ALTERNARIA LEAF SPOT OF BRASSICA CROPS: DISEASE INCIDENCE AND SUSTAINABLE MANAGEMENT

A Thesis

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by

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ABSTRACT

Alternaria leaf spot (ALS) is a fungal disease that affects all cultivated brassicas, causing small black spots that grow into large lesions with characteristic concentric rings on leaves, stems and heads. The disease is of growing concern to NYS cabbage growers because of decreasing efficacy of chemical fungicides to control the disease. Concurrent with the decline in efficacy of fungicides there has been a 406% increase in organic cabbage production in NYS between 2008 and 2011, indicating that research on organic alternatives for ALS management in NYS is warranted. The goals of the studies presented in this thesis were to answer basic questions of population structure in NYS, and to investigate disease management strategies suitable for use in organic cropping systems.

A disease survey was conducted and samples of plants showing ALS symptoms were collected from different host crops and from farms across NYS. All isolates collected were identified as *Alternaria brassicicola* using DNA-based methods, indicating that disease is not or is rarely caused by the closely related species, *A. brassicae,* which is also known to infect vegetable brassicas. Furthermore, cultural and biological disease control strategies were investigated in two field studies. First, two soil organic matter amendments and several fungicides approved for use in organic cropping systems were evaluated for their efficacy in controlling ALS in cabbage. None of the treatments evaluated had significant effects on disease severity, but further study under different application conditions is warranted. Secondly, the effects of mulches on kale growth and disease incidence were tested in a field contaminated with ALS-infected crop residues. Results of this study indicate that mulch increases plant growth while reducing disease incidence, and highlights the potential of cultural practices to reduce ALS

disease severity in organic cropping systems. Further research is necessary to confirm these results, and additional studies on epidemiology and pathogen population structure will aid in the continuing development of successful ALS management strategies for NYS growers.

BIOGRAPHICAL SKETCH

Susan Scheufele grew up in Worcester, Massachusetts and began her undergraduate career at Hampshire College in Amherst, MA, in 2002. In her first semester at Hampshire she took her one required science class, and much to her own surprise, began to cultivate a love for scientific inquiry and writing. At the end of her first year she took a road trip to the deserts and mountains of Utah and Wyoming where she became interested in the geological processes that formed those unique landscapes, and she began to concentrate her academic studies on geology. She also developed interests in chemistry, microbial ecology, and forest ecology, and incorporated these fields into her senior research project entitled, "Tracing the Chemical Influence of Ultramafic Bedrock on Plant Communities in Western Massachusetts."

She spent several years gaining work experience and had many diverse jobs including research in invasive pest biocontrol at the University of Massachusetts, at a cellulosic ethanol research and development laboratory, and then worked at an organic vegetable farm. It was on the farm that Sue became all too well acquainted with plant diseases, as late blight, caused by *Phytophthora infestans*, ravaged the Northeast and claimed the entire tomato crop. She decided then to use her scientific background and skills to help farmers manage pests and diseases more effectively and more sustainably. She did her thesis research on sustainable management of Alternaria leaf spot with Drs. Helene Dillard and Christine Smart in the Department of Plant Pathology and Plant-Microbe Biology and Dr. Stephen Reiners in the Department of Horticulture at Cornell University. Sue is planning to continue doing agricultural research and working with vegetable growers as part of the UMass Cooperative Extension vegetable team in Amherst, MA.

To my parents

& to Sara, Stephen, Joe and Doreen

for always encouraging me to learn and grow

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CHAPTER 1

INTRODUCTION

Alternaria leaf spot (ALS) is a fungal disease affecting cultivated and wild *Brassica* spp., which impacts crop yield and seed production worldwide. The disease can be caused by several fungi in the genus *Alternaria*, but the most damaging species in the production of vegetable brassicas are *A. brassicae* and *A. brassicicola*. Disease development is favored by cool temperatures and long periods of leaf wetness or high relative humidity, and ALS can be a limiting factor in the production of vegetable and seed crops in regions where these conditions are common. Infection can cause reduction in crop quality and yield through damage to seeds, seedlings, leaves, and heads, and can also spread during storage of vegetable crops like cabbage. The disease is a perennial problem for growers in New York State (NYS) and is of growing concern due to increasing disease severity and limited control by fungicides. Concurrent with the decline in efficacy of fungicides there has been a 406% increase in organic cabbage production in NYS between 2008 and 2011, indicating that research on organic alternatives for ALS management in NYS is warranted. The goals of the studies presented in this thesis were to answer basic questions of population structure in NYS, and to investigate disease management strategies suitable for use in organic cropping systems.

Biology of Alternaria spp.

Fungi belonging to the genus *Alternaria* are ubiquitous in both air and soil. Most species are saprophytes or opportunistic plant pathogens but there are also many destructive plant pathogenic species that affect a tremendously wide range of agricultural crops worldwide (Rotem, 1994). The genus belongs to the deuteromycete group of fungi, which have no known sexual stage and produce only asexual spores. These spores, known as conidia, are pigmented, oblong, club-shaped, and may have one to many transverse and longitudinal septations, making them quite characteristic of the genus (Rotem, 1994). Conidia are produced singly or in chains on simple or branched conidiophores or directly on hyphae.

There are at least a few *Alternaria* spp. causing disease on cultivated brassicas. These include *A. japonica* that infects radish (*Raphanus sativus*), *A. brassicae* that most commonly infects oil and mustard seed crops (*B. rapa* and *B. napus*) but can also infect vegetable crops (*B. oleracea*), and *A. brassicicola* that is the dominant species infecting vegetable crops but can also infect seed crops (Humpherson-Jones, 1992). *A. brassicicola* and *A. brassicae* are the most important species infecting vegetable brassicas, and the two fungi can be difficult to distinguish based on symptoms produced in the field or by their morphology in cultures grown in the laboratory (Humpherson-Jones & Maude, 1982b). Grown *in vitro* on potato dextrose agar (PDA), *A. brassicae* produces more aerial mycelia and less secondary sporulation giving it a gray, fluffy appearance compared to the dense sporulation characteristic of *A. brassicicola*, which produces a black, velvety appearance (Rotem, 1994). Much early research on ALS focused on distinguishing the two species and determining their relative abundance in fields and seed lots in the United Kingdom (Humpherson-Jones & Maude, 1982b). Furthermore, studies on the epidemiology and management of ALS have been performed largely in Europe and India,

where both seed and vegetable crops are grown and both *A. brassicicola* and *A. brassicae* are found. Very little research has been conducted on ALS epidemiology in the United States, and the relative contributions of the two species to disease is not known.

Primary inoculum - seed

Disease outbreaks are initiated primarily by overwintering spores that persist on crop residues in the soil, or by introduction of contaminated seeds or seedlings. Early studies in England determined incidence of *A. brassicicola* on seeds of *B. oleracae* could be as high as 90%, with internal infection up to 12% (Humpherson-Jones, 1983; Maude & Humpherson-Jones, 1980b). Infection of *B. rapa* and *B. napus* by *A. brassicae* is highest in India where an average of 30% of seeds are affected (Humpherson-Jones, 1992). Researchers in Australia studied seed contamination of commercial *B. oleraceae* seeds, with 24-37% surface infestation and only 4-8% internal infections (Sivapalan & Browning, 1992). External infections occur when spores and mycelia of *Alternaria* spp. adhere to crevices on the seed surface, especially the testa and hilum areas, while internal infections are caused by dormant mycelia in the micropyle of the seed (Maude & Humpherson-Jones, 1980b). These mycelia can remain viable for 7.5-8 years in the case of *A. brassicicola*, while *A. brassicae* has been shown to remain viable for only 20 months when kept at reduced temperature (0ºC) (Maude & Humpherson-Jones, 1980b). The fungi infect the fruit-bearing branches and seed pods resulting in yield loss due to premature pod ripening and shedding of seed (Maude & Humpherson-Jones, 1980b). Furthermore, infected seeds can be small, shriveled and non-viable, and oil seeds may contain a lower concentration of oil (Humpherson-Jones, 1992). Infected seeds produce diseased seedlings at rates of 5.6% and up to

35% for *A. brassicicola* and *A. brassicae*, respectively, and internal infections are more closely correlated with seedling disease than are surface infestations (Maude & Humpherson-Jones, 1980b).

In a recent study on dark leaf and pod spot epidemiology in organic cauliflower seed production, Kohl et al. (2010) found that *A. brassicicola* was present both externally (70-90%) and internally (62-80%) on cauliflower seeds, while *A. brassicae* was also present but at a much lower incidence (3%). *A. brassicicola* was found colonizing seeds and seedpods 14 days after flowering and infection increased slowly until seeds began to develop and then increased sharply during seed maturation. The quality of seeds produced was significantly lower than in control plants, as only 80% of seeds were viable. Management of ALS in production of *B. oleraceae* seed is difficult in organic systems because the plants are biennial, requiring a two year growing season without the use of synthetic fungicides to control the disease (Kohl et al., 2010). Hot water treatments, the only seed treatment approved by the Organic Materials Review Institute (OMRI) for use in organic systems in the US, did not decrease seed germinability, but were also not effective in eliminating internal infections. The authors concluded that controlling the pathogen in the field early on was important, and removing weak and infected plants may be the best way to control epidemics in seed production fields.

Primary inoculum - overwintering spores

Another important source of primary inoculum is infected crop residues left in the field or in the soil (Humpherson-Jones, 1989; Humpherson-Jones, 1992; Kohl et al., 2010). Spores and mycelia remain viable on crop debris over the winter and, at low temperatures, *A. brassicicola* and *A. brassicae* can also form microsclerotia and chlamydospores which are able to survive

freezing at -40ºC (Humpherson-Jones, 1992). These overwintering structures and hardy conidia in the soil are important sources of primary inoculum. Brassicaceous weed hosts may also provide alternative overwintering sites (Humpherson-Jones, 1992).

In a recent study of pathogen survival on crop residues of Brussels sprouts, Kohl et al. (2010) used quantitative polymerase chain reaction (QPCR) to monitor pathogen populations over time in leaf, stalk and stalk base samples left on the soil surface or buried in the topsoil. This method allowed for the direct and simultaneous identification and quantification of the pathogens based on their genotype. The authors found that on leaf tissue, *A. brassicicola* populations increased 7-fold when left on the soil surface and 3.5-fold when buried within soil over the first two months, but rapidly declined as no leaf tissue was present after 4 months under Dutch conditions. *A. brassicae* populations, which were low at the start of the experiment, decreased continuously and decreased more quickly when buried within soil than when left on the soil surface.

Leaf tissue had completely decomposed within 4 months whether left on the soil surface or buried 10 cm deep in the soil, but lignin-rich stems remained intact in the soil at the end of the two year study, whether they were left on the soil surface or were incorporated into the soil. *A. brassicicola* population densities were lower on stalk bases than on the upper stalk and increased over the first year, peaking in the summer following fall harvest, after which time they steadily decreased. At the end of the two year study *A. brassicicola* was still present on stalks left on the soil surface but not on buried stalk residues. *A. brassicae* populations also increased over the first year peaking in the summer following fall harvest when stalks were left on the soil surface but quickly decreased when buried in the soil and were no longer detectable by the end of the study on any stalks. This study confirmed results of previous studies which also showed that

populations of *A. brassicicola* and *A. brassicae* can continue to grow and produce spores on leaf tissue as long as it is present (Humpherson-Jones & Phelps, 1989) and highlights the potential of lignin-rich crop residues to serve as long term reservoirs of inoculum.

Disease spread - infection and symptom development

Spores of *Alternaria* spp. germinate very quickly—within two hours in the case of *A. dauci*— in the presence of water (Rotem, 1994). Germinated spores penetrate host leaf tissue via stomata in the case of *A. brassicae*, while *A. brassicicola* can also cause infection by direct penetration (Humpherson-Jones, 1992). These fungi are necrotrophic and so, upon infection of a host plant, secrete toxins that kill host cells, releasing plant nutrients which the fungi can then consume (Agrios, 2005). *Alternaria* spp. are prolific toxin producers and there has been much recent research focused on characterizing non-specific and host specific toxins produced by *A. brassicicola* since the sequencing of its genome was completed in 2004 (Lawrence et al., 2008). These toxins are often secondary metabolites but some are proteinaceous and, so far, it seems that extracellular lipases are important pathogenicity factors (Lawrence et al., 2008).

The initial symptoms of ALS appear as small dark spots and, because of the secreted toxins, ALS lesions are often surrounded by chlorotic haloes (Humpherson-Jones, 1992; Lawrence et al., 2008). Under the right environmental conditions, the fungus continues to grow and small spots expand into large, target-like lesions with necrotic, brown centers (Humpherson-Jones, 1992). Once the fungi have infected their host, they begin the polycyclic phase of growth and continuous production of conidia throughout the growing season, as long as conditions remain favorable for growth of the fungus (Humpherson-Jones, 1992).

ALS tends to affect foliage but all plant parts can be infected, including petioles, stems, flowers, seeds, and heads of crops like cabbage, broccoli, and cauliflower. Severe infections of leaves can lead to general chlorosis and senescence of leaves, though many vegetable brassicas are leaf crops and even a few small lesions per leaf can reduce quality and yield of the crop (Humpherson-Jones, 1992). On stem tissue ALS lesions appear dark purple to black and spread up and down the stem causing necrotic streaking (Strandberg, 1992). Infection of petioles can cause premature leaf senescence and thereby reduction in photosynthetic area and plant growth (Humpherson-Jones, 1992). If seed pods become infected the seeds within may also become infected and severe pod infections can cause pods to shatter and shed their seed, lowering seed yield (Humpherson-Jones, 1992). In the production of oilseed crops, infection of seed by *Alternaria* spp. can also lead to decreased oil quality (Humpherson-Jones, 1992). Infection of broccoli or cauliflower heads causes brown sunken lesions and presence of one or a few of these can significantly lower yield because the whole plant is then unmarketable. *A. brassicicola* and *A. brassicae* can both spread during cold storage of vegetable crops, making this disease a serious problem in the production of storage cabbage, which is often stored for months in order to take advantage of spring markets (Humpherson-Jones, 1992).

Disease spread - sporulation

Key factors contributing to the rapid disease progress of *Alternaria* spp. are their short life cycles, and flexibility in regard to climatic conditions (Strandberg, 1992). The time between leaf infection and subsequent spore production is generally 7-10 days and spores can be produced for several consecutive weeks from existing leaf lesions in the case of most *Alternaria* spp.(Humpherson-Jones & Phelps, 1989; Strandberg, 1992). *A. brassicae* and *A. brassicicola* are

able to produce spores between 5 ºC and 24 ºC, although fewer spores are produced at low temperatures. For example, *A. brassicae* can take up to 80 hours from the time of infection to spore production when grown at 5 ºC while sporulation occurs within 13 hours at 20ºC (Humpherson-Jones, 1992; Humpherson-Jones & Phelps, 1989). *A. brassicae* has slightly lower optimum temperatures for spore production and also has a much shorter time to infection interval (18-24ºC, and 6 hours respectively) than does *A. brassicicola* (20-31ºC, and 18 hours) (Humpherson-Jones, 1992).

Moisture is also a very important determinant of disease development and epidemiology. Spore production by infective mycelia of *A. brassicicola* and *A. brassicae* requires at least 90% relative humidity for 12-14 hours (Humpherson-Jones & Phelps, 1989). *Alternaria* spp. have been found to produce the most spores following a period of rain or intermittent dew, and the duration of leaf wetness has been shown to significantly affect disease incidence and severity as well as spore production and germinability, (Chen et al., 2003; Hong et al., 1996; Maude & Humpherson-Jones, 1980b). When kept at a constant temperature, disease incidence showed a logistic relationship to length of leaf wetness period, with at least two hours of leaf wetness required to produce dark leaf spot infections at 15ºC (Hong et al., 1996). Similarly, the greatest level of spore dispersal coincided with hot dry periods following a period of rain (Chen et al., 2003). While moisture is necessary for spore germination, spores which had not yet germinated were shown to be viable after 10 weeks of desiccation regardless of storage conditions, contributing to the observed flexibility of these fungi to survive despite adverse environmental conditions (Humpherson-Jones & Phelps, 1989).

Disease spread - dispersal

Spores are spread from leaf lesions throughout the field primarily by wind and rain. In a comprehensive study on spore dispersal of *A. brassicicola*, Chen et al. (2003) found that leaf wetness was negatively correlated with spore dispersal, which occurred during hot dry periods following a period of rain. Most spores were dispersed between 10am and 2pm, coinciding with high temperatures and low relative humidity, following a period of overnight and early morning dew. This confirms results of an early study conducted in England in which also found that most spores were released between 1 and 3 pm, when temperatures increased and relative humidity decreased (Humpherson-Jones, 1992). The study also showed that spores were found up to 50 cm above the soil line but the most spores were collected from 20 cm above the soil or lower. Splash dispersal increased with rain intensity but dispersal was limited to the lower 15 cm of the plant and wind-blown splash generally resulted in spore movement of less than but up to one meter. Chen et al. (2003) also found that wind speeds from 3-7.5 m/s resulted in the highest rates of spore dispersal and conidia were blown at least 3 meters with wind at speeds of 5-7 m/s. Overall this study showed that *Alternaria* spores can be moved around a field by wind and rain, but the authors did not find evidence for dispersal of great distances by these mechanisms. However, an early study on ALS epidemiology in England demonstrated that spores of *A. brassicicola* were dispersed up to 1800 m when an infected seed crop was harvested and threshed (Humpherson-Jones & Maude, 1982b). Distances of that magnitude could easily move spores from field to field or even farm to farm so prominent wind direction should be taken into account when designing rotations and field plans.

Alternaria spores can also be moved around a field by flea beetles (*Phyllotreta cruciferae*), a destructive pest affecting most brassicaceous plants. Using scanning electron

microscopy (SEM), Dillard et al. (1998) observed intact, viable conidia on wings, mouthparts, antennae and legs of flea beetles isolated from cabbage fields infected with *A. brassicicola*. Intact, viable conidia were also recovered from digestive tracts and fecal matter of flea beetles which had fed on cabbage infested with *A. brassicicola*. Isolation of *A. brassicicola* from flea beetles increased over the growing season as the crop reached maturity and feeding wounds were often the site of ingress of the fungus and subsequent disease development. When flea beetles from fields with either high or low *A. brassicicola* inoculum were transferred to healthy plants in the greenhouse, disease severity was increased in the presence of beetles from fields with higher inoculum load. This study showed the importance of insects in spreading the disease into, as well as throughout, a field.

Management - cultural control

Crop rotation is one of the simplest and most effective ways to control ALS in the field, since a major source of primary inoculum is infected crop debris in the soil (Humpherson-Jones, 1992). A three year rotation to a non-host crop is considered to be the minimum rotation length necessary to avoid infection by soil-borne inoculum (Seaman, 2012). It can be difficult for many growers, especially those with small acreage, to achieve rotations in which host crops are adequately separated in both time and space because of the large number of host crops in the *Brassica* family and their long and overlapping growing seasons. Furthermore, studies on overwintering mortality of *A. brassicicola* and *A. brassicae* have been conducted largely in Europe (Humpherson-Jones, 1989; Kohl et al., 2011), and it is possible that longer rotations may be necessary for lignin-rich stalk tissue to be broken down under NYS conditions.

Infected seed is the other main source of primary inoculum, and therefore using clean seeds and transplants is essential in preventing the introduction of *A. brassicicola* and *A. brassicae* into clean fields (Humpherson-Jones, 1992; Seaman, 2012). Hot water treatment for 18 minutes at 50°C has resulted in 98% reduction in disease incidence but if significant internal infection is present this method may be less effective and seeds may lose viability (Humpherson-Jones, 1992). Hot water treatments are the only currently available method for disinfesting seeds in organic crop production systems (Kohl et al., 2010). In conventional systems, seeds can be treated with surface active fungicides but the best control is obtained with the locally systemic fungicide, iprodione, which can also be absorbed into the seed tissues to a level sufficient for eradication of established cotyledon infections, though further movement or toxicity are limited (Humpherson-Jones, 1992; Maude & Humpherson-Jones, 1980a). Iprodione has been used as a seed treatment since at least the 1970's and continues to be a major contributor to ALS control efforts worldwide (Iacomi-Vasilescu et al., 2004b; Maude & Humpherson-Jones, 1980a).

Research on biological controls of seed infection include the use of antagonistic fungi and bacteria, most often *Nectria inventa* spp., *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., *Periconia* spp., *Chaetomium globosum* and *Streptomyces griseoviridis* (Humpherson-Jones, 1992; Sivapalan, 1993; Tahvonen & Avikainen, 1987; Vannacci & Harman, 1987). While many of these biocontrol agents were capable of controlling disease incidence, none studied to date are as effective at controlling internal seed infections as fungicides (Humpherson-Jones, 1992). Many biocontrol antagonists also have less protective effects in field settings due to narrow growth requirements or poor competition in the rhizosphere and, as a result, fungicides are still the most widely used control method worldwide (Humpherson-Jones, 1992; Iacomi-Vasilescu et al., 2004b).

