G. J. 2006. *Trichoderma*: systematics, the sexual state, and ecology. Phytopathology 96(2):195-206.

156. Sarwar, M., Kirkegaard, J. A., Wong, P. T. W., and Desmarchelier, J. M. 1998. Biofumigation potential of brassicas. III. *In-vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil 201:103-112.

157. Schaafsma, A. W., Hooker, D. C. 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. International Journal of Food Microbiology 119(1-2):116-25.

158. Scheffknecht, S., Mammerler, R., Steinkellner, S., and Vierheilg, H. 2006. Root exudates of mycorrhizal tomato plants exhibit a different effect on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants. Mycorrhiza 16(5):365-370.

159. Schreiner, R. P., and Koide, R. T. 1993. Mustards, mustard oils, and mycorrhizas. New Phytologist 123 (1):107-113.

160. Sharma, S. R., and Kolte, S. J. 1994. Effect of soil-applied NPK fertilizers on severity of black spot disease (*Alternaria brassicae*) and yield of oilseed rape. Plant and Soil 167(2):313-320.

161. Shrestha, A., Hesterman, O. B., Copeland, L. O., Squire, J. M., Fisk, J. W., and Sheafferm C. C. 1999. Annual legumes as green manure and forage crops in winter canola (*Brassica napus* L.) rotations. Canadian Journal of Plant Science 79:19-25.

162. Sivan, A., Elad, Y., and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology 74(4):498-501.

163. Siqueira, J. O., Safir, G. R., and Nair, M. G. 1991. Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. New Phytologist 118:87-93.

164. Sivan, A., and Chet, I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. Phytopathology 79(2):198-203.

165. Skovgaard, K., Nirenberg, H. I., O'Donnell, K., and Rosendahl, S. 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91(12):1231-1237.

166. Smith, S. N. 2007. An overview of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil-borne pathogenic forms. Plant Pathology Bulletin 16:97-120.

167. Smith, S. N., DeVay, J. E., Hsieh, W.-H., and Lee, H.-J. 2001. Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum,* a cotton wilt fungus in California fields. Mycologia 93(4):737-743.

168. Smolinska, U., and Horbowicz, M. 1999. Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. Phytopathology 147(2):119-124.

169. Smolinska, U., Morra, M. J., Knudsen, G. R., and James, R. L. 2003. Isothiocyanates produced by Brassicaceae species as inhibitors of *Fusarium oxysporum*. Plant Disease 87(4):407-412.

170. Snoeijers, S. S., Perez-Garcia, A., Joosten, M. H. A. J., and De Wit, P. J. G. M. 2000. The effect of nitrogen on disease development and gene expression in bacterial and fungal plant pathogens. European Journal of Plant Pathology 106:493-506.

171. Snyder, W. C., and Hansen, H. N. 1940. The species concept in *Fusarium.* American Journal of Botany 27:64-67.

172. Sorensen, J. N., Larsen, J., and Jakobsen, I. 2005. Mycorrhiza formation and nutrient concentration in leeks (*Allium porrum*) in relation to previous crop and cover crop management on high P soils. Plant and Soil 273(1-2):101-114.

173. Srivastava, R., Khalid, A., Singh, U. S., and Sharma, A. K. 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Psuedomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biological Control 53(1):24-31.

174. Steinmaus, S., Elmore, C. L., Smith, R. J., Donaldson, D., Weber, E. A., Roncoroni, J. A., and Miller, P. R. M. 2008. Mulched cover crops as an alternative to conventional weed management systems in vineyards. Weed Research 48(3):273-281.

175. Suman, A., Shahi, H. N., Pushpa, S., and Guar, A. 2002. Allelopathic influence of *Vigna mungo* (black gram) seeds on germination and radical growth of some crop plants. Plant Growth Regulation 38(1):69-74.

176. Sundaresan, P., Raja, N. U., and Gunasekaran, P. 1993. Induction and accumulation of phytoalexins in cowpea roots infected with a mycorrhizal fungus *Glomus fasciculatum* and their resistance to *Fusarium* wilt disease. Journal of Biosciences 18(2):291-301.