Cultural practices can also be important in minimizing secondary spread of disease. Cull piles containing infected plant debris can act as inoculum sources, as *A. brassicicola* and *A. brassicae* can continue to grow and reproduce as long as host tissue is present (Kohl et al., 2011). Management of crop residues in cull piles is therefore important, and brassica crops should be grown in fields as far from these repositories as possible (Seaman, 2012). Weed management is critical in any vegetable production system and can be a yield-limiting factor in the productions of cabbage in NYS (Dillard et al., 2004). Many weed species can also serve as hosts for common diseases of brassica crops such as white and gray molds (*Sclerotinia sclerotiorum* and *Botrytis cinerea*) and control of brassicaceous weed hosts such as field pennycress (*Thlaspi arvense*), which are common in NYS, can be an important component of an integrated approach to ALS management (Cobb & Dillard, 1998; Humpherson-Jones, 1992; Seaman, 2012).

As was previously discussed, flea beetles can move spores of *A. brassicicola* from plant to plant within a field as well as from field to field so controlling these insects can be an important part of an integrated disease control effort. Flea beetles are difficult to control and are a major limiting factor in organic production of brassica vegetables and greens. Their control in these systems is principally through use of floating row-covers to provide a physical barrier between beetle and crop foliage (Andersen et al., 2006; Seaman, 2012). Efficacy of OMRI approved products for control of Flea beetles in organic systems have typically shown inadequate or inconsistent control, but newer materials containing spinosad provide sufficient control (Andersen et al., 2006; Seaman, 2012). There are many insecticides available for conventional growers, and those containing pyrethroids or carbamates provide the best control, though Flea beetles are still a major pest in brassica production (Weiss et al., 1991).

Often resistant cultivars are used to control crop diseases, but this strategy is not usually effective against necrotrophs, and there are no resistant varieties of brassica crops commercially available (Iacomi-Vasilescu et al., 2004b; Lawrence et al., 2008). However, some brassicaceous weed species, including the model plant *Arabidopsis thaliana*, do show variability in tolerance to *A. brassicicola* (Lawrence et al., 2008). This plant tolerance seems to be controlled by the jasmonic acid signaling pathway and production of camalexin, a phytoalexin with antimicrobial activity, is implicated in disease suppression (Lawrence et al., 2008). Current research in this model pathosystem is aimed at determining the *A. thaliana* genes responsible for tolerance to *A. brassicicola* and subsequently breeding for tolerance in commercial cultivars (Lawrence et al., 2008).

Management - chemical control

Chemical control of ALS on seeds and in the field has remained the most common method of managing the disease worldwide. ALS in brassica crops has historically been controlled using iprodione, a dicarboximide interfering with osmotic signal transduction, as a foliar spray and as a seed treatment (Babadoost et al., 1993; Humpherson-Jones & Maude, 1982a). Chlorothalonil, a broad spectrum fungicide with multiple sites of action, has also been used extensively as a foliar spray to control ALS (Babadoost et al., 1993), and there are several newer fungicides which have become widely used to control ALS in NYS. The most important of these new fungicides in NYS is azoxystrobin, a quinone outside inhibitor (QoI) with a very specific mode of action that exerts very high selection pressure for resistant isolates within the pathogen populations.

Indeed, fungicide resistance has been reported in *A. brassicicola* for several major fungicides in other locations and in other plant pathogenic *Alternaria* spp. (Huang & Levy, 1995; Iacomi-Vasilescu et al., 2004b; Ma & Michailides, 2004a). Field isolates of *A. brassicicola* with resistance to iprodione, the most widely used chemical control of ALS, was first reported in the mid-2000's (Iacomi-Vasilescu et al., 2004b). Cross resistance to both dicarboximides (including iprodione) and phenylpyrroles was also present in these populations (Huang & Levy, 1995; Iacomi-Vasilescu et al., 2004b). Resistance to azoxystrobin has been documented in many *Alternaria* pathosystems including early blight of potato caused by *A. solani* (Rosenzweig et al., 2008), and late blight of pistachio caused by *A. tenuissima*, *A. alternata*, and *A. arborescens* (Ma & Michailides, 2004b). Resistance to azoxystrobin has not been demonstrated for *A. brassicicola* or *A. brassicae*, but its development in other *Alternaria* spp. (as well as in many other plant pathogenic fungi) indicates that resistance management procedures should be followed carefully.

Management – biological control

Some biological control methods are known, especially as seed inoculants, as discussed previously. Sivapalan et al. (2003) tested the efficacy of several microbes isolated from broccoli seeds or from the rhizosphere of broccoli seedlings in reducing ALS incidence on seeds and seedlings. The authors found that *Gliocladium roseum* and *Trichoderma harzianum* were effective in reducing incidence of seed infection, increasing seedling emergence, and decreasing leaf infections by *A. brassicicola* (Sivapalan, 1993). An early study on treatment of cabbage seeds infected with *A. brassicicola* with seed-borne antagonists indicated that a *Periconia* sp., a

Pencillium sp., and a strain of *Chaetomium globosum* Kunze increased healthy seedling emergence equivalently to iprodione (Vannacci & Harman, 1987).

While there has been much interest in biocontrol during the seed-borne phase of *A. brassicicola* and *A. brassicae*, very little is known about the effects of biocontrol organisms on the foliar disease phase. One study demonstrated potential protection of oilseed rape (*B. napus*) foliage from *A. brassicae* by seed treatment with *Bacillus amyloliquefaciens*, indicating an induction of plant defense responses (Danielsson et al., 2007). Induction of systemic plant defense responses by *Trichoderma* spp. has been implicated in protection of tomato from *Alternaria solani*, causing early blight in that crop (Fontenelle et al., 2011) and may be worth investigating for *A. brassicicola*. There are a number of biocontrol agents which are registered for foliar application use in organic production and are listed for control of ALS in NYS, but the efficacy of these products in reducing disease severity is not known (Seaman, 2012), and this is an area in need of more research attention in the future.

Conclusion

The present studies were undertaken in order to address gaps in our knowledge about Alternaria leaf spot biology in NYS, and to investigate sustainable disease management strategies for use in organic cropping systems in the Northeastern US. A disease survey was conducted in which isolates of the pathogen were collected from a variety of crop hosts from farms across the NYS. Molecular biological techniques were used to positively identify these isolates in order to confirm that *A. brassicicola* is the major cause of ALS in NYS. This work lays the foundation for future studies that may focus on pathogen population structure, presence of sexual recombination, and presence and genetic basis for fungicide resistance.

Furthermore, cultural and biological disease control strategies were investigated in two field studies. First, two soil organic matter amendments and several fungicides approved for use in organic cropping systems were evaluated for their efficacy in controlling ALS in cabbage. None of the biological treatments evaluated had significant effects on disease severity, but further study under different application conditions is warranted. Secondly, the effects of mulches on kale growth and disease incidence were tested in a field contaminated with ALS-infected crop residues. Results of this study indicate that mulch increases plant growth while reducing disease incidence, and highlights the potential of cultural practices to reduce ALS disease severity in organic cropping systems. Further research is necessary to both expand and confirm these results, and additional studies on epidemiology and pathogen population structure will aid in the continuing development of successful ALS management strategies for NYS growers.

REFERENCES

Agrios GN, 2005. *Plant Pathology*. Burlington, MA: Elsevier Academic Press.

Andersen CL, Hazzard R, Van Driesche R, Mangan FX, 2006. Alternative management tactics for control of *Phyllotreta cruciferae* and *Phyllotreta striolata* (Coleoptera : Chrysomelidae) on *Brassica rapa* in Massachusetts. *Journal of Economic Entomology* **99**, 803-10.

Babadoost M, Gabrielson RL, Olson SA, Mulanax MW, 1993. Control of Alternaria diseases of brassica seed crops caused by *Alternaria brassicae* and *Alternaria brassicicola* with ground and aerial fungicide applications. *Seed Science and Technology* **21**, 1-7.

Chen LY, Price TV, Park-Ng Z, 2003. Conidial dispersal by *Alternaria brassicicola* on Chinese cabbage (*Brassica pekinensis*) in the field and under simulated conditions. *Plant Pathology* **52**, 536-45.

Cobb AC, Dillard HR, 1998. *Thlaspi arvense*, a new host for *Alternaria brassicicola*. *Plant Disease* **82**, 960.

Danielsson J, Reva O, Meijer J, 2007. Protection of oilseed rape (*Brassica napus*) toward fungal pathogens by strains of plant-associated *Bacillus amyloliquefaciens*. *Microb Ecol* **54**, 134-40.

Dillard HR, Bellinder RR, Shah DA, 2004. Integrated management of weeds and diseases in a cabbage cropping system. *Crop Protection* **23**, 163-8.

Dillard HR, Cobb AC, Lamboy JS, 1998. Transmission of *Alternaria brassicicola* to cabbage by flea beetles (*Phyllotreta cruciferae*). *Plant Disease* **82**, 153-7.

Fontenelle ADB, Guzzo SD, Lucon CMM, Harakava R, 2011. Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by *Trichoderma* spp. *Crop Protection* **30**, 1492-500.

Hong CX, Fitt BDL, Welham SJ, 1996. Effects of wetness period and temperature on development of dark pod spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus*). *Plant Pathology* **45**, 1077-89.

Huang RG, Levy Y, 1995. Characterization of iprodione-resistant isolates of *Alternaria brassicicola*. *Plant Disease* **79**, 828-33.

Humpherson-Jones FM, 1983. The occurrence of *Alternaria brassicicola, Alternaria brassicae* and *Leptosphaeria maculans* in brassica seed crops in Southeast England between 1976 and 1980. *Plant Pathology* **32**, 33-9.

Humpherson-Jones FM, 1989. Survival of *Alternaria brassicae* and *Alternaria brassicicola* on crop debris of oilseed rape and cabbage. *Annals of Applied Biology* **115**, 45-50.

Humpherson-Jones FM, 1992. Epidemiology and Control of Dark Leaf Spot of Brassicas. In: Chelkowski J, Visconti A, eds. *Alternaria Biology, Plant Diseases and Metabolites.* New York, NY: Elsevier, 267-88.

Humpherson-Jones FM, Maude RB, 1982a. Control of dark leaf spot (*Alternaria brassicicola*) of *Brassica oleracea* seed production crops with foliar sprays of iprodione. *Annals of Applied Biology* **100**, 99-104.

Humpherson-Jones FM, Maude RB, 1982b. Studies on the epidemiology of *Alternaria brassicicola* in *Brassica oleracea* seed production crops. *Annals of Applied Biology* **100**, 61-71.

Humpherson-Jones FM, Phelps K, 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola*. *Annals of Applied Biology* **114**, 449-58.

Iacomi-Vasilescu B, Avenot H, Bataillé-Simoneau N, Laurent E, Guénard M, Simoneau P, 2004. In vitro fungicide sensitivity of *Alternaria* species pathogenic to crucifers and identification of *Alternaria brassicicola* field isolates highly resistant to both dicarboximides and phenylpyrroles. *Crop Protection* **23**, 481-8.

Kohl J, Van Tongeren CaM, Groenenboom-De Haas BH, Van Hoof RA, Driessen R, Van Der Heijden L, 2010. Epidemiology of dark leaf spot caused by *Alternaria brassicicola* and *A. brassicae* in organic seed production of cauliflower. *Plant Pathology* **59**, 358-67.

Kohl J, Vlaswinkel M, Haas B, *et al.*, 2011. Survival of pathogens of Brussels sprouts (*Brassica oleracea* Gemmifera Group) in crop residues. *Plant Pathology* **60**, 661-70.

Lawrence CB, Mitchell TK, Craven KD, Cho Y, Cramer RA, Kim KH, 2008. At death's door: *Alternaria* pathogenicity mechanisms. *Plant Pathology Journal* **24**, 101-11.

Ma ZH, Michailides TJ, 2004a. Characterization of iprodione-resistant *Alternaria* isolates from pistachio in California. *Pesticide Biochemistry and Physiology* **80**, 75-84.

Ma ZH, Michailides TJ, 2004b. A real-time PCR assay for the detection of azoxystrobin-resistant *Alternaria* populations from pistachio orchards in California. *Crop Protection* **23**, 1259-63.

Maude RB, Humpherson-Jones FM, 1980a. The effect of iprodione on the seed-borne phase of *Alternaria brassicicola*. *Annals of Applied Biology* **95**, 321-7.

Maude RB, Humpherson-Jones FM, 1980b. Studies on the seed-borne phases of Dark leaf-spot (*Alternaria brassicicola*) and Grey leaf spot (*Alternaria brassicae*) of brassicas. *Annals of Applied Biology* **95**, 311-9.

Rosenzweig N, Olaya G, Atallah ZK, Cleere S, Stanger C, Stevenson WR, 2008. Monitoring and tracking changes in sensitivity to azoxystrobin fungicide in *Alternaria solani* in Wisconsin. *Plant Disease* **92**, 555-60.

Rotem J, 1994. *The Genus Alternaria: Biology, Epidemiology, and Pathogenicity*. St. Paul, MN: APS Press.

Seaman A, 2012. *2012 Production Guide for Organic Cole Crops: Cabbage, Cauliflower, Broccoli, and Brussels Sprouts.* Geneva, NY: NYS IPM.

Sivapalan A, 1993. Fungi associated with broccoli seed and evaluation of fungal antagonists and fungicides for the control of seed-borne *Alternaria brassicicola*. *Seed Science and Technology* **21**, 237-45.

Sivapalan A, Browning JW, 1992. Incidence of *Alternaria brassicicola* (Schw) Wiltsh on *Brassica oleracea* seeds. *Australian Journal of Experimental Agriculture* **32**, 535-7.

Strandberg JO, 1992. Alternaria Species That Attack Vegetable Crops: Biology and Options for Disease Management. In: Chelkowski J, Visconti A, eds. *Alternaria Biology, Plant Diseases and Metabolites.* New York, NY: Elsevier, 175-208.

Tahvonen R, Avikainen H, 1987. The biological control of seed-borne *Alternaria brassicicola* of cruciferous plants with a powdery preparation of *Streptomyces* spp. *Journal of Agricultural Science in Finland* **59**, 199-208.

Vannacci G, Harman GE, 1987. Biocontrol of seed-borne *Alternaria raphani* and *Alternaria brassicicola*. *Canadian Journal of Microbiology* **33**, 850-6.

Weiss MJ, Mcleod P, Schatz BG, Hanson BK, 1991. Potential for insecticidal management of flea beetle (Coleoptera, Chrysomelidae) on canola. *Journal of Economic Entomology* **84**, 1597-603.

CHAPTER 2

SURVEY AND GENETIC CHARACTERIZATION OF ALTERNARIA LEAF SPOT IN NEW YORK STATE

Introduction

Alternaria leaf spot (ALS) is a perennial problem for producers of vegetable brassicas in New York State (NYS). Over the last several years, NYS growers have been reporting increasing disease severity and decreasing efficacy of control by fungicides. These two trends point to the importance of characterizing the genetic structure of the pathogen population in the NYS region. A disease survey was therefore conducted to collect pathogen isolates from across the state and an effort was made to include isolates from a geographically diverse range, as well as from a variety of crop hosts, and from farms using different disease control strategies in order to adequately sample the extant diversity.

Genetic characterization of pathogen populations can be critical to understanding disease epidemiology in any system, but it is especially important in the case of ALS because symptoms can be caused by more than one species. The genus *Alternaria* is composed of many species which are found ubiquitously in both air and soil and they exist somewhere along a continuum between saprophytes, opportunistic plant pathogens, or highly host specific necrotrophic pathogens (Rotem, 1994). There are a few *Alternaria* spp. that are capable of causing disease on cultivated *Brassica* spp. These include *A. japonica* that infects radish (*Raphanus sativus*), *A. brassicae* that tends to infect oil and mustard seed crops (*B. rapa* and *B. napus*) but can also

infect vegetable crops (*B. oleracea*), and *A. brassicicola* that is most commonly found infecting vegetable crops but can also infect seed crops (Humpherson-Jones, 1992).

A. brassicicola and *A. brassicae* are the most prevalent and cause nearly identical symptoms on cultivated brassicas. The two fungi can be difficult to distinguish by their symptoms in the field as well as by their morphology in the lab. Fungi in the genus *Alternaria* have no known sexual stage and are only known to produce asexual spores, known as conidia—though recent analyses of genetic diversity in non-agricultural *A. brassicicola* populations indicate that there may be a cryptic sexual stage present (Linde et al., 2010; Rotem, 1994). These conidia are highly characteristic of the genus but it can be difficult for the untrained eye to differentiate species based on spore morphology alone (Humpherson-Jones & Maude, 1982b). Grown in lab cultures on potato dextrose agar (PDA), *A. brassicae* produces more aerial mycelia and less secondary sporulation giving it a gray, fluffy appearance compared to the dense sporulation characteristic of *A. brassicicola* which produces a black, velvety appearance (Rotem, 1994). DNA based methods are preferable for identifying *Alternaria* spp. with confidence due to the similarity in morphology, symptoms and overlapping host ranges among species. Genetic characterization of field isolates in this survey was undertaken in order to ensure that variability in disease severity and fungicide sensitivity were not being caused by the presence of mixed populations of both species within a field.

Because ALS has historically been easy to control with synthetic fungicides, it has not been of great concern to growers or researchers in the US. Studies on the biology, epidemiology, and management of ALS in brassica hosts have been performed largely in Europe and India, where both seed and vegetable crops are grown and both *A. brassicicola* and *A. brassicae* are found. Very little research has been conducted on the epidemiology or population structure of

the causal organisms in the US, and the presence and relative abundance of the two species here has, to our knowledge, not been studied.

While canola and mustard are not often grown in NYS, there is a large canola producing region just to the North in the greenbelt region of Canada in southern Ontario and Quebec, where, in 2012, 64,000 ha was produced in Ontario alone (Canola Council of Canada, 2011). The greenbelt region is also a major production area of cabbage (87% of the Canadian national production area), as well as Chinese cabbage (*B. campestris*) and other vegetable *Brassicas* (*B. oleracea, B. rapa, B. napus*) with over 10,000 ha in production of these crops in 2011 (Statistics Canada, 2011). ALS is considered one of the five most economically important diseases of canola in Canada (Kharbanda & Tewari, 1996) and is also often cited as an important disease of cruciferous vegetables there so presence of both *A. brassicicola* and *A. brassicae* is assumed (Cerkauskas et al., 1998; Tolman et al., 2004). It is not known if *A. brassicae* and *A. brassicicola* could migrate to NYS from these production regions and cause infection and mixed populations in NYS cabbage fields. Since growers have been reporting increased severity of ALS in their fields over the last several years, we hypothesize that mixed populations of *A. brassicae* and *A. brassicicola* may be present in NY fields.

Another reason for an observed increase in ALS severity could be a decreasing efficacy of fungicides used for disease control due to development of fungicide resistance in the pathogen population, which has been reported in *A. brassicicola* for several major fungicides in other locations, or in other plant pathogenic *Alternaria* spp. (Huang & Levy, 1995; Iacomi-Vasilescu et al., 2004b; Ma & Michailides, 2004a). ALS in brassica crops has historically been controlled using iprodione, a dicarboximide interfering with osmotic signal transduction, as a foliar spray or seed treatment (Babadoost et al., 1993; Humpherson-Jones & Maude, 1982a).

Chlorothalonil, a broad spectrum fungicide with multiple sites of action, has also been used extensively as a foliar spray to control ALS (Babadoost et al., 1993), and there are several newer fungicides which have become widely used to control ALS in NYS including quinone outside inhibitors, sterol biosynthesis inhibitors, and phenylpyrroles (Iacomi-Vasilescu et al., 2004b). However, the ability of *A. brassicicola* isolates to develop cross resistance to both dicarboximides and phenylpyrroles was demonstrated through continuous culture of these isolates on fungicide amended media in lab studies (Huang & Levy, 1995). Then in 2004, Iacomi-Vasilescu et al. discovered cross-resistant field isolates that were capable of growing on fungicide amended media directly out of the field, indicating *A. brassicicola* populations resistant to these two chemistries were present in radish and cabbage fields in France.

Perhaps the most widely used fungicide for controlling ALS in NYS is azoxystrobin, a quinone outside inhibitor (QoI) with a very specific mode of action that exerts very high selection pressure for resistant isolates within pathogen populations. Resistance to azoxystrobin has been documented in many *Alternaria* pathosystems including early blight of potato caused by *A. solani* (Rosenzweig et al., 2008), and late blight of pistachio caused by *A. tenuissima*, *A. alternata*, and *A. arborescens* (Ma & Michailides, 2004b). Given that other *Alternaria* spp. (as well as numerous plant-pathogenic fungi in other genera) have overcome sensitivity to azoxystrobin, we hypothesize that resistance may be occurring in NYS fields, resulting in increased disease severity and decreased or erratic efficacy of disease control with fungicides.

Another possible explanation for the increasing variation in disease severity may be that sexual recombination is occurring within the pathogen population, increasing the frequency of new genotypes that are more aggressive or could overcome sensitivity to fungicides. Recent research on populations of *A. brassicicola* infecting wild brassica hosts (*Cakile maritima*) in

Australia has provided evidence for sexual recombination in the fungus, which has not been previously reported (Bock et al., 2005; Linde et al., 2010). These studies used either amplified fragment length polymorphism (AFLP) or microsatellite markers to determine genotypic diversity of *A. brassicicola* populations and determined that the high levels of diversity observed could not be achieved through asexual reproduction alone.

In the present study, isolates of ALS causing *Alternaria* spp. were collected from a variety of brassica hosts across NYS. DNA extraction and polymerase chain reaction (PCR) using specific primers were conducted in order to reliably identify the species of the isolates. This work lays the foundation for future genetic characterization of the pathogen population that may elucidate the causes of increasing disease severity observed by NYS brassica growers.

Materials & Methods

Survey. Leaf tissue from brassica hosts showing ALS symptoms were collected from farms across NYS during the 2010 and 2011 growing seasons. Sample collection was accomplished through cooperation with Cornell Cooperative Extension personnel and other researchers who were notified of the present survey and submitted samples from farms across the state. An effort was made to collect samples from both conventional and organic systems and from as many host crops as possible. The final collection included 46 samples from 11 different counties and six different crop hosts.

Isolations. Pathogen isolations were performed for each sample received. This was accomplished by cutting a small square (about 1.5 cm) from the margin of leaf lesions using a sterile scalpel. The plant material was then surface sterilized by soaking in a 10% bleach solution for three minutes and was then rinsed by soaking for three minutes in deionized water.

The sterilized plant sample was then placed on a Petri dish containing a thin layer of Potato Dextrose Agar (PDA) and was incubated at room temperature for 7-10 days to allow the fungus to grow to fill the entire surface of the agar. If the culture was not pure, a 1.5 cm square containing only growth of *A. brassicicola* was transferred to a fresh plate in order to remove any contaminants. Once a pure culture was achieved, fungal material was transferred to slants prepared by adding 7 ml of PDA to 20 mm x 150 mm glass test tubes using a sterile, wire inoculating loop. The inoculated slant tubes were kept at room temperature for 7-10 days and were then sealed with parafilm and maintained at 2° C for long-term storage.

Single conidial cultures. Single conidial cultures were obtained from each field isolate in order to assure that the cultures did not contain mixed genotypes. These cultures were isolated by removing a 1.5 cm agar plug from each pure culture plate and placing it in a disposable, 1.5 ml microcentrifuge tube containing one ml of deionized water. The tube was inverted several times in order to create an aqueous suspension of fungal spores and mycelia. This suspension was diluted 1:10 and 1:100 in microcentrifuge tubes containing deionized water. A 50-100µl drop of each dilution was spotted onto a PDA plate and spread with a sterile, single-use cell spreader (LabScientific Inc., Livingston, NJ, USA) to achieve an even distribution of conidia across the agar surface. After 24 hours growth at room temperature, plates were observed using a stereomicroscope and individual, germinated conidia were removed, transferred to new PDA plates and maintained at room temperature for 7-10 days until growth of the fungus expanded across the entire plate. Three replicate single conidial cultures were made from each field isolate, and these are demarcated as #11XX-1, -2, and -3. Slants of each single conidial culture were prepared as described above for long-term storage of the isolate collection at 2°C.

DNA Extractions. DNA was extracted from each single conidial culture using the Qiagen DNEasy Plant MiniKit (Qiagen Inc., Valenica, CA, USA). Fresh culture plates of each single conidial isolate were made and 20 mg of fungal material was scraped from PDA plates into autoclaved, disposable, microcentrifuge tubes, each containing two 4.5 mm stainless steel beads which had been cleaned and autoclaved. Tubes containing fungal material were centrifuged for one minute at 13,000 rpm and were then shaken at 30 beats per second for one minute using a Qiagen TissueLyser II (Qiagen Inc., Valenica, CA, USA) bead beater in order to improve cell lysis. The Qiagen DNEasy mini protocol for purification of total DNA from plant tissue was followed according to manufacturer's instructions, except that only 1µl of RNAse A was used per tube and DNA was eluted in 50µl ultra-pure water after incubating the spin filter for 5 minutes at 65°C.

PCR. The extracted genomic DNA was used as template and amplified by polymerase chain reaction (PCR) using species specific primers which were designed and tested for specificity by Iacomi-Vasilescu et al. (2002). The primer pairs ABRA1/ABRA2 (5'-aaggcgagtctccagcaaactg-3'/5'-actcacctcagcagcatctgctgt-3') and ABRE1/ABRE3(5'-aaggcgagtctccagcaaacta-3'/5'-tgaaatctctcgagacgacg-3') were used to amplify *A. brassicicola* and *A. brassicae*, respectively. Conventional PCR was performed using 1 µl of undiluted DNA preparations in 25 µl reactions under the following conditions: 1X GoTaq Green Reaction Buffer containing 1.5 mM $MgCl_2$ (Promega Corp., Madison, WI, USA)., 200 µM of each deoxyribonucleotide triphosphate, 0.2 mM each primer, and 1.25 units of thermostable DNA polymerase (Promega Corp., Madison, WI, USA). Reactions were performed using an Eppendorf Mastercycler Gradient thermocycler (Eppendorf, Hauppauge, NY, USA) with an initial denaturation step of 3 min at 94°C; followed by 35 cycles of 30 s of denaturation at 94°C, 50 s of

annealing at 55°C for *A. brassicicola* or 60°C for *A. brassicae*, and 1 min of elongation at 72°C; and a final elongation step of 10 min at 72°C. The amplification results were visualized after electrophoresis of an aliquot (5 µl) of the reaction product on a 1% agarose gel with 1% ethidium bromide stain.

Results

The final collection of field isolates included 46 samples from 11 different counties and six different crop hosts (Table 1, Figure 1). Samples were collected from all of the major cabbage producing regions—Monroe, Genesee, Orleans, Ontario, and Niagara Counties—except for Niagara County (Figure 1). While most of the samples collected in the survey came from Ontario County (43.5% of the total), the distribution of samples was widespread, with samples collected from far Eastern NY—including Suffolk, Ulster, Rensselaer, and Washington counties—and several samples collected from organic farms located in Tompkins County in South Central NY. Samples from organically managed fields made up 28.3% of the surveyed isolates and these were collected from Ontario, Tompkins, and Washington counties.

Five samples (#1134, 1145, 1148, 1152, and 1156)were not successfully purified in the lab so the culture collection consisted of 41 isolates from which single conidial cultures were successfully isolated. Results of PCR amplification of these 123 samples (three single conidial isolates of each of the 41 field isolates) with both ABRA1/ABRA2 and ABRE1/ABRE3 primer sets positively identified all samples as *A. brassicicola* (Figure 2). An amplicon was produced from DNA of isolate 1150 using the ABRE1/ABRE3 primer set specific to *A. brassicae*. This sample also had a unique growth habit, being somewhat lighter in color and producing more aerial mycelia giving it a fluffier appearance, which is often how *A. brassicae* is described.

However, the PCR was repeated several times and in all subsequent attempts DNA of isolate 1150 only produced an amplicon using the ABRA1/ABRA2 primer set specific to *A. brassicicola*. Therefore sample #1150 was considered to have been positively identified as *A. brassicicola.*

Table 1.1. Field isolates collected from farms across NYS during 2010-12.

Isolate #	Collected By	Collection Date	Host (Variety)	County	Cropping System
1121	J. Kikkert	8/24/2010	Cabbage	Ontario	Conventional
1122	J. Kikkert	8/27/2010	Cabbage	Orleans	Conventional
1123	C. Smart	8/30/2012	Cabbage	Wayne	Conventional
1126	J. Strauss	9/24/2010	Cabbage	Ontario	Conventional
1128	H. Dillard	10/14/2010	Cauliflower	Ontario	Organic
1129	H. Dillard	10/14/2010	Cabbage	Ontario	Organic
1130	H. Dillard	10/14/2010	Cauliflower	Ontario	Organic
1133	H. Dillard	10/14/2010	Cabbage (Constellation)	Orleans	Conventional
1134	H. Dillard	10/14/2010	Cabbage (Novatore)	Monroe	Conventional
1135	H. Dillard	10/14/2010	Cauliflower	Monroe	Conventional
1136	H. Dillard	10/14/2010	Cabbage	Monroe	Conventional
1137	H. Dillard	10/14/2010	Cabbage (Amtrak)	Monroe	Conventional
1138	J. Strauss	11/2/2010	Cabbage	Ontario	Conventional
1139	J. Strauss	11/2/2010	Cabbage	Ontario	Conventional
1140	J. Strauss	11/2/2010	Cabbage	Ontario	Conventional

Table 1.1 (Continued)

Isolate #	Collected By	Collection Date	Host (Variety)	County	Cropping System
1141	J. Strauss	11/2/2010	Cabbage	Ontario	Conventional
1142	J. Strauss	3/27/2011	Brussels Sprouts	Seneca	Conventional
1144	T. Rusinek	8/19/2011	Kale (Red Russian)	Ulster	Conventional
1145	H. Dillard	9/13/2011	Cabbage	Ontario	Organic
1146	M. McGrath	10/3/2011	Brussels Sprouts	Suffolk	Conventional
1147	H. Dillard	9/13/2011	Cauliflower	Ontario	Conventional
1148	H. Dillard	9/13/2011	Cauliflower	Ontario	Conventional
1149	H. Dillard	9/29/2011	Broccoli	Ontario	Organic
1150	H. Dillard	9/13/2011	Cabbage	Ontario	Conventional
1151	H. Dillard	9/13/2011	Cabbage	Ontario	Conventional
1152	H. Lange	8/16/2011	Cabbage	Ontario	Conventional
1153	A. Dunn	9/27/2011	Cabbage	Rensselaer	Conventional
1155	J. Strauss	9/27/2011	Red Cabbage	Ontario	Conventional
1156	C. Hoepting	10/7/2011	Cabbage (Constellation)	Monroe	Conventional

Table 1.1 (Continued)

Isolate #	Collected By	Collection Date	Host (Variety)	County	Cropping System
1157	J. Strauss	9/27/2011	Cabbage	Ontario	Conventional
1158	J. Strauss	9/27/2011	Cabbage	Ontario	Conventional
1160	A. Seaman	11/7/2011	Kale	Ontario	Conventional
1161	L. McDermott	10/13/2011	Cabbage (Reaction)	Washington	Conventional
1162	C. Hoepting	11/4/2011	Brussels sprouts	Monroe	Conventional
1163	C. Hoepting	11/22/2011	Cabbage	Orleans	Conventional
1164	C. Hoepting	11/22/2011	Cabbage	Orleans	Conventional
1165	C. Hoepting	11/22/2011	Broccoli	Genessee	Conventional
1166	C. Hoepting	11/22/2011	Cauliflower	Genessee	Conventional
1167	C. Hoepting	11/22/2011	Cabbage	Genessee	Conventional
1168	S. Scheufele	12/4/2011	Brussels Sprouts	Tompkins	Organic
1169	S. Scheufele	12/4/2011	Brussels sprouts	Tompkins	Organic
1170	S. Scheufele	12/10/2011	Cabbage	Tompkins	Organic
1171	S. Scheufele	12/4/2011	Kale (Toscano)	Tompkins	Organic

Table 1.1 (Continued)

Isolate #	Collected By	Collection Date	Host (Variety)	County	Cropping System
1172	S. Scheufele	12/10/2011	Kale (Winterbor)	Tompkins	Organic
1173	S. Scheufele	12/10/2011	Cabbage (Deadon)	Tompkins	Organic
1174	S. Scheufele	12/04/2011	Kale (Winterbor)	Tompkins	Organic

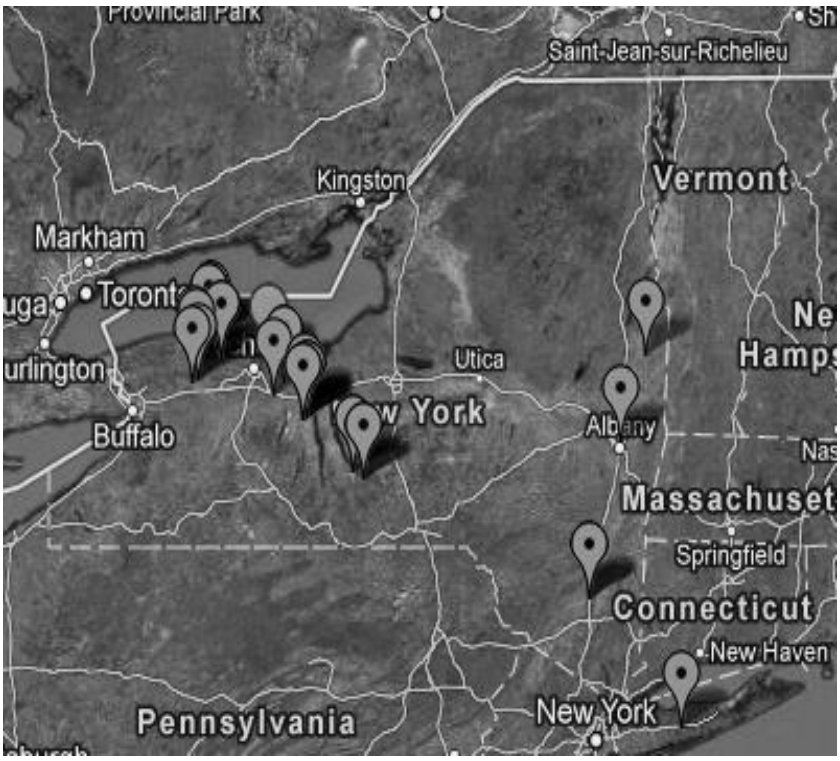

Figure 1.1. Map showing sampling locations of field isolates collected from across NYS during the 2010 and 2011 growing seasons.

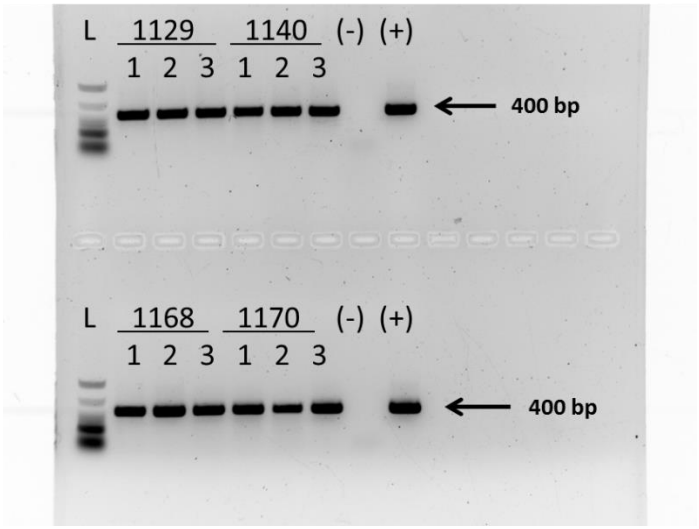

Figure 1.2. Gel showing examples of PCR amplicons produced using template DNA extracted from isolates 1129, 1140, 1168 and 1170 with the primer pair ABRA1/ABRA2. Low molecular weight ladder (L) was used to confirm size of bands was approximately 400 bp. Ultra-pure DI water was used as a negative control and sample 1123-1 was used as a positive control.

Discussion

ALS disease severity and efficacy of fungicides to control disease outbreaks has been increasingly variable in NYS over the last several years. Therefore, the current survey was conducted in order to confirm the species of the causal organism as *A. brassicicola* as a first step in the process of characterizing the genetic diversity of the pathogen population. The survey resulted in 46 samples from 11 counties across NYS and samples were collected from at least six different crop hosts during the 2010 and 2011 growing seasons. Of the field samples collected, 41 were successfully cultured in the lab and genomic DNA was extracted from single conidial cultures. The results of PCR tests using primers specific for *A. brassicicola* and another species capable of causing similar symptoms on brassica crops, *A. brassicae*, positively identified all 41 isolates as *A. brassicicola*. This confirmed our hypothesis that only *A. brassicicola* is found infecting vegetable brassicas in NYS.

This finding was expected because, while *A. brassicae* can infect vegetable brassicas (*B. oleracea*), it is typically found infecting oilseed and mustard crops (*B. rapa, B. juncea* and *B. napus*)—crops that are not commonly grown in NYS (Humpherson-Jones, 1983). This indicates that the variation we see in the field is not caused by mixed populations of the two fungi, but rather, is an effect of genetic diversity within the *A. brassicicola* population. Evaluation pathogenicity, virulence and fungicide sensitivity of each isolate is currently being carried out. These assays will provide important information about variability in virulence and fungicide sensitivity of *A. brassicicola* populations in NYS, and in fact, have already begun to provide evidence that there are significant differences in both parameters among the surveyed isolates. Characterizing the genetic basis for these observed differences is the next step in understanding

the diversity and importantly, whether or not fungicide resistance has developed or if sexual recombination is occurring within the population of *A. brassicicola* in NYS.

There are many tools available for analyzing genetic diversity of this pathogen because *A. brassicicola* has become a model organism in the field of plant-microbe interactions due to its extensive production of host-specific toxins (HSTs). The infection biology and HST production has been widely studied in the model host *Aribidopsis thaliana*, a member of the *Brassicaceae* (Lawrence et al., 2008). Furthermore, the genome of *A. brassicicola* has been sequenced and microsatellite primers have been developed, allowing for easier comparisons of genetic and genotypic diversity between isolates to be made (Avenot et al., 2005a; Lawrence et al., 2008). These types of investigations will allow researchers to determine if variation in disease severity experienced by growers in NYS is a result of fungicide resistance or is due to increasing diversity in virulence of isolates. A study by Avenot et al. (2005) made use of these genetic tools and identified the *A. brassicicola* gene that confers resistance or sensitivity to dicarboximides and phenylpyrroles. Once the genetic cause for differences in fungicide sensitivity among isolates is known, it can be used to develop detection techniques that can be used to help manage fungicide resistance in the field. This was recently accomplished in Alternaria leaf blight of pistachio in California, where researchers developed a quantitative method of detecting azoxystrobin-resistant isolates in the field using real-time PCR with primers specific to the resistance-causing mutation in the cytochrome b gene (Ma & Michailides, 2004b).

The present study has served as a first step in the process of characterizing *A. brassicicola* population structure and diversity in NYS. Future experiments could elucidate population genetic structure and, specifically, whether or not sexual recombination is occurring

or if fungicide resistance has developed in these populations. This kind of information could help to design more successful management systems to control Alternaria leaf spot in NYS.

REFERENCES

Avenot H, Dongo A, Bataille-Simoneau N, *et al.*, 2005a. Isolation of 12 polymorphic microsatellite loci in the phytopathogenic fungus Alternaria brassicicola. *Molecular Ecology Notes* **5**, 948-50.

Avenot H, Simoneau P, Iacomi-Vasilescu B, Bataille-Simoneau N, 2005b. Characterization of mutations in the two-component histidine kinase gene AbNIK1 from Alternaria brassicicola that confer high dicarboximide and phenylpyrrole resistance. *Current Genetics* **47**, 234-43.

Babadoost M, Gabrielson RL, Olson SA, Mulanax MW, 1993. Control of Alternaria diseases of brassica seed crops caused by *Alternaria brassicae* and *Alternaria brassicicola* with ground and aerial fungicide applications. *Seed Science and Technology* **21**, 1-7.

Bock C, Thrall P, Burdon J, 2005. Genetic structure of populations of *Alternaria brassicicola* suggests the occurrence of sexual recombination. *Mycological Research* **109**, 227-36.

Canada CCO, 2011a.The Economic Impact of Canadian Grown Canola and its End Products on the Canadian Economy. March 7, 2013.

Canada S, 2011b.2011 Census of Agriculture, Farm and Farm Operator Data. March 13, 2013.June 05 2012. <http://www29.statcan.gc.ca/ceag-web/eng/geo-geo>

Cerkauskas RF, Stobbs LW, Lowery DT, Van Driel L, Liu W, Vanschagen J, 1998. Diseases, pests, and abiotic problems associated with oriental cruciferous vegetables in southern Ontario in 1993-1994. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* **20**, 87-94.

Huang RG, Levy Y, 1995. Characterization of iprodione-resistant isolates of *Alternaria brassicicola*. *Plant Disease* **79**, 828-33.

Humpherson-Jones FM, 1983. The occurrence of *Alternaria brassicicola, Alternaria brassicae* and *Leptosphaeria maculans* in brassica seed crops in Southeast England between 1976 and 1980. *Plant Pathology* **32**, 33-9.

Humpherson-Jones FM, 1992. Epidemiology and Control of Dark Leaf Spot of Brassicas. In: Chelkowski J, Visconti A, eds. *Alternaria Biology, Plant Diseases and Metabolites.* New York, NY: Elsevier, 267-88.

Humpherson-Jones FM, Maude RB, 1982a. Control of dark leaf spot (*Alternaria brassicicola*) of *Brassica oleracea* seed production crops with foliar sprays of iprodione. *Annals of Applied Biology* **100**, 99-104.