177. Tabaglio, V., Gavazzi, C., Schulz, M., and Marocco, A. 2008. Alternative weed control using the allelopathic effect of natural benzoxazinoids from rye mulch. Agron. Sustain. Dev. 28:397-401.

178. Teasdale, C. 2009. Disease management in organic blueberries. E. S. Cropconsult Ltd. p1-11. Available at: http://www.certifiedorganic.bc.ca/programs/osdp/I-104%20Organic%20Blueberry%20Disease_final.pdf

179. Teasdale, J. R., and Pillai, P. 2005. Contribution of ammonium to stimulation of smooth pigweed (*Amaranthus hybridus* L.) germination by extracts of hairy vetch (*Vicia villosa* Roth) residue. Weed Biology and Management 5(1):19-25.

180. Tenuta, M., and Lazarovits, G. 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology 92(3):255-264.

181. Termorshuizen, A. J., and Jeger, M. J. 2008. Strategies of soilborne plant pathogenic fungi in relation to disease suppression. Fungal Ecology 1(4):108-114.

182. Tobar, R. M., Azcon, R., and Barea, J. M. 1994. The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorhizae. Mycorrhiza 4:105-108.

183. Tokala, R. K., Strap, J. L., Jung, C. M., Crawford, D. L., Salove, M. H., Deobald, L. A., Bailey, J. F., and Morra, M. J. 2002. Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl. Environ. Microbiol. 68(5):2161-2171.

184. Tomaso-Peterson, M., and Perry, D. H. 2007. The role of biofungicides and organic fertilizer in the management of dollar spot in Bermudagrass. Online. Applied Turfgrass Science doi:10.1094/ATS-2007-0911-01-RS.

185. Tsuneo, N. 1991. Diversity of root fungal floras: Its implications for soil-borne diseases and crop growth. Japanese Agricultural Research Quarterly 25(1):6-11.

186. USDA-ERS. 2011. Fresh watermelon: U.S. imports by country, 1978-2010. National Agricultural Statistics service. http://usda.mannlib.cornell.edu. USDA Economics, Statistics, and Market Information System. Viewed 20 Feb 2013.

187. USDA-ERS. 2012. Vegetables 2012 Summary. National Agricultural Statistics service. http://usda.mannlib.cornell.edu. USDA Economics, Statistics, and Market Information System. Viewed 20 Feb 2013.

188. Vallad, G. E., Huang, C.–H. 2011. Evaluation of biopesticides and fungicides for management of Fusarium wilt and southern blight on tomato, 2010. Plant Disease Management Reports 5:V064. Online publication. doi:10.1094/PDMR05.

189. Van Os, G. J., and van Ginkel, J. H. 2001. Suppression of *Pythium* root rot in bulbous *Iris* in relation to biomass and activity of the soil microflora. Soil Biology and Biochemistry 33(11):1447-1454.

190. Vaz, A. F. M., Costa, R. M. P. B., Melo, A. M. M. A., Olivia, M. L. V., Santana, L. A., Silva-Lucca, R. A., Coelho, L. C. B. B., and Correia, M. T. S. 2010. Biocontrol of *Fusarium* species by a novel lectin with low ecotoxicity isolated from *Sebastiania jacobinensis*. Food Chemistry 119:1507-1513.

191. Vinale, F., Ghisalberti, E. L., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., Woo, S., and Lorito, M. 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Letters in Applied Microbiology 48:705-711.

192. Weller, D. M., Raajimakers, J.-M., McSpadden Gardener, B. B., Thomashow, L. S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Phytopathology 40:309-348.

193. Wells, L. 2009. Clover management in pecan orchards. The University of Georgia Cooperative Extension. Bulletin (1):1-6. Available at: http://athenaeum.libs.uga.edu/bitstream/handle/10724/12335/B1360.pdf?sequence=1

194. White, C. M., and Weil, R. R. 2010. Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. Plant and Soil 328(1-2):507-521.

195. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76(5):518-521.

196. Woltz, S. S., and Engelhard, A. W. 1973. Fusarium wilt of chrysanthemum: Effect of nitrogen source and lime on disease development. Phytopathology 63:155-157.