Humpherson-Jones FM, Maude RB, 1982b. Studies on the epidemiology of *Alternaria brassicicola* in *Brassica oleracea* seed production crops. *Annals of Applied Biology* **100**, 61-71.

Iacomi-Vasilescu B, Avenot H, Bataillé-Simoneau N, Laurent E, Guénard M, Simoneau P, 2004. In vitro fungicide sensitivity of *Alternaria* species pathogenic to crucifers and identification of *Alternaria brassicicola* field isolates highly resistant to both dicarboximides and phenylpyrroles. *Crop Protection* **23**, 481-8.

Kharbanda PD, Tewari JP, 1996. Integrated management of canola diseases using cultural methods. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* **18**, 168-75.

Lawrence CB, Mitchell TK, Craven KD, Cho Y, Cramer RA, Kim KH, 2008. At death's door: *Alternaria* pathogenicity mechanisms. *Plant Pathology Journal* **24**, 101-11.

Linde CC, Liles JA, Thrall PH, 2010. Expansion of Genetic Diversity in Randomly Mating Founder Populations of Alternaria brassicicola Infecting Cakile maritima in Australia. *Applied and Environmental Microbiology* **76**, 1946-54.

Ma ZH, Michailides TJ, 2004a. Characterization of iprodione-resistant *Alternaria* isolates from pistachio in California. *Pesticide Biochemistry and Physiology* **80**, 75-84.

Ma ZH, Michailides TJ, 2004b. A real-time PCR assay for the detection of azoxystrobin-resistant *Alternaria* populations from pistachio orchards in California. *Crop Protection* **23**, 1259-63.

Rosenzweig N, Olaya G, Atallah ZK, Cleere S, Stanger C, Stevenson WR, 2008. Monitoring and tracking changes in sensitivity to azoxystrobin fungicide in *Alternaria solani* in Wisconsin. *Plant Disease* **92**, 555-60.

Rotem J, 1994. *The Genus Alternaria: Biology, Epidemiology, and Pathogenicity*. St. Paul, MN: APS Press.

Tolman JH, Mcleod DGR, Harris CR, 2004. Cost of crop losses in processing tomato and cabbage in southwestern Ontario due to insects, weeds and/or diseases. *Canadian Journal of Plant Science* **84**, 915-21.

CHAPTER 3

EFFICACY OF BIO-FUNGICIDES AND SOIL AMENDMENTS TO CONTROL ALTERNARIA LEAF SPOT OF CABBAGE

Introduction

Alternaria leaf spot (ALS) is a perennial disease in New York State (NYS) affecting all crop hosts in the family *Brassicaceae*. The disease, caused by *Alternaria brassicicola*, is of growing concern to NYS cabbage growers because of decreasing efficacy of synthetic fungicides to control the disease (Dillard, 2011; Iacomi-Vasilescu et al., 2004a). There has also been a surge of interest in organic vegetable production, as consumer concerns over the effects of conventional agricultural practices on human health and the environment continue to grow. The Census of Agriculture conducted by the NASS showed that, in 2007 there were 91,465 acres of cropland in organic production in NYS, with an additional 30,687 acres in transition to organic production. Organic cabbage production in NYS has increased 406% from 2008 to 2011. (NASS 2011 Organic Production Survey-NY). These two trends highlight the need for new strategies to control ALS that can be used by organic growers and those seeking alternatives to synthetic fungicides.

Control practices available to organic growers are essentially limited to cultural methods such as crop rotation, use of clean seeds, control of brassicaceous weeds, and control of insect pests that spread disease (Dillard et al., 2004; Dillard et al., 1998; Humpherson-Jones, 1992; Kharbanda & Tewari, 1996; Seaman, 2012). There are a number of fungicides registered for use in organic production listed for control of ALS in NYS but the efficacy of these products is

not known (Seaman, 2012). The active ingredients of these products are biological control organisms or plant defense activators, which have direct activity against plant pathogens or affect the plants ability to ward off pathogens. The present studies were undertaken in order to test the efficacy of some of these products, as well as two soil amendments with disease suppressive potential. All of the materials tested are registered for use in certified organic systems, and are listed by the Organic Materials Review Institute (OMRI).

Biological control is defined by Baker and Cook (1974) as: "the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists" (Baker & Cook, 1974). Underlying mechanisms of biological control, as reviewed extensively by Whipps (2001), are: (i) competition for nutrients and space in the rhizosphere; (ii) competition for iron by production of iron-chelating siderophores; (iii) inhibition of the pathogen by antimicrobial compounds; (iv) direct parasitism; (v) degradation of toxins produced by the pathogen; and (vi) induction of jasmonate and ethylene mediated systemic defense pathways. The relative importance of each of these mechanisms varies depending on the biocontrol organism, but it is believed that multiple mechanisms work simultaneously to provide plant protection. Biocontrol organisms have the potential to provide durable protection from plant disease because of these complex and non-specific modes of action, which exert less selection pressure on pathogen populations than do fungicides with more specific modes of action (Baker & Cook, 1974; Cook & Baker, 1983; Whipps, 2001).

The realization that induced systemic resistance (ISR) is an underlying mechanism of biological control and the availability of new methods for studying the physiological and

biochemical processes involved in ISR have resulted in a proliferation of research on the subject over the past two decades (De Vleesschauwer & Hofte, 2009; Vallad & Goodman, 2004; Van Loon, 1997; Whipps, 2001). ISR is "the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (inducing agents)" (Kloepper et al., 1992). The ISR response is mediated by the plant hormones jasmonate and ethylene (Pieterse et al., 2002; Vallad & Goodman, 2004) and is often incited by plant growth promoting rhizobacteria (PGPR) in the soil (De Vleesschauwer & Hofte, 2009; van Loon et al., 1998; Whipps, 2001). These microbes foster plant health by: reducing effects of minor soil-borne pathogens; by producing auxin, a plant hormone that stimulates plant growth; or by decreasing ethylene production in soil, thereby increasing root growth (Heil & Walters, 2009; Whipps, 2001).

Soil management practices such as tillage, crop rotation, and organic matter amendments are known to cause shifts in density and composition of microbial communities, and can therefore influence microbially-mediated plant defense responses (Hoitink & Boehm, 1999; Hoitink & Fahy, 1986; Lehmann et al., 2011; Vallad & Goodman, 2004; Whipps, 2001). Efficacy of soil-applied treatments in the present studies will indicate an induction of systemic plant defenses since the treatments will never come in physical contact with the foliar pathogen. Microbes applied to foliage can also trigger a systemic plant defense response known as systemic acquired resistance (SAR) (Durrant & Dong, 2004; Vallad & Goodman, 2004). The SAR response is controlled by another signaling pathway (the salicylic acid —SA—pathway) which induces production of pathogenesis-related (PR) proteins, causing a hypersensitive response (Durrant & Dong, 2004; Hammerschmidt, 2009; Vallad & Goodman, 2004).

Of the ten materials tested in this study (Table 3.1), Regalia, Serenade and Double Nickel are all labeled for control of ALS in cole crops, though efficacy data is not available for any of these materials (Seaman, 2012). Regalia is an extract of Giant knotweed (*Reynoutria sachalinensis*) which controls foliar and root diseases by activating plant defense systems (Daayf et al., 2000; Daayf et al., 1997; Konstantinidou-Doltsinis et al., 2006; Konstantinidou-Doltsinis & Schmitt, 1998), while Serenade and Double Nickel are biological control organisms belonging to the bacterial genus *Bacillus* and they protect plants from disease by inducing plant defenses, promoting plant growth, and producing antibiotic secondary metabolites (Wulff et al., 2002b)

Sonata, Rootshield, and Mycostop are commonly used pesticides that are not registered specifically for ALS control, but are listed for use in controlling other diseases on vegetable brassicas. Sonata is another *Bacillus* sp. (*Bacillus pumilis* strain QST 2808) that has been shown to protect plants from disease by inducing systemic resistance and promoting plant growth (Benhamou et al., 1996; Lian et al., 2011; Murphy et al., 2000; Ryu et al., 2004; Sari et al., 2007). Rootshield is formulated from a proprietary strain (KRL-AG2) of the fungus *Trichoderma harzianum* which controls soil and foliar diseases by outcompeting pathogenic microbes for space or nutrients, directly parasitizing other microbes, and inducing plant defenses (Ahmad & Baker, 1987; Dandurand et al., 2000; De Meyer et al., 1998; Elad, 2000; Whipps, 2001; Yedidia et al., 1999). The active ingredient of Mycostop is a commercial preparation of the soil bacterium *Streptomyces griseoviridis* strain K61 which is thought to protect plants from soil-borne root and stem rotting pathogens by outcompeting other soil microbes for space and nutrients in the rhizosphere, by producing antifungal metabolites, and by promoting plant growth (Kortemaa et al., 1997; Minuto et al., 2006; Tuomi et al., 1994).

Both Rootshield and Mycostop are only labeled for soil applications and are not intended for use on edible foliage such as cabbage heads. Successful disease control in these treatment groups would indicate an induction of plant defense responses and show that rhizosphere microorganisms are able to prime plants for defense against foliar pathogens in cabbage (De Meyer et al., 1998; Elad, 2000; Elad et al., 2010; Elmer & Pignatello, 2011; Kolton et al., 2011; Whipps, 2001; Yedidia et al., 1999).

Two of the treatments investigated were soil organic matter amendments—biochar and vermicompost—with potential disease suppressive properties. Biochar produced from Southern Yellow Pine woodchips was obtained from Waste to Energy Solutions Inc, Destin, FL. Chemical analysis and a greenhouse study on effects of this material on cabbage growth and ALS severity demonstrated that it is comparable to other biochars made from wood substrates (see Appendix B) and has no phytotoxic effects on cabbage. Vermicompost was obtained from WormPower (Avon, NY). This particular compost, with NPK ratio of 1.5-0.7-1.5, has been used experimentally in several vegetable systems and has been shown to increase plant growth and yield, as well as suppress root rotting pathogens of pea and turf grass (Chen et al., 2012; Jack et al., 2011). Biochar that is made from burning of plant or animal material, and vermicompost are both approved by the Organic Materials Review Institute (OMRI), and are listed for use by certified organic growers as soil amendments.

The goals of the present studies were to address the lack of options available to organic cabbage growers in NYS for controlling Alternaria leaf spot by testing the efficacy of (i) OMRI-listed biological control organisms and (ii) OMRI-listed soil organic amendments for control of the disease. Efficacy of these materials was tested in a field experiment in which the biocontrol organisms were applied to the foliage (except in the cases of Mycostop and Rootshield), and also

in a greenhouse study in which all materials were applied to the soil. The greenhouse study was undertaken in order to determine whether any of the materials were able to elicit an ISR or PGPR response in cabbage. If disease control could be achieved in this fashion it would make application of the biocontrol organisms easier for growers since they could be used as transplant dips or soil drenches as opposed to having to make repeated sprays throughout the growing season. These studies were not designed to directly elucidate underlying mechanisms of disease control, but induced systemic resistance is hypothesized in the case of soil-applied biological treatments or organic matter amendments.

Materials and Methods

Greenhouse Study

Experimental Design. The experiment was conducted in a greenhouse at the New York State Agricultural Experiment Station in Geneva, NY where plants were grown under natural light and a maximum temperature of 24 °C. A randomized complete blocks design was used with each treatment replicated six times. Soil organic matter amendments were mixed with Cornell Mix potting soil —a mixture of peat, perlite, and vermiculite at a ratio of 4:1:1— at the rates given in Table 1. These rates have been used in previous studies and have shown improved plant health and yield (Jack et al., 2011; Rajkovich et al., 2011). For all other treatments pots were filled with Cornell Mix potting soil.

Cabbage seeds ("Gonzales", Johnny's Selected Seeds, Waterville, ME) were sown on 17 May into 2.54 cm square plastic pots and plants were maintained in the greenhouse for about 8 weeks. The plants were fertilized once on 22 June with Miracle Grow All Purpose Plant Food

(24-8-16) at 3.95 g/L (Scotts Company, LLC, Marysville, OH, USA). Treatments were applied as soil drenches on 17 July at the application rates given by the manufacturers (Table 1) and were distributed in a volume of 150 ml per pot. After a 24 hour latent period, plants were moved into a moist chamber (relative humidity of 100%, temperature of 21 °C, and 12 hour light/dark periods) where they were inoculated with a suspension of *A. brassicicola* spores.

The fungal inoculum was produced using *A. brassicicola* isolate #1122, which was collected from cabbage growing in Orleans County, NY. A single conidial culture of this isolate (#1122-3) was grown in 100mm by 15 mm Petri dishes containing Difco Bacto Potato Dextrose Agar (BD Biosciences, Franklin Lakes, NJ, USA). A conidial suspension was made by flooding each culture plate with sterilized de-ionized water, gently scraping the agar surface with a rubber policeman, and straining the suspension through cheesecloth to remove mycelial fragments. The concentration of conidia was determined using a haemocytometer, the inoculum was diluted to achieve 10^4 spores/ml, and Tween 20 was added at 0.05% in order to attain an even distribution of spores on waxy cabbage leaves. The inoculum was sprayed on cabbage foliage on 18 July using handheld aerosol spray bottles (Thermo Fisher Scientific, Waltham, MA, USA) and plants were kept in the moist chamber for 48 hours in order to encourage successful infection. Plants were returned to the greenhouse where they were monitored every 2-3 days for two weeks and disease severity was measured by estimating the percent leaf area covered in ALS lesions.

Statistical analyses. Disease severity data was used to calculate area under the disease progress curve (AUDPC), a method for quantifying disease development over time. AUDPC was calculated for each set of time-points, and was then summed over all time-points to yield an overall AUDPC. Efficacy of bio-fungicides and soil amendments to reduce disease severity was evaluated using a mixed linear model, with AUDPC as the dependent variable, and treatment and

random block as the independent variables. One sample (Double Nickel treatment in block 4) never showed signs of disease and including it in the analysis caused the residuals to be non-normally distributed. Therefore, this sample was considered an outlier and was left out of all statistical tests. Tukey's honestly significant difference was used to separate treatment means. All analyses were performed using JMP Pro version 10.0.0 (SAS Institute Inc, Cary, NC, USA).

Table 3.1. Rates, application methods, and active ingredients for treatments used in the greenhouse study.

Treatment	Rate	Active Ingredient	Application Method
Control…….	--	None	drench
Regalia…..……...……..	1%	*Reynoutria sachalinensis* extract	drench
Double Nickel 55 WDG.…….	0.1 kg/100 L	*Bacillus amyloliquefaciens* D747	drench
Rootshield WP……...…….	0.15ml/380 L	*Trichoderma harzianum* strain KRL-AG2	drench
Mycostop……..…….	0.10%	*Streptomyces griseovirdis* Strain K61	drench
Serenade ASO..…..……..……	9.4 L/ha	*Bacillus subtilis* strain QST 713	drench
Sonata ASO..……..……	9.4 L/ha	*Bacillus pumilus* strain QST 2808	drench
Vermicompost……..……...……	20% v/v	Vermicompost	soil
Biochar……...…….……..	0.2% w/w	Southern Yellow Pine Chip Biochar	soil
Biochar……..…….……..	2.0%w/w	Southern Yellow Pine Chip Biochar	soil

Field Study

Plant materials. Cabbage seeds ("Gonzales", Johnny's Selected Seeds, Waterville, ME) were sown on 21 May in 72-cell flats containing Cornell Mix potting soil-a mixture of peat, perlite, and vermiculite at a ratio of 4:1:1. Seedling flats were maintained for five weeks under greenhouse conditions with natural light and maximum daytime temperature of 24 C° and were fertilized once, on June 22, with Miracle Grow All Purpose Plant Food (24-8-16) at 3.95 g/L (Scotts Company, LLC, Marysville, OH, USA). On 25 May, seedling flats were treated with a soil drench of imidacloprid (Admire Pro, Bayer CropScience, Research Triangle Park, NC) at 550 ml/ha and were then moved into outdoor cold frames to harden off for one week before being transplanted.

Field preparation. The experiment was carried out at the New York State Agricultural Experiment Station in Geneva, NY in a field with soil classified as Lima silt loam. A randomized complete block design was used, with four replicates of 7.62 m x 0.76 m for each of the ten treatments. Five foot buffers were planted between replicate plots and around the perimeter of the experimental area.

The experimental area was measured and flagged so that the two organic matter amendments could be incorporated into the soil before seedlings were transplanted into the field. Biochar was applied at a rate of two tons per acre (corresponding to about 0.2% w/w (Rajkovich et al., 2011)), and vermicompost was applied at a rate of 3.2 tons per hectare. In order to prevent loss of material and soil under extremely dry and windy conditions, the materials were weighed out in the lab and then applied by hand on 1 July. Manual incorporation of soil amendments was accomplished by spreading the material evenly across each replicate plot, mixing into the top 15 cm of soil with a spade, and raking the area to smooth out the amended soil.

Seedlings were transplanted on 3 July using a two-row transplanter at 0.76 m row centers and 0.46 m plant spacing. A water wheel was used to irrigate and fertilize transplants with Peter's Excel 15-5-15 (N-P-K) starter fertilizer at a rate of 0.68 kg/190 L. S-metalochlor (Dual Magnum EC, Syngenta Crop Protection LLC, Greensboro, NC, USA) was applied after transplanting (5 July) at a rate of 1.16 L/ha to control weeds and one application of zeta-cypermethrin plus bifenthrin (Hero EW, FMC Corp, Philadelphia, PA) at a rate of 440 ml/ha was used on 27 July to control Flea beetles (*Phyllotreta cruciferae*) and lepidopteran pest populations which were quite high in this field. Overhead irrigation was used (1.25 cm rain per application) on 10 and 18 July and 6, 13, 20, 27 and 31 August in order to maintain plant health and encourage disease development during an extremely hot and dry growing season.

Treatments. Pesticides approved for organic production and a conventional synthetic fungicide, Quadris (Syngenta Crop Protection LLC, Greensboro, NC, USA) were applied on 2 August at the recommended labeled rates (Table 3.2). Most treatments were applied to the foliage using a CO_2 sprayer equipped with three 8003 flat fan nozzles delivering 2 L de-ionized water per treatment (470 L/ha) at 50psi. However, two of the treatments (Mycostop and Rootshield) which are not registered for use on leafy vegetables were applied to the soil as drenches using 30 L of de-ionized water per treatment. All materials were measured out in the laboratory and transported to the field site in disposable centrifuge tubes so that they could be mixed with de-ionized water immediately preceding application.

Table 2.2. Rates, application methods, and pesticide labeling information for treatments used in the field study.

Treatment, Rate/ha	Application Method	Active Ingredient	Labeled for ALS	Labeled for Cole Crops
Control……..	foliar	Deionized H_2O	--	--
Regalia, 7.0 L……	foliar	*Reynoutria sachalinensis* extract	X	X
Mycostop, 2.23L…….	drench	*Streptomyces griseovirdis* Strain K61	--	X
Serenade ASO, 14.1 L…….	foliar	*Bacillus subtilis* strain QST 713	X	X
Sonata ASO, 9.40 L…….	foliar	*Bacillus pumilus* strain QST 2808	--	X
Double Nickel 55 WDG, 1.1 kg	foliar	*Bacillus amyloliquefaciens* D747	X	X
Rootshield WP, 2.35 L…….	drench	*Trichoderma harzianum* strain KRL-AG2	--	X
Quadris, 1.0 L...…….……	foliar	Azoxystrobin	X	X
Vermicompost , 3.21 t……..	soil	Vermicompost	--	--
Biochar, 4.95 t…..……..……	soil	Southern Yellow Pine Chip Biochar	--	--

Pathogen inoculation. Fungal inoculum was produced using *A. brassicicola* isolate #1153, which was collected from cabbage plants growing in Rensselaer County, NY (see Appendix A). A single conidial culture of this isolate (#1153-3) was grown in 100mm by 15 mm Petri dishes containing Difco Bacto Potato Dextrose Agar (BD Biosciences, Franklin Lakes, NJ, USA). A conidial suspension was made by flooding each culture plate with de-ionized water, gently scraping the agar surface with a rubber policeman, and straining the suspension through cheesecloth to remove mycelial fragments. The concentration of conidia was determined using a haemocytometer and the inoculum was then diluted to achieve 10^6 spores/ml. The inoculum was

sprayed on cabbage plants on 3 August using a SP Systems SP1 backpack sprayer at a rate of 235 L/ha.

Efficacy of treatments to control Alternaria leaf spot on cabbage. Ten cabbage plants in the center of each replicate plot were observed weekly over a 34 day period and were rated for severity of ALS symptoms. Disease severity was measured by estimating the percent leaf area affected. On 4 September, once the disease had progressed and the cabbage heads had reached marketable size (10-15 cm in diameter), the experiment was harvested and yield data was taken. Ten cabbage heads per replicate plot were harvested by hand and their total weight was measured. Marketable yield was assessed by removing any outer leaves showing ALS symptoms from each of the ten cabbage heads and then re-weighing them.

Statistical analyses. Disease severity data was used to calculate area under the disease progress curve (AUDPC), a method for quantifying disease development over time. AUDPC was calculated by determining the average disease severity between each set of time-points, and summing over all time-points. Efficacy of each treatment to reduce disease severity was evaluated using a mixed linear model, with AUDPC or yield as the dependent variable, and treatment and random block as the independent variables. Tukey's honestly significant difference was used to separate treatment means. All analyses were performed using JMP Pro version 10.0.0 (SAS Institute Inc, Cary, NC, USA).

Results

Greenhouse Study

Efficacy of treatments to control Alternaria leaf spot on cabbage. Disease symptoms spread quickly and evenly on all cabbage plants except one plant in block four which had received the Double Nickel treatment. This was likely due to inadequate coverage with the inoculum and therefore, this sample was not included in the statistical analyses. No data was collected on plant growth or vigor, but cabbage plants in the vermicompost treatment group were observably larger than those from the control group (Figure 3.1).

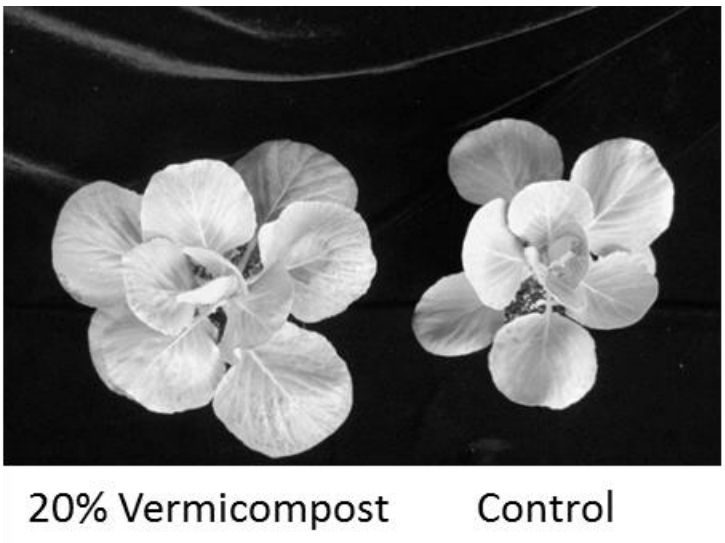

Figure 3.1. Photograph of a vermicompost amended cabbage plant showing increased growth compared to an untreated control plant.

None of the treatments studied had a significant effect on ALS disease severity compared to the untreated control (Table 3.3, Figure 3.2). There were, however, statistical differences between ALS severity on plants from the best treatment —vermicompost— and those from the worst treatments—biochar at 2.0% and 0.2% (w/w), Serenade, Sonata, and Double Nickel (Table 3.3, Figure 3.2).

Table 3.3. Efficacy of bio-fungicides and organic matter amendments to control ALS in the greenhouse.

	Mean Disease Severity[z]					
Treatment, Rate/ha	2 dpi[y]	5 dpi	8 dpi	12 dpi	15 dpi	AUDPC
Control……..	0.07 a	0.35 ab	0.47 bc	0.58 ab	0.61 ab	6.38 ab
Regalia, 7.0 L…..……..	0.08 a	0.40 ab	0.52 abc	0.56 ab	0.63 ab	6.71 ab
Double Nickel 55 WDG, 1.1 kg	0.08 a	0.41 ab	0.53 abc	0.49 a	0.66 b	6.98 a
Rootshield WP, 2.35 L…..……	0.06 a	0.37 ab	0.50 abc	0.54 ab	0.58 ab	6.33 ab
Mycostop, 2.23 L……..…	0.08 a	0.35 ab	0.53 abc	0.58 ab	0.63 ab	6.68 ab
Serenade ASO, 14.1 L…..……	0.10 a	0.45 b	0.55 abc	0.62 b	0.67 b	7.35 a
Sonata ASO, 9.40 L…..……	0.13 a	0.42 b	0.50 abc	0.59 ab	0.63 ab	6.95 a
Vermicompost , 3.21 t…..……	0.08 a	0.22 a	0.38 a	0.43 a	0.48 a	4.88 b
Biochar, 0.495 ton……..……..	0.11 a	0.47 b	0.58 ab	0.58 ab	0.63 ab	7.32 a
Biochar, 4.95 ton……..……	0.08 a	0.50 b	0.67 b	0.68 b	0.73 b	7.32 a
	p = 0.0729	p = 0.0010	p = 0.0006	p = 0.0033	p = 0.0027	p = 0.0004

[z]Values not connected by a letter are significantly different as determined using Tukey's HSD (α=0.05).
[y]Days post inoculation.

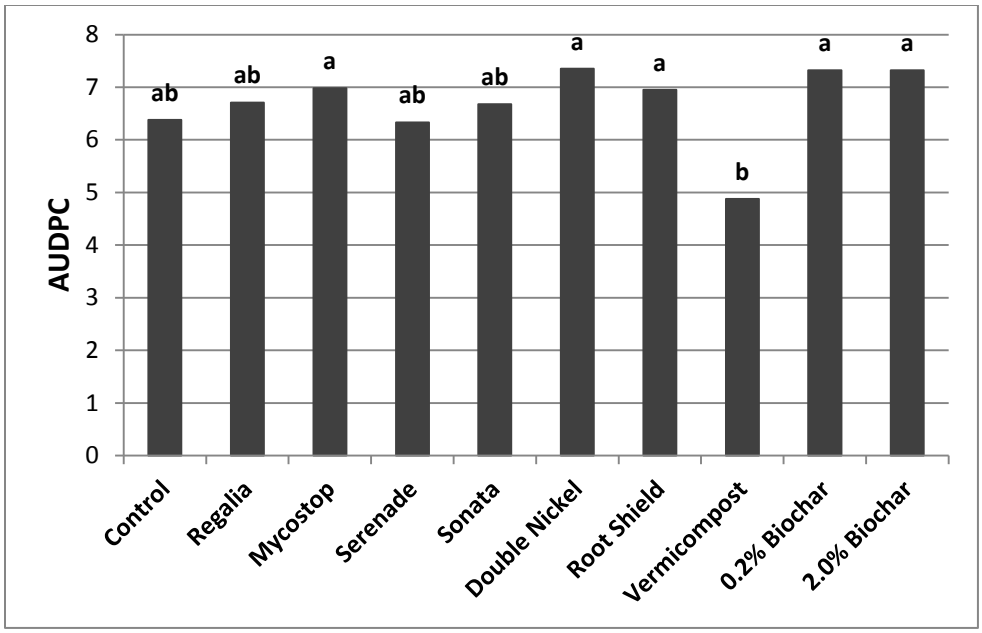

Figure 3.2. Efficacy of bio-fungicides and soil amendments to control ALS in a greenhouse study.

Field Study

Disease Development. The 2012 growing season was very hot and dry with average maximum temperatures of 29.0 and 27.4 °C, and total rainfall of 7.11 and 5.8 cm in July and August, respectively. Overhead irrigation was applied but disease symptoms were slow to develop and the maximum disease severity observed for any plant was quite low, only 55%, making it more difficult to differentiate treatment effects. No phytotoxic effects were observed for any treatments.

Efficacy of treatments control Alternaria leaf spot on cabbage. None of the treatments studied significantly reduced ALS severity compared to the untreated control, except for the chemical fungicide—Quadris—that was used as a positive control (Table 3.4). The statistical analysis was performed excluding the Quadris treatment in order to determine whether

or not there were slight differences in efficacy between any of the biological treatments but this model was not significant ($p = 0.2335$). However, when disease severity for individual time-points were analyzed several of the experimental treatments —Mycostop, Serenade, Sonata, Vermicompost, and Biochar— did show slightly lower disease severity than the untreated control at the first time-point (7 days post inoculation). Though these findings were not significant, they do indicate that effective disease control may be achieved with these materials under slightly different conditions of use. There were no significant differences in total weight or marketable weight of cabbage from any treatment, including the positive control, Quadris (Table 3.5).

Table 3.4. Efficacy of bio-fungicides and soil amendments on reducing ALS severity of cabbage in a field study.

Treatment, Rate/ha	Mean Disease Severity[z]				AUDPC[x]
	7 dpi[y]	14 dpi	23 dpi	31 dpi	
Control……..	0.15 a	0.25 a	0.29 a	0.37 a	8.34 a
Regalia, 7.0 L…..……...	0.14 a	0.28 a	0.32 a	0.36 a	8.85 a
Double Nickel 55 WDG, 1.1 kg	0.14 a	0.26 a	0.32 a	0.38 a	7.89 a
Rootshield WP, 2.35 L…..…….	0.17 a	0.24 a	0.26 a	0.34 a	8.295 a
Mycostop, 2.23 L…….……..	0.13 ab	0.23 a	0.30 a	0.36 a	7.77 a
Serenade ASO, 14.1 L…..……..	0.13 ab	0.26 a	0.30 a	0.37 a	8.61 a
Sonata ASO, 9.40 L…..……..	0.09 ab	0.27 a	0.29 a	0.36 a	8.01 a
Vermicompost , 3.21 t…..……..	0.13 ab	0.21 a	0.27 a	0.34 a	7.23 a
Biochar, 4.95 ton…….……..	0.11 ab	0.23 a	0.29 a	0.37 a	7.575 a
Quadris, 1.0 L…..……..	0.02 b	0.02 b	0.03 b	0.14 b	0.723 b
	p = 0.0097	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

[z]Values not connected by a letter are significantly different as determined using Tukey's HSD (α=0.05). [y]Days post inoculation. [x]Area under the disease progress curve.

Table 3.5. Efficacy of bio-fungicides and soil amendments on cabbage yield.

	Total Yield (kg/Rep)[z]	Marketable Yield (kg/Rep)
Control…….	12.83	11.765
Regalia, 7.0 L…..…….	11.455	10.395
Mycostop, 2.23 L…….……	10.92	9.835
Serenade ASO, 14.1 L…..…….	12.13	11.02
Sonata ASO, 9.40 L…..…….	11.78	10.75
Double Nickel 55 WDG, 1.1 kg……	12.685	11.6
Rootshield WP, 2.35 L…..…….	11.79	10.74
Quadris, 1.0 L…..……..	13.055	11.99
Vermicompost , 3.21 t…..…….	11.895	10.855
Biochar, 4.95 ton…….……..	11.86	10.75
	p = 0.6784	0.6362

[z]Total and marketable yields were measured as weight per replicate plot, each rep consisting of ten cabbage heads. Values presented are the average yield across all four reps.

Discussion

In light of the recent decrease in efficacy of conventional, synthetic fungicides that has been observed, and increasing acreage and interest in organic vegetable production, new strategies for controlling Alternaria leaf spot in brassica crops are essential. There are currently several fungicides listed for control of ALS on cabbage in organic production, but the efficacy of these materials had not been tested. In the present studies, efficacy of these products was tested, along with other bio-fungicides and soil amendments that are not registered for ALS control. Unfortunately, none of the treatments evaluated had a significant effect on ALS severity or crop yield in either the greenhouse or field studies when compared to the untreated control. Biological control of plant diseases is known to be somewhat unpredictable, especially in field settings where survival and efficacy of biocontrol organisms are influenced by factors such as climatic conditions, soil physicochemical and biological properties, and extant microbial environment, which are all highly variable, both temporally and spatially, in field settings (Cook & Baker, 1983; Whipps, 2001).

The 2012 growing season was exceptionally hot and dry with only a few minor rain events (0.30, 0.53, and 0.81 cm) in the three weeks following application of the treatments. Overhead irrigation was used twice (each application consisting of approximately 1.25 cm) in the three weeks following biological treatment application in order to maintain plant health and promote growth of the pathogen, but the soil remained observably dry throughout the experiment. Efficacy of biocontrol organisms is known to be negatively impacted by dry conditions (Baker & Cook, 1974). A study by Peng et al. (2011) demonstrated that, under controlled environmental conditions, efficacy of both *B. subtilis* and *Gliocladium catenulatum* in controlling clubroot (*Plasmodiophora brassicae*) on canola (*Brassica napus*) declined

substantially after two weeks of simulated drought conditions. It is also known that antibiotic production by antagonists is decreased in cases of water stress (Baker & Cook, 1974). It is likely that under environmental conditions more conducive to microbial growth we would have seen a greater and more durable beneficial effect of the biological treatments in the field.

Biocontrol may be relatively difficult to achieve in crucifer crops because of the great diversity of phytoalexins, antimicrobial secondary metabolites produced by plants in response to biotic or abiotic stress, that are produced by these plants (Pedras & Yaya, 2010; Pedras et al., 2011). All plants make antimicrobial defense compounds of some kind, but *Brassica* spp. are especially inhospitable to microbes (Jetiyanon & Kloepper, 2002), as evidenced by the fact that they do not form mycorrhizal associations while 80% of land plants do form relationships with mycorrhizae (DeMars & Boerner, 1996). Brassicaceous plants are capable of synthesizing 44 unique defense chemicals which are differentially produced depending on the type of stress encountered (Pedras et al., 2009; Pedras et al., 2011). Furthermore, brassicaceous plant tissues contain glucosinolates which, upon hydrolysis produce isothiocyanates that are also toxic or inhibitory to many microorganisms. As such, brassica crop residues are now being used as biofumigants to disinfest soils contaminated by pathogens (Angus et al., 1994; Cohen et al., 2005; Kirkegaard et al., 2000; Mazzola & Mullinix, 2005; Sarwar et al., 1998; Weerakoon et al., 2012).

Meanwhile, pathogens of brassica crops have evolved mechanisms to metabolize these plant defense chemicals, allowing them to survive and grow while most microbes remain sensitive and are killed (Pedras et al., 2009; Pedras & Yaya, 2010; Pedras et al., 2011). For example, *A. brassicicola* is capable of detoxifying brassinin, a potent cruciferous phytoalexin and precursor molecule in the synthesis of other phytoalexins, by hydrolysis to indolyl-3-

methanamine (Pedras et al., 2011). Similarly, oilseed rape breeding lines with higher glucosinolate concentrations have been shown to stimulate the pathogen and cause increased ALS symptoms (Giamoustaris & Mithen, 1997; Macdonald & Ingram, 1986).

Nevertheless, there is a precedent for successful biological control in brassica crops, especially with *Bacillus* spp. and *Trichoderma* spp. (Dandurand et al., 2000; Danielsson et al., 2007; Fiddaman & Rossall, 1995; Massomo et al., 2004; Peng et al., 2011; Ryu et al., 2004; Wulff et al., 2002a). In the field study, there was evidence that some of the biological treatments were capable of reducing ALS symptoms, though no biological treatments were significantly different from the untreated control and the observed disease control did not last beyond the first time-point (Table 3.4). It is important to note that the biological treatments were applied only once —24 hours prior to inoculation with the pathogen— during this experiment so that they could be compared directly to the efficacy of the positive control, Quadris, which is a systemic chemical fungicide requiring few repeated sprays that was able to provide significant ALS control throughout this study with just one application. Induced systemic resistance, a likely mechanism underlying the observed reduction in ALS severity by these biocontrol organisms, causes a temporary induction of plant defenses that is maintained only in the continued presence of the inducing microbe (Heil & Walters, 2009). It is likely that the biocontrol organisms had difficulty establishing due to hot, dry conditions, and had these organisms been applied repeatedly during the experiment, a significant level of disease control may have been achieved.

Another factor which may have contributed to the inefficacy of some biological treatments is the latent period: the amount of time between application of the biocontrol agents and inoculation with the pathogen. While no reductions in ALS severity by biocontrol organisms were observed in the greenhouse study, several biocontrol treatments did show slight

disease control effects in the field trial. The three biological treatments which showed no sign of efficacy at all in the field study were the extract of *Reynoutria sachalinensis* (Regalia), *B. amyloliquefaciens* D747 (Double Nickel), and *T. harzianum* strain KRL-AG2 (Rootshield) (Table 3.3).

Most of the biocontrol studies on *Reynoutria sachalinensis* extracts have been conducted in the cucumber-Powdery mildew (*Sphaerotheca fuliginea*) pathosystem, so there is no information about the efficacy of this product on the defense system of cabbage plants (Daayf et al., 2000; Daayf et al., 1995; Daayf et al., 1997; Konstantinidou-Doltsinis et al., 2006; Konstantinidou-Doltsinis & Schmitt, 1998). However, successful control of downy mildew (*Peronospora parasitica*) by induction of systemic resistance using the plant defense activator benzothiadiazole (BTH) has been documented in the related vegetable brassica, cauliflower (*Brassica oleracea* var. *botyritis*) (Godard et al., 1999; Ziadi et al., 2001). Studies on latent periods required for successful disease control by plant defense activators show that, while there is some variation, a latent period of 0-2 days is usually optimal for successful disease control (Daw et al., 2008; Godard et al., 1999; Ziadi et al., 2001). Systemic disease control was achieved for at least 30 days after pathogen inoculation when the chemical was applied 1 or more days before pathogen challenge in the case of BTH and 2-8 days before pathogen challenge for ASM on cauliflower (Godard et al., 1999; Ziadi et al., 2001). ASM and another plant defense activator, salicylic acid (SA), have been shown to provide effective disease control within the range of 0-2 days (Daw et al., 2008; Ziadi et al., 2001). When SA was used to protect rice plants against *Magnaportha grisea* the best control was achieved when a latent period of 2-48 hours was used and the effectiveness of control only began to decline with a latent period of 72 hours or greater (Daw et al., 2008). Another formulation of *R. sachalinensis* extract, Milsana, was

more effective in reducing severity of downy mildew (*Leveillula taurica*) on tomato when it was applied on the same day as the pathogen, as opposed to one day before challenge with the pathogen (Konstantinidou-Doltsinis et al., 2006). These studies, though they focused on other pathosystems, indicate that the time interval between treatment application and pathogen inoculation of 24 hours used in this study should have been appropriate. However, a greenhouse experiment aimed at determining the appropriate timing and rate of application of Regalia, as well as comparing its efficacy with that of other plant defense activators such as SA and BTH in this pathosystem would be warranted.

B. amyloliquefaciens has been used most often as seed or root treatment and when studied, has been shown to be less effective when applied to foliage (Abdullah et al., 2008; Danielsson et al., 2007; Massomo et al., 2004; Simonetti et al., 2012a; Simonetti et al., 2012b; Wulff et al., 2002b). Massomo et al. (2004) showed that root treatment with a two-week latent period provided significant black rot (*Xanthomonas campestris* pv. *campestris*) control in cabbage, regardless of the *Bacillus* spp. studied (included *B. subtilis*, *B. pumilis*, and *B. amyloliquefaciens*). When foliar or seed application in addition to root application were studied no increased benefit was observed, indicating that a soil drench is sufficient to provide disease control of black rot in cabbage (Massomo et al., 2004). However, no effect on disease severity was observed when *Bacillus* spp. were applied as soil drenches in the greenhouse study (Table 3.3). Future studies using *Bacillus* spp. as biocontrol agents in cabbage cropping systems should investigate the appropriate latent periods and rates for these organisms when applied to the root zone of cabbage.

Trichoderma harzianum (RootShield) has been successfully used to control disease in many pathosystems (Ahmad & Baker, 1987; De Meyer et al., 1998; Elad, 2000; Hoitink &

Boehm, 1999; Whipps, 2001; Yedidia et al., 1999). De Meyer et al. (1998) showed that *T. harzianum*, whether applied to foliage or as a soil drench, was effective in reducing grey mold (*Botrytis cinerea*) in pepper, bean, tobacco, tomato, and lettuce when applied 1, 3, or 7 days before being challenged with the pathogen but was ineffective when plants were co-inoculated. Ahmed et al. (2000) achieved successful disease control through induced plant resistance when a latent period of 7 days between soil drench with *T. harzianum* and inoculation with *Phytophthora capsici* was used in a greenhouse study on pepper. Many researchers have used longer latent periods, as *T. harzianum* is often used as a seed treatment and thus is applied at sowing while challenge with a pathogen would likely not take place until the crop is well established after several weeks (Ahmad & Baker, 1987). It may be that 24 hours was not a long enough latent period for *T. harzianum* strain KRL-AG2 to colonize the root zone and stimulate an ISR response in cabbage. Before future field experiments are planned in this pathosystem, a greenhouse study investigating the appropriate latent period for this product should be performed.

The two soil amendments tested provided a slight reduction of ALS severity in the field study while in the greenhouse study biochar caused a slight increase in disease severity but vermicompost still reduced disease severity slightly (Table 3.3). Composts have been known for decades to provide disease suppressive effects but these effects can be inconsistent due, in large part, to differences in substrate and preparation (Bonanomi et al., 2010; Bonanomi et al., 2007; Hoitink & Boehm, 1999; Hoitink & Fahy, 1986; Jack et al., 2011; Lehmann et al., 2011; Termorshuizen et al., 2006). The vermicompost used in this study has been previously shown to suppress root rots caused by *Pythium* spp. and its highly regulated indoor production system ensures a consistent product (Chen et al., 2012; Chen & Nelson, 2008; Craft & Nelson, 1996). In

the greenhouse trial, vermicompost at 20% v/v reduced ALS severity slightly, though not significantly, and this effect was maintained throughout the experiment (Table 3.3). It is therefore disappointing that the treatment was not more effective in the field. It may be that the dry conditions limited decomposition of the compost and release of soil carbon, lowering its ability to induce microbially-mediated disease suppression. It would be of value to repeat this treatment under more typical field conditions, and to increase the rate to 2 ton/A.