197. Wu, H.-S., Luo, J., Raza, W., Liu, Y.-X., Gu, M., Chen, G., Hu, X.-F., Wang, J.-H., Mao, Z.-S., and Shen, Q.-R. 2010. Effect of exogenously added ferulic acid on *in vitro Fusarium oxysporum* f. sp. *niveum*. Scientia Horticulturae 124(4):448-453.

198. Wu, H.-S., Raza, W., Fan, J.-Q., Sun, Y.-G., Bao, W., Shen, Q.-R. 2008. Cinnamic acid inhibits growth but stimulates production of pathogenesis factors by *in vitro* cultures of *Fusarium oxysporum* f. sp. *niveum.* Journal of Agriculture and Food Chemistry 56(4):1316-132.

199. Wu, H.-S., Wang, Y., Zhang, C.-Y., Bao, W., Ling, N., Liu, D.-Y. and Qi-Rong, S. 2009a. Growth of *in vitro Fusarium oxysporum* f. sp. *niveum* in chemically defined media amended with gallic acid. Biological Research 42(3):297-304.

200. Wu, H.-S., Yang, X.-N., Fan, J.-Q., Miao, W.-G., Ling, N., Xu, Y.-C., Huang, Q-W., and Shen, Q. 2009b. Suppression of Fusarium wilt of watermelon by a bio-organic fertilizer containing combinations of antagonistic microorganisms. BioControl 54:287-300.

201. Wu, Q.-S., and Xia, R.-X., 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal of Plant Physiology 163:417-425.

202. Wyse, D. L. 1994. New technologies and approaches for weed management in sustainable agriculture systems. Weed Technology 8(2):403-407.

203. Yang, X., Chen, L., Yong, X., and Shen, Q. 2011. Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against Fusarium wilt of cucumbers. Biology and Fertility of Soils 47(3):239-248.

204. Yedidia, I., Benhamou, N., and Chet, I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied and Environmental Microbiology 65(3):1061-1070.

205. Yuan, M. W. and Crawford, D. L. 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. Applied and Environmental Microbiology 61(8):3119-3128.

206. Zasada, I. A., Ferris, H., Elmore, C. L., Roncoroni, J. A., and MacDonald, J. D. 2003. Field application of brassicaceous amendments for control of soilborne pests and pathogens. Plant Health Progress. doi:10.1094/PHP- 2003-1120-01-RS.

207. Zhang, S. 2011. Evaluation of microbial products for management of powdery mildew on summer squash and cantaloupe in Florida. Plant Disease 95(4):461-468.

208. Zhou, X. G., and Everts, K. L. 2003a. Carryover effect of hairy vetch and urea soil amendments on Fusarium wilt of watermelon in the following year, 2003. B&C Tests.19:V006. DOI:10.1094/FN58

209. Zhou, X. G., and Everts, K. L. 2003b. Races and inoculum density of *Fusarium oxysporum* f. sp. *niveum* in commercial watermelon fields in Maryland and Delaware. Plant Disease 87(6):692-698.

210. Zhou, X. G., and Everts, K. L. 2004. Suppression of Fusarium wilt of watermelon by soil amendment with hairy vetch. Plant Disease 88(12):1357-1365.

211. Zhou, X. G., and Everts, K. L. 2006. Suppression of Fusarium wilt of watermelon enhanced by hairy vetch green manure and partial cultivar resistance. Online. Plant Health Progress doi:110.1094/PHP-2006-0405-01-RS.

212. Zhou, X. G., and Everts, K. L. 2007. Effects of host resistance and inoculum density on the suppression of Fusarium wilt of watermelon induced by hairy vetch. Plant Disease 91:92-96.

213. Zhou, X. G., Everts, K. L., and Armentrout, D. K. 2002. Hairy vetch and urea soil amendments: A novel strategy for management of Fusarium wilt of watermelon, 2002. B&C Tests. 18:V006. doi:10.1094/FN57

214. Zhou, X. G., Everts, K. L., and Bruton, K. L. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f . sp . *niveum* causing Fusarium wilt in watermelon. Plant Disease 94(1):92-98.

215. Zimand, G., Elad, Y., and Ilan, C. 1996. Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology 86(11):1255-160.

216. Zitter, T. A., Hopkins, D. L., and Thomas, C. E. Compendium of cucurbit diseases. St. Paul: American Phytopathological Society, 1996.