The mechanisms underlying biochar-mediated suppression of plant diseases and its effects on crop growth in the field remain largely untested however, it is likely that these materials act similarly to other soil organic matter amendments such as composts (Lehmann et al., 2011; Rajkovich et al., 2011). Composts have been shown to promote the growth of antagonistic microbes, promote improved plant nutrition and health, and induce systemic resistance to disease signals (Bonanomi et al., 2010; Bonanomi et al., 2007; Elad et al., 2011; Elad et al., 2010; Elmer & Pignatello, 2011; Graber et al., 2010; Harel et al., 2012; Jack et al., 2011; Kolton et al., 2011; Lehmann et al., 2011; Termorshuizen et al., 2006). In addition, because of its adsorptive surface quality, biochar influences communication between plants and pathogens in the rhizosphere by intercepting chemical signals (Elad et al., 2011; Lehmann et al., 2011). Biochar has been successfully used to control disease in controlled environment and microplot studies and, while several mechanisms are likely responsible for the observed disease suppression, induced systemic resistance has been documented in several pathosystems (Elad et al., 2010; Elmer & Pignatello, 2011; Graber et al., 2010; Harel et al., 2012; Kolton et al., 2011; Lehmann et al., 2011). A greenhouse study by Elad et al. in 2010 showed significant reduction in disease severity caused by the foliar pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) on pepper and tomato with application of biochar made from citrus

wood at 3 and 5% (w/w) to coconut fiber potting mix (Elad et al., 2010). In a similar greenhouse study by Harel et al. (2012), significant reductions in disease severity caused by three foliar diseases of strawberry (*Botrytis cinerea*, *Colletotrichum acutatum* and *Podosphaera apahanis*) were observed when plants were grown in 3% biochar (w/w) (Harel et al., 2012). In the present studies, soil amendment with biochar did not have a significant effect on disease severity whether in the field or the greenhouse. However, the biochar treatment in the greenhouse study actually caused a slight yet insignificant increase in ALS severity when compared to the untreated control and the mean AUDPC increased with the higher application rate (Table 3.3). Previous research has shown that application rates of 1-5% (w/w) biochar were sufficient to achieve disease control under greenhouse conditions, so the 2% (w/w) biochar used in this study should have been adequate but future studies could investigate other application rates or biochars made from different feedstocks or under different pyrolysis conditions.

The efficacy of biocontrol agents in the field is inconsistent, and this is often attributed to unsuccessful establishment in the presence of extant microbial communities (Hoitink & Boehm, 1999; Lehmann et al., 2011; Whipps, 2001). There is a relatively recent surge in interest in using soil organic amendments—such as vermicompost or biochar—as inoculant carriers, as they provide a food source, and physical protection in the case of biochar, for biocontrol organisms (Hoitink & Boehm, 1999; Lehmann et al., 2011). Thus, another interesting study would be a factorial analysis of interactions between biochar or vermicompost and the more effective biocontrol organisms tested. While none of the treatments evaluated in this study provided significant and sustained control of Alternaria leaf spot in cabbage, several treatments did show potential and warrant further research aimed at identifying the optimal application conditions.

REFERENCES

Abdullah MT, Ali NY, Suleman P, 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. *Crop Protection* **27**, 1354-9.

Ahmad JS, Baker R, 1987. Rhizosphere competence of *Trichoderma harzianum*. *Phytopathology* **77**, 182-9.

Angus JF, Gardner PA, Kirkegaard JA, Desmarchelier JM, 1994. Biofumigation - Isothiocyanates released from brassica roots inhibit growth of the take-all fungus. *Plant and Soil* **162**, 107-12.

Baker KF, Cook RJ, 1974. *Biological Control of Plant Pathogens*. San Francisco, CA: W.H. Freeman.

Benhamou N, Kloepper JW, Quadthallman A, Tuzun S, 1996. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. *Plant Physiology* **112**, 919-29.

Bonanomi G, Antignani V, Capodilupo M, Scala F, 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology & Biochemistry* **42**, 136-44.

Bonanomi G, Antignani V, Pane C, Scala E, 2007. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* **89**, 311-24.

Chen M-H, Jack ALH, Mcguire IC, Nelson EB, 2012. Seed-Colonizing Bacterial Communities Associated with the Suppression of Pythium Seedling Disease in a Municipal Biosolids Compost. *Phytopathology* **102**, 478-89.

Chen MH, Nelson EB, 2008. Seed-colonizing microbes from municipal biosolids compost suppress *Pythium ultimum* damping-off on different plant species. *Phytopathology* **98**, 1012-8.

Cohen MF, Yamasaki H, Mazzola M, 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. *Soil Biology & Biochemistry* **37**, 1215-27.

Cook RJ, Baker KF, 1983. *The nature and practice of biological control of plant pathogens*. St. Paul, Minn.: American Phytopathological Society.

Craft CM, Nelson EB, 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. *Applied and Environmental Microbiology* **62**, 1550-7.

Daayf F, Ongena M, Boulanger R, El Hadrami I, Belanger RR, 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. *Journal of Chemical Ecology* **26**, 1579-93.

Daayf F, Schmitt A, Belanger RR, 1995. The effects of plant-extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long english cucumber. *Plant Disease* **79**, 577-80.

Daayf F, Schmitt A, Belanger RR, 1997. Evidence of phytoalexins in cucumber leaves infected with powdery mildew-following treatment with leaf extracts of Reynoutria sachalinensis. *Plant Physiology* **113**, 719-27.

Dandurand LM, Mosher RD, Knudsen GR, 2000. Combined effects of *Brassica napus* seed meal and *Trichoderma harzianum* on two soilborne plant pathogens. *Canadian Journal of Microbiology* **46**, 1051-7.

Danielsson J, Reva O, Meijer J, 2007. Protection of oilseed rape (*Brassica napus*) toward fungal pathogens by strains of plant-associated *Bacillus amyloliquefaciens*. *Microb Ecol* **54**, 134-40.

Daw BD, Zhang LH, Wang ZZ, 2008. Salicylic acid enhances antifungal resistance to *Magnaporthe grisea* in rice plants. *Australasian Plant Pathology* **37**, 637-44.

De Meyer G, Bigirimana J, Elad Y, Hofte M, 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology* **104**, 279-86.

De Vleesschauwer D, Hofte M, 2009. Rhizobacteria-Induced Systemic Resistance. In: Vanloon LC, ed. *Plant Innate Immunity*. 223-81. (Advances in Botanical Research; vol. 51.)

Demars BG, Boerner REJ, 1996. Vesicular arbuscular mycorrhizal development in the *Brassicaceae* in relation to plant life span. *Flora* **191**, 179-89.

Dillard HR, Bellinder RR, Shah DA, 2004. Integrated management of weeds and diseases in a cabbage cropping system. *Crop Protection* **23**, 163-8.

Dillard HR, Cobb AC, Lamboy JS, 1998. Transmission of *Alternaria brassicicola* to cabbage by flea beetles (*Phyllotreta cruciferae*). *Plant Disease* **82**, 153-7.

Durrant WE, Dong X, 2004. Systemic acquired resistance. *Annual Review of Phytopathology* **42**, 185-209.

Elad Y, 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection* **19**, 709-14.

Elad Y, Cytryn E, Harel YM, Lew B, Graber ER, 2011. The Biochar Effect: plant resistance to biotic stresses. *Phytopathologia Mediterranea* **50**, 335-49.

Elad Y, David DR, Harel YM, *et al.*, 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. *Phytopathology* **100**, 913-21.

Elmer WH, Pignatello JJ, 2011. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. *Plant Disease* **95**, 960-6.

Fiddaman PJ, Rossall S, 1995. Selection of bacterial antagonists for the biological control of *Rhizoctonia solani* in oilseed rape (*Brassica napus*). *Plant Pathology* **44**, 695-703.

Giamoustaris A, Mithen R, 1997. Glucosinolates and disease resistance in oilseed rape (*Brassica napus* ssp *oleifera*). *Plant Pathology* **46**, 271-5.

Godard JF, Ziadi S, Monot C, Le Corre D, Silue D, 1999. Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. *Crop Protection* **18**, 397-405.

Graber ER, Harel YM, Kolton M, *et al.*, 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant and Soil* **337**, 481-96.

Hammerschmidt R, 2009. Systemic Acquired Resistance. In: Vanloon LC, ed. *Plant Innate Immunity.* 173-222. (Advances in Botanical Research; vol. 51.)

Harel YM, Elad Y, Rav-David D, *et al.*, 2012. Biochar mediates systemic response of strawberry to foliar fungal pathogens. *Plant and Soil* **357**, 245-57.

Heil M, Walters DR, 2009. Ecological Consequences of Plant Defence Signalling. In: Vanloon LC, ed. *Plant Innate Immunity.* 667-716. (Advances in Botanical Research; vol. 51.)

Hoitink HaJ, Boehm MJ, 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology* **37**, 427-46.

Hoitink HaJ, Fahy PC, 1986. Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**, 93-114.

Humpherson-Jones FM, 1992. Epidemiology and Control of Dark Leaf Spot of Brassicas. In: Chelkowski J, Visconti A, eds. *Alternaria Biology, Plant Diseases and Metabolites.* New York, NY: Elsevier, 267-88.

Iacomi-Vasilescu B, Avenot H, Bataille-Simoneau N, Laurent E, Guenard M, Simoneau P, 2004. In vitro fungicide sensitivity of Alternaria species pathogenic to crucifers and identification of Alternaria brassicicola field isolates highly resistant to both dicarboximides and phenylpyrroles. *Crop Protection* **23**, 481-8.

Jack ALH, Rangarajan A, Culman SW, Sooksa-Nguan T, Thies JE, 2011. Choice of organic amendments in tomato transplants has lasting effects on bacterial rhizosphere communities and crop performance in the field. *Applied Soil Ecology* **48**, 94-101.

Jetiyanon K, Kloepper JW, 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological Control* **24**, 285-91.

Kharbanda PD, Tewari JP, 1996. Integrated management of canola diseases using cultural methods. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* **18**, 168-75.

Kirkegaard JA, Sarwar M, Wong PTW, Mead A, Howe G, Newell M, 2000. Field studies on the biofumigation of take-all by brassica break crops. *Australian Journal of Agricultural Research* **51**, 445-56.

Kloepper JW, Tuzun S, Kuc JA, 1992. Proposed definitions related to induced disease resistance. *Biocontrol Science and Technology* **2**, 349-51.

Kolton M, Harel YM, Pasternak Z, Graber ER, Elad Y, Cytryn E, 2011. Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants. *Applied and Environmental Microbiology* **77**, 4924-30.

Konstantinidou-Doltsinis S, Markellou E, Kasselaki AM, *et al.*, 2006. Efficacy of Milsana (R), a formulated plant extract from *Reynoutria sachalinensis*, against powdery mildew of tomato (*Leveillula taurica*). *Biocontrol* **51**, 375-92.

Konstantinidou-Doltsinis S, Schmitt A, 1998. Impact of treatment with plant extracts from *Reynoutria sachalinensis* (F. Schmidt) *Nakai* on intensity of powdery mildew severity and yield in cucumber under high disease pressure. *Crop Protection* **17**, 649-56.

Kortemaa H, Haahtela K, Smolander A, 1997. Effect of soil-spraying time on root-colonization ability of antagonistic *Streptomyces griseoviridis*. *Agricultural and Food Science in Finland* **6**, 341-8.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D, 2011. Biochar effects on soil biota - A review. *Soil Biology & Biochemistry* **43**, 1812-36.

Lian LL, Xie LY, Zheng LP, Lin QY, 2011. Induction of systemic resistance in tobacco against Tobacco mosaic virus by *Bacillus* spp. *Biocontrol Science and Technology* **21**, 281-92.

Macdonald MV, Ingram DS, 1986. Towards the selection in vitro for resistance to *Alternaria brassicicola* (Schw) Wilts, in *Brassica napus* ssp. *oleifera* (Metzg) Sinsk, Winter oilseed rape. *New Phytologist* **104**, 621-9.

Massomo SMS, Mortensen CN, Mabagala RB, Newman MA, Hockenhull J, 2004. Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage in Tanzania with *Bacillus* strains. *Journal of Phytopathology* **152**, 98-105.

Mazzola M, Mullinix K, 2005. Comparative field efficacy of management strategies containing *Brassica napus* seed meal or green manure for the control of apple replant disease. *Plant Disease* **89**, 1207-13.

Minuto A, Spadaro D, Garibaldi A, Gullino ML, 2006. Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization. *Crop Protection* **25**, 468-75.

Murphy JF, Zehnder GW, Schuster DJ, Sikora EJ, Polston JE, Kloepper JW, 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against Tomato mottle virus. *Plant Disease* **84**, 779-84.

Pedras MS, Minic Z, Sarma-Mamillapalle VK, 2009. Substrate specificity and inhibition of brassinin hydrolases, detoxifying enzymes from the plant pathogens *Leptosphaeria maculans* and *Alternaria brassicicola*. *FEBS J* **276**, 7412-28.

Pedras MS, Yaya EE, 2010. Phytoalexins from *Brassicaceae*: news from the front. *Phytochemistry* **71**, 1191-7.

Pedras MS, Yaya EE, Glawischnig E, 2011. The phytoalexins from cultivated and wild crucifers: chemistry and biology. *Nat Prod Rep* **28**, 1381-405.

Peng G, Mcgregor L, Lahlali R, *et al.*, 2011. Potential biological control of clubroot on canola and crucifer vegetable crops. *Plant Pathology* **60**, 566-74.

Pieterse CMJ, Van Wees SCM, Ton J, Van Pelt JA, Van Loon LC, 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biology* **4**, 535-44.

Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J, 2011. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils* **48**, 271-84.

Ryu CM, Murphy JF, Mysore KS, Kloepper JW, 2004. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against Cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant Journal* **39**, 381-92.

Sari E, Etebarian HR, Aminian H, 2007. The effects of *Bacillus pumilus*, isolated from wheat rhizosphere, on resistance in wheat seedling roots against the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. *Journal of Phytopathology* **155**, 720-7.

Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM, 1998. Biofumigation potential of brassicas - III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* **201**, 103-12.

Seaman A, 2012. *2012 Production Guide for Organic Cole Crops: Cabbage, Cauliflower, Broccoli, and Brussels Sprouts.* Geneva, NY: NYS IPM.

Simonetti E, Carmona MA, Scandiani MM, *et al.*, 2012a. Evaluation of indigenous bacterial strains for biocontrol of the frogeye leaf spot of soya bean caused by *Cercospora sojina*. *Letters in Applied Microbiology* **55**, 170-3.

Simonetti E, Hernandez AI, Kerber NL, Pucheu NL, Carmona MA, Garcia AF, 2012b. Protection of canola (*Brassica napus*) against fungal pathogens by strains of biocontrol rhizobacteria. *Biocontrol Science and Technology* **22**, 111-5.

Termorshuizen AJ, Van Rijn E, Van Der Gaag DJ, *et al.*, 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. *Soil Biology and Biochemistry* **38**, 2461-77.

Tuomi T, Laakso S, Rosenqvist H, 1994. Indole-3-acetic-acid (IAA) production by a biofungicide *Streptomyces griseoviridis* strain. *Annales Botanici Fennici* **31**, 59-63.

Vallad GE, Goodman RM, 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* **44**, 1920-34.

Van Loon LC, 1997. Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology* **103**, 753-65.

Van Loon LC, Bakker P, Pieterse CMJ, 1998. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* **36**, 453-83.

Weerakoon DMN, Reardon CL, Paulitz TC, Izzo AD, Mazzola M, 2012. Long-term suppression of *Pythium abappressorium* induced by *Brassica juncea* seed meal amendment is biologically mediated. *Soil Biology & Biochemistry* **51**, 44-52.

Whipps JM, 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52**, 487-511.

Wulff EG, Mguni CM, Mansfeld-Giese K, Fels J, Lubeck M, Hockenhull J, 2002a. Biochemical and molecular characterization of Bacillus amyloliquefaciens, B-subtilis and B-pumilus isolates with distinct antagonistic potential against Xanthomonas campestris pv. campestris. *Plant Pathology* **51**, 574-84.

Wulff EG, Mguni CM, Mansfeld-Giese K, Fels J, Lubeck M, Hockenhull J, 2002b. Biochemical and molecular characterization of *Bacillus amyloliquefaciens, B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. *Plant Pathology* **51**, 574-84.

Yedidia I, Benhamou N, Chet I, 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Applied and Environmental Microbiology* **65**, 1061-70.

Ziadi S, Barbedette S, Godard JF, Monot C, Le Corre D, Silue D, 2001. Production of pathogenesis-related proteins in the cauliflower (*Brassica oleracea* var. *botrytis*)-downy mildew (*Peronospora parasitica*) pathosystem treated with acibenzolar-S-methyl. *Plant Pathology* **50**, 579-86.

CHAPTER 4

EFFICACY OF MULCHES IN REDUCING ALTERNARIA LEAF SPOT INFECTION IN KALE

Introduction

Cultural controls serve as a first line of defense against plant disease in any production system, but in some cases they may be the only options available for reducing disease severity in organic systems. Most cultural controls are aimed at reducing the amount of a pathogen in the field, reducing contact between plant and pathogen, and creating an environment unfavorable for disease development (Agrios, 2005). Cultural practices that can be effective in reducing severity of Alternaria leaf spot (ALS) in *brassica* crops include using pathogen-free seed or transplants, using crop rotations of at least three years, managing infected crop residues, reducing populations of brassicaceous weeds that can serve as alternate hosts, and managing flea beetle (*Phyllotreta cruciferae*) populations, as these insects can spread spores of the pathogen, *Alternaria brassicicola* throughout a field.

Alternaria leaf spot of brassica crops is primarily spread by infested seed or transplants and by infected crop residue in the field (Humpherson-Jones, 1989; Humpherson-Jones, 1992; Humpherson-Jones & Maude, 1982b). To prevent infection by soil-borne *A. brassicicola* spores, crop rotations of three years or longer are recommended (Humpherson-Jones, 1989; Humpherson-Jones & Maude, 1982b; Kohl et al., 2011). However, it can often be difficult for many farmers, especially those with small acreage, to achieve rotations in which host crops are

adequately separated in both time and space because of the large number of host crops in the *Brassicaceae* family and their long and overlapping growing seasons. Therefore, the use of mulches to provide a physical barrier between host tissue and overwintering *A. brassicicola* spores in the soil was investigated. Several mulch materials were tested in order to determine their effects on growth of kale (*Brassica oleracea* Acephala group) and on ALS disease incidence. Mulches tested included low density polyethylene (LDPE) plastic film, Biotelo biodegradable plastic film, and wheat straw, and these materials were compared to a bare ground control.

Mulches have been used in vegetable production for centuries because of their myriad effects on soil and plant health. Mulches can impart changes in soil temperature, increase moisture and water use efficiency, increase microbial and microarthropod activity, contribute to management of weeds, insect pests and diseases, increase soil carbon and tilth, and reduce soil erosion (Abdul-Baki et al., 2002; Lamont, 2005; Tiquia et al., 2002; Waterer, 2010). All of these factors can contribute to earlier crop production, increased yields, and higher fruit quality (Lamont, 2005; Monks et al., 1997; Waterer, 2010). Development and use of low-density polyethylene (LDPE) plastic sheet mulches in agriculture began in the 1950's and has been tremendously successful in increasing crop earliness, productivity and extending the growing season and range of many heat-loving crops, especially those belonging to the *Solanaceae* and *Cucurbitaceae* (Lamont, 2005). As a result, this system—known as plasticulture—has become the dominant cropping system for many high-value vegetable crops such as tomatoes and melons (Kasirajan & Ngouajio, 2012; Lamont, 2005; Ngouajio et al., 2008).

Although there are clearly many benefits to vegetable production using plastic mulches, there are also significant costs and environmental concerns associated with the removal and

disposal of the plastic from the field at the end of the season (Kasirajan & Ngouajio, 2012; Lamont, 2005; Ngouajio et al., 2008; Waterer, 2010). It is estimated that the cost of removing and disposing of used plastic mulch is $250/ha when labor, equipment, and landfill fees are taken into consideration (Ngouajio et al., 2008; Waterer, 2010). Though it is possible to recycle used plastic films, those used in vegetable production can contain 40-50% (w/w) contaminants such as soil and plant debris, fertilizers, and pesticides by the end of the season and plastic films containing greater than 5% contamination are considered unsuitable for recycling (Kasirajan & Ngouajio, 2012). Therefore, interest in degradable plastic mulches that can be broken down in the field by photo-initiated reactions (photodegradable) or by the action of soil moisture and microorganisms (biodegradable) has been increasing (Kasirajan & Ngouajio, 2012; Lamont, 2005). However, developing new degradable sheet mulches that are easy to implement, maintain field performance, and also break down within the appropriate time-frame has proven to be a challenge. Perhaps the most important factor is that the biodegradable mulch materials persist long enough to provide adequate soil warming and prevent weed growth, but these mulches must also not degrade so slowly that they cannot be plowed in easily or that plastic fragments remain in the field in the following season—a balance which has been difficult to achieve (Feuilloley et al., 2005; Kasirajan & Ngouajio, 2012). Thus far, biodegradable mulches made from polyesters and starch-based polymers have shown the most promise in providing field performance comparable to standard PE mulches as well as good biodegradation within the necessary time-frame (Kasirajan & Ngouajio, 2012).

One such material, Mater-Bi (Novamont, Italy), is made from a blend of polycaprolactone co-polyester and starch, and has been shown to have good field performance and biodegradability (Feuilloley et al., 2005; Kasirajan & Ngouajio, 2012). Mater-Bi is the

principal component of the agricultural sheet mulch Biotelo (Dubois Agrinovation, St.Remi, QC, CAN), which has been shown to raise soil temperature, maintain soil moisture, control weeds, and increase crop yields comparably to the standard LDPE mulches in tomato, pepper, eggplant, cantaloupe, zucchini, and sweet corn production systems (Cirujeda et al., 2012; Ngouajio et al., 2008; Waterer, 2010). One benefit of biodegradable plastic sheet mulches like Biotelo is that, though more prone to tearing than the standard LDPE plastic films, these materials can be used with traditional plasticulture equipment, making them more likely to be implemented (Kasirajan & Ngouajio, 2012; Ngouajio et al., 2008; Waterer, 2010). Biotelo has also been shown to be a cost-effective alternative to mulching with LDPE because, though the material can be 15% more expensive than PE mulch, labor costs and disposal or recycling fees are avoided (Cirujeda et al., 2012; Waterer, 2010).

While biodegradable mulches address the environmental concerns surrounding use of LDPE mulch, none of these materials have yet been certified for use in organic systems in the US, though many are allowed in organic production within the European Union. Therefore, environmentally friendly alternatives to plastic mulching for use in organic systems in the US are very limited. Mulches made from organic matter such as straw or paper have been used in agriculture for centuries as they also provide benefits for soil and plant health, but their effects can differ slightly from those provided by LDPE plastics (Cirujeda et al., 2012; Monks et al., 1997; Trdan et al., 2008). For example, straw mulches also increase soil moisture but can actually lower soil temperature compared to bare ground controls (Monks et al., 1997; Trdan et al., 2008). Cooler soil temperatures increase activity of microorganisms, microarthropods, and earthworms which can contribute to increased plant and soil health, but would not provide the season extending warming effect of black plastic (Kasirajan & Ngouajio, 2012; Tiquia et al.,

2002). One of the major advantages of organic mulches over plastics is that they can be easily tilled in at the end of the season, thereby increasing soil carbon, tilth, and microbial activity (Cirujeda et al., 2012; Monks et al., 1997; Tiquia et al., 2002; Trdan et al., 2008).

Cole crops have previously been shown to benefit from use of mulches in terms of crop earliness, yield, and quality (Lamont, 2005; Trdan et al., 2008). Management of ALS in organic systems depends on preventing introduction of the fungus into a field by infected seeds or transplants, and preventing infection of the crop from soil-borne overwintering spores. Therefore, the hypothesis that mulches can reduce ALS incidence by providing a physical barrier between infested soil and susceptible crop tissue was tested. The effects of standard LDPE plastic, Biotelo biodegradable plastic, and wheat straw mulches on ALS incidence and plant growth were measured and compared to a bare-ground control.

Materials and Methods

In-field presence of *A. brassicicola*. The experiment was conducted at the New York State Agricultural Experiment Station (NYSAES) in Geneva, NY. Field 31 of the Research North Agricultural Research Farm had been used for ALS research trials for several previous growing seasons and infested crop residue was predicted to be found in the soil there. In order to confirm that the pathogen was present in the field, rotorod spore traps were used to take air samples from which DNA was extracted and presence of *A. brassicicola* was determined by conventional PCR.

The traps (Figure 4.1) consisted of a small electric motor powered by a 30 cm square solar panel. The motor spins a metal rod into which pieces of stainless steel welding rod which

have been cut to 3.8 cm, washed and sterilized, and coated in a thin layer of high vacuum grease (Dow Corning, Midland, Michigan, USA) are placed. These small rods collect particulate matter suspended in the air, thereby catching any spores that may be present. The trap also contains a battery-powered Watchdog A-series datalogger (Spectrum Technologies, INC, Plainfield, IL) that records temperature and relative humidity once every hour. The trap with datalogger and solar panel was mounted on a wooden frame that was secured by heavy-duty zip-ties to fence posts that were sunk into the soil.

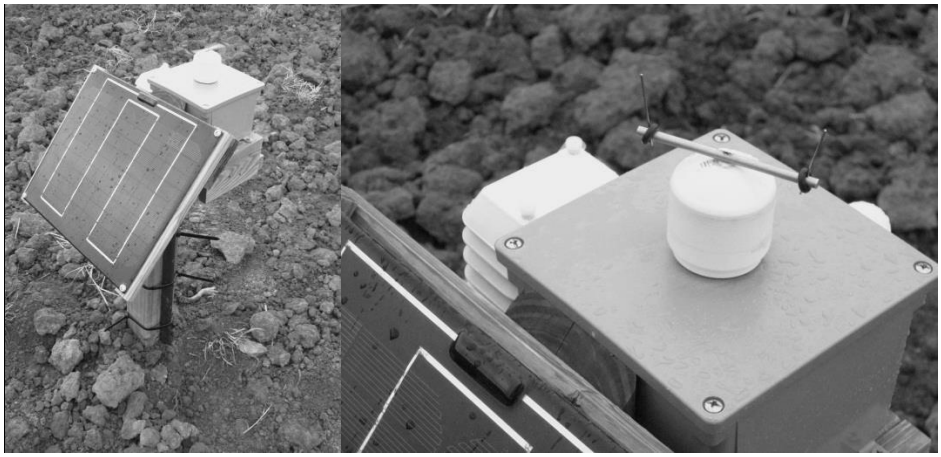

Figure 4.1. Rotorod spore traps were used to confirm the presence of *A. brassicicola* in the research plot and to monitor the presence of spores throughout the growing season.

The two traps were placed at the North and South ends of the experiment and the traps were positioned such that the collection rods were 80 cm above the soil surface and were 20 m apart. Air-borne dispersal of *A. brassicicola* was monitored from 04 June until 18 June in Field 31 and then the traps were moved across the research site to an apple orchard, where no *A. brassicicola* should have been present in the soil, as a negative control. Presence of air-borne *A. brassicicola* spores was monitored in the orchard site from 21 June until 09 July when the spore

traps were moved back to Field 31 until 19 October when the crop in the experiment was harvested.

The sampling rods were replaced twice a week from 06 June until 19 October and were stored in sterilized disposable culture tubes at 4 °C until DNA extractions could be performed (every one-two weeks). The two sampling rods from each spore trap were pooled and extracted as one sample in order to increase DNA yield. DNA was extracted using the MoBio Ultraclean Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to manufacturer's instructions, but with the addition of polyvinyl pirrolidone at 0.002 g/sample during the cell lysis step in order to remove impurities.

The extracted genomic DNA was used as the template amplified by conventional polymerase chain reaction (PCR) using species specific primers—ABRA1/ABRA2 (5'-aaggcgagtctccagcaaactg-3'/5'-actcacctcagcagcatctgctgt-3')—which had been previously designed and tested for specificity to *A. brassicicola* (Iacomi-Vasilescu et al., 2002). PCR reactions were performed using 5 µl of undiluted DNA preparations in 25 µl reactions under the following conditions: 1X GoTaq Green Reaction Buffer containing 1.5 mM $MgCl_2$ (GoTaq, Promega Corp., Madison, WI, USA)., 200 µM of each deoxyribonucleotide triphosphate, 0.2 mM each primer, and 1.25 units of thermostable DNA polymerase (GoTaq, Promega Corp., Madison, WI, USA). Reactions were performed using an Eppendorf Mastercycler Gradient thermocycler (Eppendorf, Hauppauge, NY, USA) with an initial step of 3 min of denaturation at 94°C; followed by 35 cycles of 30 s of denaturing at 94°C, 50 s of annealing at 55°C, and 1 min of elongation at 72°C; and a final elongation step of 10 min at 72°C. The amplification results were visualized after electrophoresis of an aliquot (5 µl) of the reaction product on a 2% agarose gel.

Plant Material. Kale seeds ("Winterbor", Johnny's Selected Seeds, Waterville, Maine) were sown on 23 May in 72-cell trays containing Cornell Mix potting soil —a mixture of peat, perlite, and vermiculite at a ratio of 4:1:1. Seedlings were maintained under greenhouse conditions with a maximum daily temperature of 24°C for about five weeks. Seedlings were fertilized once on 22 June with Miracle Grow All Purpose Plant Food (24-8-16) at 3.95 g/L (Scotts Company, LLC, Marysville, OH, USA). On 25 June, seedling flats were treated with a soil drench of imidacloprid (Admire Pro, Bayer CropScience, Research Triangle Park, NC) at 550 ml/ha and were then moved into outdoor cold frames to harden off for one week before being transplanted.

Field Preparation. The experiment was conducted in Field 31 of the New York State Agricultural Experiment Station (NYSAES) Research North farm, with soil classified as Lima silt loam. A randomized complete blocks design was used, with four replicate plots (7.62 m x 1.52 m) for each of the four treatments. On 27 June drip irrigation tape was set with metal staples just off-center of each row and the two sheet mulches were then laid out and secured with soil. Mulch treatments were established by hand due to the small size of the plot and the need to randomize the treatments.

On 05 July kale seedlings were transplanted by hand in single rows at 0.46 cm spacing into bare ground or into the sheet mulches. For straw mulch plots, wheat straw was spread around transplanted kale seedlings to a depth of five to ten cm, or until the soil surface was not visible through the straw (~5 ton/ha). Seedlings were fertilized with Miracle Grow All Purpose Plant Food (Scotts Company, LLC, Marysville, OH) and drip irrigated with one 380-L tank of water (equivalent to ~1 cm of rain) at transplanting and two more 380-L tanks (~2 cm of rain) on 06 July. The rest of the field surrounding the experiment was then cover cropped with sudex

(*Sorghum bicolor* × *S. bicolor* var. Sudanese) to reduce wind dispersal of soil-borne inoculum into the experimental area. One application of zeta-cypermethrin plus bifenthrin (Hero EW, FMC Corp, Philadelphia, PA) at a rate of 440 ml/ha was used on 27 July to control flea beetles (*Phyllotreta cruciferae*) and lepidopteran pest populations, which were quite high in this field. The field was drip irrigated throughout the experiment so as to receive at least ~1.25 cm rain every week and additional irrigation was used as needed to maintain plant health.

Assessment of plant growth and ALS incidence. Ten healthy plants in the middle of each replicate plot were observed every one-two weeks from 24 July until 26 August. At each rating, plant height—measured as centimeters from the soil surface to the base of the shoot apical meristem—and plants were examined for ALS symptoms. Disease pressure was very low across the entire region and no disease symptoms were observed until the end of the experiment in mid-October. Therefore, disease incidence—the number of plants out of the ten observed that showed any symptom of ALS—rather than disease severity was recorded for each replicate plot at the time of harvest. The experiment was harvested on 19 October by cutting stems of ten plants per replicate plot at their base, recording the total weight, removing senescent or diseased leaves that were unsaleable, and recording the marketable weight.

Statistical analysis. The effects of mulches on plant height, yield, and disease incidence were evaluated using a mixed linear model, with the above mentioned measures as dependent variables and with treatment and random block as the independent variables. Tukey's honestly significant difference was used to separate treatment means. All analyses were performed using JMP Pro version 10.0.0 (SAS Institute Inc, Cary, NC, USA).

Results

In-field presence of *A. brassicicola*. Rotorod spore traps were established on 04 June in order to detect spores of *A. brassicicola* that would be present in the field providing natural inoculum for the experiment. PCR analysis of DNA extracted from the rotorods yielded positive results, thereby confirming that *A. brassicicola* was present in the field (Figure 2).

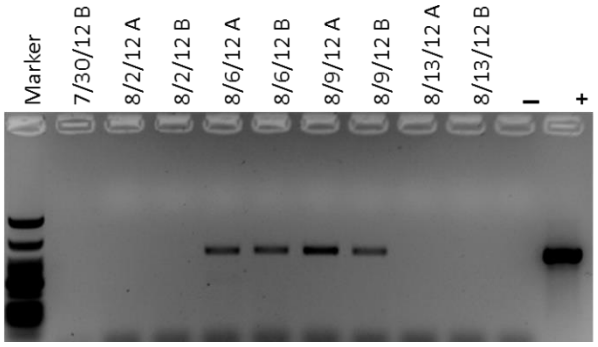

Figure 4.2. Products of PCR performed using genomic DNA extracted from rotorod sporetraps and amplified using *A. brassicicola*-specific primers were visualized after electrophoresis on a 2% Agarose gel. A positive and a negative control, and an ultra-low molecular weight lane marker were included on the gel. Amplification bands are at ~400bp, as expected from previously published results with this primer pair (Iacomi-Vasilescu et al., 2002).

However, when the spore traps were moved to the negative control site in the orchard, spores of the pathogen were still detected, indicating that there was considerable wind-dispersal of *A. brassicicola* spores (Figure 4.3). Importantly, on three out of the four collection dates from the orchard control site the rods had been dislodged from one but not both of the traps, likely a result of windy conditions during early July, and there was therefore no replication on those dates. Furthermore, on one orchard control site collection date (05 July) rods were collected

from both replicate traps but there was disagreement between them, and this disagreement between replicate traps also occurred on 21 September in Field 31. Spore traps were moved back into Field 31 on 12 July and the presence of air-borne *A. brassicicola* was monitored there for the remainder of the experiment. Results from PCR of these rotorod spore trap samples showed that *A. brassicicola* spores were captured during somewhat distinct episodes in early July and early August, but for much of the season no spores were collected (Figure 4.3). These episodes are difficult to link directly to weather events or field operations like cultivation or mowing which might disturb the soil and cause major spore releases. Nonetheless, spores of the pathogen were detected continuously beginning 18 September until 25 September, corresponding to lower temperatures and higher frequency of rain events. Traps were removed from the field on 25 September for the remainder of the experiment.

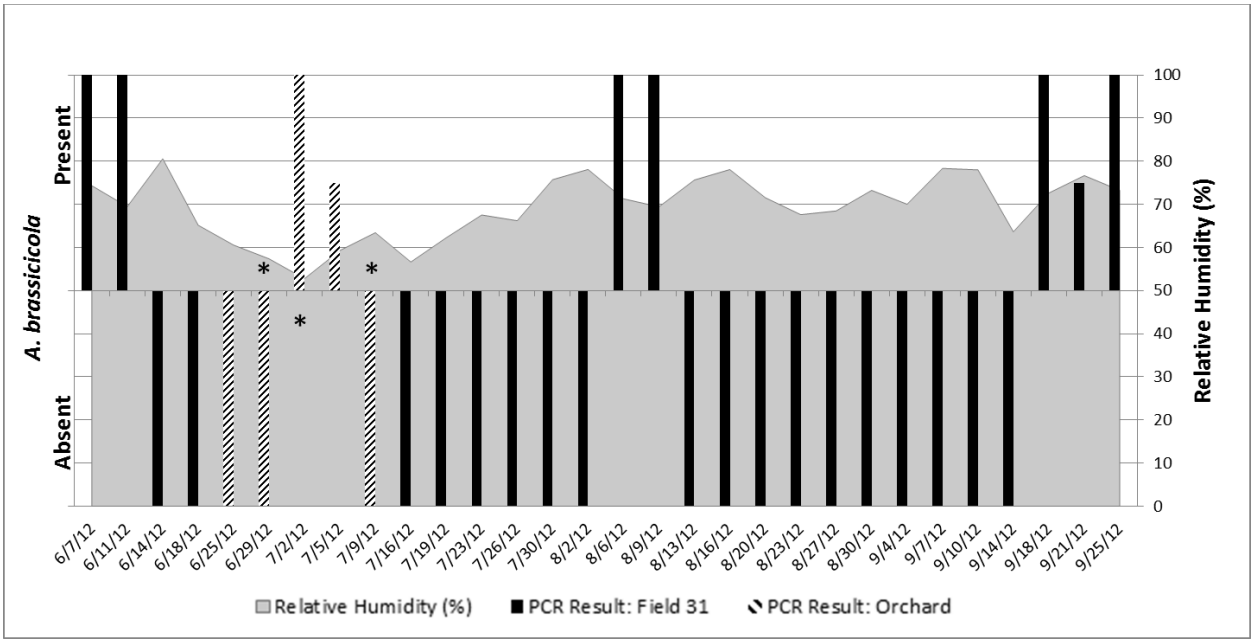

Figure 4.3. Rotorod spore traps were used to sample air for spores of *A. brassicicola* throughout the course of the experiment. Black bars show positive or negative results of PCR of samples taken from Field 31 while lined bars indicate samples from the orchard control site. Short bars represent samples for which there was disagreement between the two replicate spore traps.

Asterisks denote samples for which only one sample was collected. Average relative humidity (%) for the collection period is depicted by the blue shaded area.

Effects of mulches on plant growth and yield. It was clear from the first rating that plants in the straw mulch group were much larger and healthier than plants in the other treatment groups. Plant height was therefore measured throughout the study in order to document this observation and determine whether or not it was a statistically significant trend. Indeed, significant differences between treatment groups were found and these differences remained significant for the duration of the experiment (Table 4.1). All of the mulches tested caused significant increases in plant height compared to the bare ground control, with straw mulch providing the greatest increase in growth and the two sheet mulches performing intermediately. Unfortunately, increases in plant growth resulting from mulch use did not cause significant increases in total or marketable yields (Table 4.2). Mixed linear models of marketable yield by treatment and random block indicated that this relationship was not significant (p-value = 0.1168). The quantile plot of the residuals showed that there was one outlier and when this data point was excluded the p-value for the F-test was reduced to 0.0543, but was still not quite significant. However, it is clear that there was positive relationship between mulch and yield (Table 4.2).

Table 4.1. Effect of mulch on plant height (cm).

	Plant Height (cm)[z]						
Mulch Type	24-Jul	1-Aug	13-Aug	26-Aug	14-Sep	1-Oct	19-Oct
Control……	6.3 c	9.9 b	16.0 c	22.6 c	33.8 c	40.4 c	44.3 c
Plastic…….	7.6 b	11.8 b	18.5 b	27.2 b	39.3 b	45.6 b	49.1 b
Biotelo…….	7.7 b	11.2 b	17.8 bc	26.1 b	38.7 b	45.0 b	48.4 b
Straw……..	11.2 a	14.7 a	22.0 a	30.7 a	43.8 a	50.3 a	53.5 a
	p < 0.0001	p = 0.0002	p = 0.0002	p < 0.0001	p < 0.0001	p = 0.0006	p = 0.0005

[z]Plant height (cm) was measured from soil surface to base of shoot apical meristem. Ten plants per replicate plot were measured and averages across all 4 reps are presented.

Table 4.2. Effects of mulch on plant yield (kg) were analyzed using mixed linear models but no significant differences were found.

Mulch Type	Total Yield (kg)	Marketable Yield (kg)
Control…….	13.7	11.7
Plastic……..	15.9	13.5
BioTelo……	16.3	13.9
Straw……	16.4	13.7
	p = 0.1788	p = 0.1168

Effects of mulches on ALS incidence. The 2012 growing season was characterized by dry, hot conditions, with average daily maximum temperatures of 29° and 27.4° C and total precipitation of 7.1 cm and 5.75 cm in July and August, respectively. These dry, hot conditions are not conducive to development of ALS and as a result, disease severity was not detectable until the very end of the experiment. Therefore, disease was rated at the time of harvest on 19 October as incidence of ALS symptoms on ten plants per replicate plot. This data showed a significant reduction in disease incidence for plants grown in straw mulch compared to plants grown in bare ground (Figure 4.4). Plants grown in either of the two sheet mulches had intermediate levels of disease but were not significantly different from the control group.

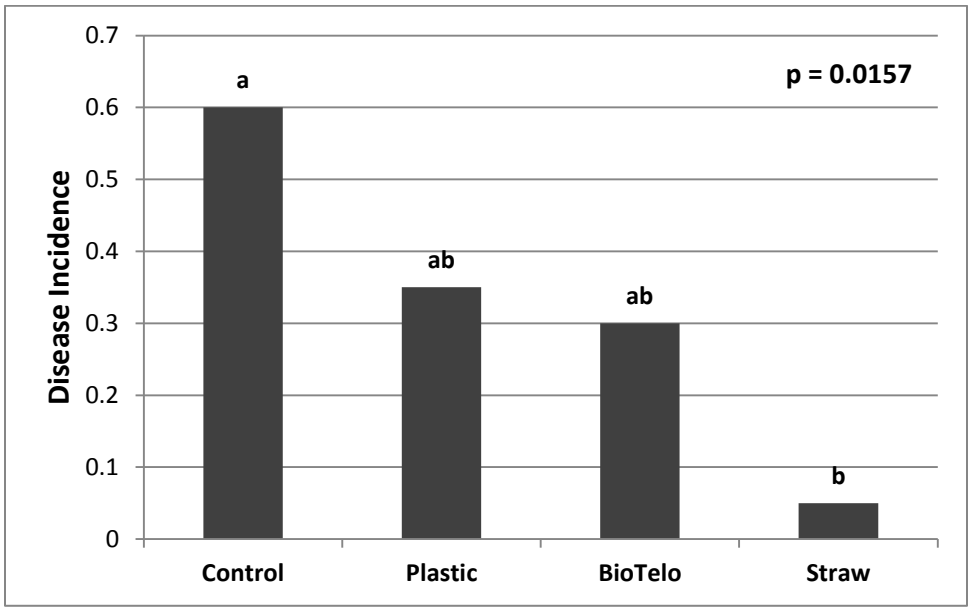

Figure 4.4. Effect of mulch on ALS disease incidence.

Discussion

In light of the increasing acreage and interest in organic cole crop production, new strategies for controlling Alternaria leaf spot in organic brassica cropping systems are essential. Cultural controls provide a first line of defense against plant pathogens and serve to minimize the effects of disease by preventing introduction of or infection by pathogens, or by manipulating the environment to reduce conduciveness to disease. In the present study, efficacy of mulches to protect a kale crop from ALS by providing a physical barrier between soil-borne inoculum and plant tissue was investigated. The results indicate that mulching can increase plant growth significantly, and that straw mulch can significantly reduce incidence of Alternaria leaf spot of kale. These findings support previous research which has shown beneficial effects of mulches on plant health and disease protection (Lamont, 2005; Waterer, 2010).

In-field presence of *A. brassicicola*. Rotorod spore traps were used to confirm the presence of the pathogen within the field and to collect and identify air-borne spores of *A. brassicicola* throughout the season. This is the first time the rotorod spore traps have been used in conjunction with DNA-based identification of samples in this pathosystem and these results, while preliminary, indicate that this technique could be used to further characterize movement and spread of *A. brassicicola* both temporally and spatially. Expanded deployment of traps and use of quantitative PCR methods to determine the amount of air-borne *A. brassicicola* inoculum present at a given time could help elucidate underlying questions of epidemiology of ALS in NY where it has not been well-studied previously.

One of the challenges to successful use of this pathogen monitoring technique that was encountered in this study was in setting up a good negative control. The rotorod spore traps were moved out of Field 31 and into another area of the NYSAES Research North farm that had been established as an apple orchard for many years. However, the distance between the orchard site

and the infested Field 31 (approximately 215 m) may not have been great enough to prevent wind dispersal of *A. brassicicola* spores into the orchard, as Humpherson-Jones and Maude (1982) showed that while spores of *A. brassicicola* are usually maintained within a field they can be spread up to 1800 meters during harvest of seed crops (Humpherson-Jones & Maude, 1982b). Additionally, there was another field planted to cabbage (*Brassica oleraceae*) about 155 m from the orchard site and 125 m from the experiment site which could have served as a source of ALS, increasing the chances that *A. brassicicola* spores would be found in the orchard control site.

Because of the failure of the negative control, it is difficult to assess whether spores that were collected in Field 31 in early July and again in early August originated within the field or were blown into the field from nearby or previous brassica plantings. There is also a concern that spores may have been wind dispersed into the experimental area from within Field 31, which had been shown to be infested with the pathogen. This effect was a concern in setting up this study, and was the reason that the remaining area of Field 31, outside the experimental area, was cover cropped with Sudex. However, the positive PCR results attained within Field 31 in July and August may have been a result of this within field wind dispersal—especially in July when the cover crop was not fully matured. Another explanation for these positive PCR results observed within the growing season is that the mulches did not successfully prevent soil-borne spores from being dispersed. Small gaps between mulch treatments as well as the bare ground control plots would be areas where soil-borne spores could disperse from. Despite the presence of air-borne *A. brassicicola* spores in Field 31 during the growing season, there was a significant effect of mulch treatment on ALS incidence, as described below, indicating that further investigation of *A. brassicicola* spore dispersal may be warranted.

Disease Incidence. The hot and dry conditions that characterized the 2012 season were not favorable to infection and growth of *A. brassicicola*. As a result, disease pressure was very low across the region and no symptoms of ALS were observed in this study until the experiment was harvested in mid-October. Thus there was only one disease rating, on 19 October, to analyze. However, this time-point is arguably the most important one in addressing the hypothesis that mulches can act as physical barriers and prevent soil-borne spores from infecting the host foliage. This is because once an infection has occurred, *A. brassicicola* can continue to grow and produce spores as long as the climatic conditions remain favorable so the disease can be spread by spores produced from extant lesions on crop foliage, as well as from inoculum in the soil.

All three mulches studied reduced ALS incidence, but only the straw mulch significantly reduced disease incidence compared to the bare ground control. There may be several reasons for the observed differences in efficacy of the three mulches but soil coverage at the plant base, tearing of sheet mulches over the duration of the growing season, and surface moisture likely play important roles. In the case of the sheet mulches, a bulb planter or trowel was used to make a small hole into which the seedling was transplanted, thus leaving an area of exposed soil around the base of the plant. This is not true for straw mulch, which was spread closely around seedlings until they were nearly buried. In this regard, the physical barrier provided by the straw mulch was likely much more complete than those of the plastic sheet mulches. Furthermore, the sheet mulches can be ripped or torn by the user, animals, or even by wind whipping over the field creating more areas where the soil surface is exposed. This is especially true in the case of Biotelo, which is quite fragile and was indeed torn in the process of laying out the mulch and had many large tears in it by the end of the experiment. Incomplete soil coverage would likely

reduce the efficacy of the mulch in serving as a physical barrier to soil-borne inoculum and would also lead to an increase in weed pressure, which must then be managed by hand and can become a labor-intensive and thus costly task.

Another possible explanation for the difference in performance between the sheet mulches and the straw mulch is related to moisture at the soil surface. Spores of *A. brassicicola* can be spread throughout the field by wind, rain and rainsplash (Chen et al., 2003; Humpherson-Jones & Phelps, 1989). While for much of the growing season conditions remained hot and dry, in September and October the temperatures reduced and there were more frequent and heavier rain events. During these wetter periods, water could be seen pooled on the surface of both the LDPE and Biotelo sheet mulches while rainwater was able to percolate through the straw mulch and no standing water collected at the soil surface. These puddles may have caused splash dispersal of spores or provided free moisture and leaf wetness (low hanging leaves of kale were observed dangling in these pools at times) needed to induce spores to germinate, cause infection, and sporulate (Humpherson-Jones, 1992). Perhaps if a mechanical plastic-layer had been used to form raised beds underneath the plastic we would have had better soil-mulch contact (fewer air pockets that later became sunken areas) and water may have rolled off the film and away from the crop host. This was not done because the experimental area was so small that randomization of the mulch treatments would have been difficult to achieve with the tractor-mounted plasticulture equipment.

Plant Growth and Yield. At the first time-point, plants in the straw mulch treatment group were observably larger and more robust than those in any other treatment group. Therefore, plant height was monitored over the course of the growing season, starting on 24 July—about three weeks after transplanting. Indeed all three mulches increased plant height

significantly compared to a bare ground control. The two sheet mulches performed equivalently while the straw mulch provided a significantly greater growth advantage than the two plastic mulches (Table 4.2). Mulching with plastic or straw can increase plant growth through impacts on soil temperature, water retention and evaporation, and absorptivity vs. reflectivity of the soil surface (Lamont, 2005). Black plastic mulches are known to increase soil temperature throughout the season while organic mulches such as straw can actually reduce soil temperatures (Monks et al., 1997). Both plastic and organic mulches can also increase soil moisture by limiting water loss through evaporation, providing a more constant moisture regime and favoring higher activity of soil micro- and macro-biota (Kasirajan & Ngouajio, 2012; Lamont, 2005; Monks et al., 1997; Tiquia et al., 2002).

Kale is one of the many cultivated varieties of cole crops belonging to the species *Brassica oleracea.* These are cool season crops with optimal daily growth temperatures between 15.5° and 21° C, while average daily maximum temperatures in 2012 were 29° and 27.4° C in July and August, respectively. Thus, it seems likely that under these hot and dry conditions, kale plants benefitted more from the cooling effects of straw mulch than from the warming effects of the black sheet mulches. This would have been especially true for kale seedlings at transplant in early July, when daily maximum temperatures were consistently above 26.7° C and the high for 05 July was 33.3° C. Kale seedlings transplanted into the black plastic mulches would have experienced much greater heat stress than those mulched with straw, and this was evidenced by the greater mortality and necessary replacement of those seedlings compared to seedlings in the straw mulch treatments.

It seems clear that under hot, dry conditions organic mulch may provide greater benefits to growth of cole crops than would plastic mulches which increase soil warming, but it is not

clear what the effects of these mulches would be in a cooler year or during spring or fall production when temperatures are lower. Increased soil warming provided by black plastic is often seen as a benefit to production in many cropping systems, as it allows for earlier spring transplanting (Kasirajan & Ngouajio, 2012; Lamont, 2005). During the more rainy spring and fall seasons, plastic mulch may keep excess moisture away from the crop, lowering disease incidence and effects of rot (Lamont, 2005; Monks et al., 1997; Waterer, 2010). The results of the present study indicate that organic mulches such as straw may be useful in growing cool season crops under hot, dry conditions, but more research would be necessary to determine their effects under other climatic conditions.

Mulches may have also provided a soil barrier for the insect pest, crucifer flea beetle (*Phyllotreta cruciferae*). Adult flea beetles feed on green tissue of host plants during the day and at night they burrow under the soil (Burgess, 1977). Adult females lay their eggs in moist soil at the base of host plants and when larvae emerge they live within the soil where they feed on host roots until they pupate and emerge as adults after about two-three weeks (Andersen et al., 2005; Burgess, 1977). While damage to kale plants by flea beetles was not measured in the present study, plants in the bare ground control treatments had observably more "shot-hole" feeding injury from flea beetles than did plants in mulched treatments. Flea beetles can also be important contributors to severity of ALS outbreaks in that these insects are able to physically transport *A. brassicicola* spores on their bodies and in their feces, spreading ALS from plant to plant and field to field (Dillard et al., 1998). Thus, if mulches can reduce flea beetle numbers in brassica plantings, they may also reduce ALS severity concomitantly. There is also reason to believe that there may be differences in flea beetle behavior depending on the type of mulch used, as populations of *Phyllotreta pusilla* (Western black flea beetle) were shown to be influenced by

mulch color and reflectivity (Demirel & Cranshaw, 2005). Future studies using mulches in brassica cropping systems should include measurements of insect damage so that these effects can be compared statistically, since there seemed to be a strong influence of mulch on flea beetle feeding damage in the present study.

The present study demonstrated that mulches have the potential to reduce ALS incidence, perhaps by reducing the initial infection of kale by overwintering *A. brassicicola* spores. Mulches also increased plant growth and, in a hot and dry year, straw mulch provided the greatest benefit to crop growth. Future studies are warranted to confirm these results under varying field conditions and with greater disease pressure. Subsequent studies should include additional measurements such as soil temperature, soil moisture, and severity of insect damage. These parameters would further our understanding of why the three mulches caused different effects on kale growth and ALS incidence and allow us to make better recommendations for brassica producers in NY.

REFERENCES

Abdul-Baki AA, Teasdale JR, Goth RW, Haynes KG, 2002. Marketable yields of fresh-market tomatoes grown in plastic and hairy vetch mulches. *Hortscience* **37**, 878-81.

Agrios GN, 2005. *Plant Pathology*. Burlington, MA: Elsevier Academic Press.

Andersen CL, Hazzard R, Van Driesche R, Mangan FX, 2005. Overwintering and seasonal patterns of feeding and reproduction in Phyllotreta cruciferae (Coleoptera : Chrysomelidae) in the northeastern United States. *Environmental Entomology* **34**, 794-800.

Anonymous. <Meena et al_2004.pdf>.

Burgess L, 1977. Flea beetles (Coleoptera-Chrysomelidae) attacking rape crops in Canadian prairie provinces. *Canadian Entomologist* **109**, 21-32.

Chen LY, Price TV, Park-Ng Z, 2003. Conidial dispersal by *Alternaria brassicicola* on Chinese cabbage (*Brassica pekinensis*) in the field and under simulated conditions. *Plant Pathology* **52**, 536-45.

Cirujeda A, Aibar J, Anzalone A, *et al.*, 2012. Biodegradable mulch instead of polyethylene for weed control of processing tomato production. *Agronomy for Sustainable Development* **32**, 889-97.

Demirel N, Cranshaw W, 2005. Colonization of cabbage by the western black flea beetle (*Phyllotreta pusilla*) as affected by mulch and time of day. *Phytoparasitica* **33**, 309-13.

Dillard HR, Cobb AC, Lamboy JS, 1998. Transmission of *Alternaria brassicicola* to cabbage by flea beetles (*Phyllotreta cruciferae*). *Plant Disease* **82**, 153-7.

Feuilloley P, Cesar G, Benguigui L, *et al.*, 2005. Degradation of polyethylene designed for agricultural purposes. *Journal of Polymers and the Environment* **13**, 349-55.

Humpherson-Jones FM, 1989. Survival of *Alternaria brassicae* and *Alternaria brassicicola* on crop debris of oilseed rape and cabbage. *Annals of Applied Biology* **115**, 45-50.

Humpherson-Jones FM, 1992. Epidemiology and Control of Dark Leaf Spot of Brassicas. In: Chelkowski J, Visconti A, eds. *Alternaria Biology, Plant Diseases and Metabolites.* New York, NY: Elsevier, 267-88.

Humpherson-Jones FM, Maude RB, 1982. Studies on the epidemiology of *Alternaria brassicicola* in *Brassica oleracea* seed production crops. *Annals of Applied Biology* **100**, 61-71.

Humpherson-Jones FM, Phelps K, 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola*. *Annals of Applied Biology* **114**, 449-58.

Iacomi-Vasilescu B, Blancard D, Guenard M, Molinero-Demilly V, Laurent E, Simoneau P, 2002. Development of a PCR-based diagnostic assay for detecting pathogenic *Alternaria* species in cruciferous seeds. *Seed Science and Technology* **30**, 87-95.

Kasirajan S, Ngouajio M, 2012. Polyethylene and biodegradable mulches for agricultural applications: a review. *Agronomy for Sustainable Development* **32**, 501-29.

Kohl J, Vlaswinkel M, Haas B, *et al.*, 2011. Survival of pathogens of Brussels sprouts (*Brassica oleracea* Gemmifera Group) in crop residues. *Plant Pathology* **60**, 661-70.

Lamont WJ, 2005. Plastics: Modifying the microclimate for the production of vegetable crops. *Horttechnology* **15**, 477-81.

Monks CD, Monks DW, Basden T, Selders A, Poland S, Rayburn E, 1997. Soil temperature, soil moisture, weed control, and tomato (Lycopersicon esculentum) response to mulching. *Weed Technology* **11**, 561-6.

Ngouajio M, Auras R, Fernandez RT, Rubino M, Counts JW, Kijchavengkul T, 2008. Field performance of aliphatic-aromatic copolyester biodegradable mulch films in a fresh market tomato production system. *Horttechnology* **18**, 605-10.

Tiquia SM, Lloyd J, Herms DA, Hoitink HaJ, Michel FC, 2002. Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes. *Applied Soil Ecology* **21**, 31-48.

Trdan S, Žnidarčič D, Kač M, Vidrih M, 2008. Yield of early white cabbage grown under mulch and non-mulch conditions with low populations of onion thrips (Thrips tabaci Lindeman). *International Journal of Pest Management* **54**, 309-18.

Waterer D, 2010. Evaluation of biodegradable mulches for production of warm-season vegetable crops. *Canadian Journal of Plant Science* **90**, 737-43.

APPENDIX I

BIOCHAR: CHEMICAL ANALYSES AND EFFECTS ON CABBAGE GROWTH AND ALTERNARIA LEAF SPOT SEVERITY

Introduction

Organic matter amendments are known to cause shifts in density and composition of microbial communities, and can therefore influence microbially-mediated plant defense responses (Hoitink & Boehm, 1999; Hoitink & Fahy, 1986; Lehmann et al., 2011; Vallad & Goodman, 2004; Whipps, 2001). Biochar is the product of thermal degradation of organic materials in the absence of air, a process known as pyrolysis, and is being extensively studied worldwide for its potential as a soil organic matter amendment to improve soil fertility, and water and nutrient retention, as well as to sequester carbon and mitigate climate change (Lehmann et al., 2011). While biochar has begun to be studied widely in agronomic settings, little is known about their effects on plant disease.

A few recent studies report that biochar has been successfully used to reduce disease in controlled environment and microplot studies and, while several mechanisms are likely responsible for the observed disease suppression, induced systemic resistance has been documented in several pathosystems (Elad et al., 2010; Elmer & Pignatello, 2011; Graber et al., 2010; Harel et al., 2012; Kolton et al., 2011; Lehmann et al., 2011). A greenhouse study by Elad et al. in 2010 showed significant reduction in disease severity caused by the foliar pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) on pepper and tomato with application of biochar made from citrus wood at 3 and 5% (w/w) to coconut fiber potting

mix (Elad et al., 2010). In a similar greenhouse study by Harel et al. (2012), significant reductions in disease severity caused by three foliar diseases of strawberry (*Botrytis cinerea, Colletotrichum acutatum* and *Podosphaera apahanis*) were observed when plants were grown in 3% biochar (w/w) (Harel et al., 2012). Furthermore, biochar that is made from burning of plant or animal material is approved by the Organic Materials Review Institute (OMRI), and are listed for use by certified organic growers as soil amendments.

Therefore the effects of biochar on growth of brassica crops and Alternaria leaf spot severity were investigated. Biochar produced from Southern Yellow Pine woodchips was obtained from Waste to Energy Solutions Inc, Destin, FL, and used in greenhouse and field studies investigating the sustainable management of ALS in brassica crop production. A detailed chemical analysis was conducted in order to determine the elemental composition and relevant physical and biochemical parameters of the biochar used in greenhouse and field trials so that it could be compared to other biochars that have been studied in agronomic settings, and so that any toxic components could be identified (Table A1.1 and Table A1.2). Furthermore, a greenhouse study on the effects of biochar amendment on plant growth and ALS severity was investigated in a greenhouse study. Rates tested included 2.0% (w/w0 biochar because previous research has shown that application rates of 1-5% (w/w) biochar were sufficient to achieve disease control under greenhouse conditions (Elad et al., 2010), while 0.2% (w/w) biochar gave the best benefit to crop yield in a field study of corn yield (Rajkovich et al., 2011). A high rate (20% w/w) was also included in order to test for phytotoxicity.

Chemical Analyses

Samples of the southern yellow pine biochar used throughout the present thesis were analyzed for pH at the New York State Agricultural Experiment Station (NYSAES) and all additional chemical characterization of the material was performed by the Cornell Nutrient Analysis Lab in Ithaca, NY. pH was determined by adding 1 g of biochar sieved to 2mm to 20 ml deionized water, shaking at 30°C for 1.5 hours, and measuring pH with a calibrated pH electrode. All CNAL analyses follow the standard operating procedures of the soil survey laboratory methods manual developed by the Natural Resources Conservation Service and methodological details can be found therein. Briefly, cation exchange capacity, a measure of nutrient retention capability and common measure of soil fertility, was measured by ammonium acetate extraction (CNAL test number 2032); total carbon (%), total nitrogen (%) were measured by dry combustion (CNAL test number 2735); ammonium and nitrate (mg/kg) were measured by a colorimetric analysis of potassium chloride extracted material (CNAL test number 2511); and total elemental analysis was performed by analysis of nitric acid/perchloric acid digested material using inductively coupled plasma atomic emission spectroscopy (ICAP) (CNAL test number 2021). Results from these tests were compared to available data on biochars produced from similar feedstocks, as well as to a compost-amended potting mix in order to provide a basis for comparison to other soil organic matter amendments.

Table A1.1. Biochemical analysis of Southern Yellow Pine (SYP) biochar compared to biochars made from other feedstocks and to a rich compost.

Material	pH	CEC	Total C (%)	Total N (%)	NH_4 (mg/kg)	NO_3 (mg/kg)
SYP	9.17	17.71	39.34	0.28	1.18	0.39
Pine[z]	-	-	67.2-91.1	0.1	-	-
Mixed Woodchips[y]	-	-	85.9	0.4	-	-
Vermicompost Soil Mix[x]	-	-	34.55	1.33	81.5	1017

[z]Range of total carbon or total nitrogen of biochars made from a pine feedstock and pyrolized at varying temperatures (Rajkovich et al., 2011). [y]Total carbon and nitrogen of biochar produced from mixed woodchips (Rajkovich et al., 2011). [x]Total carbon and nitrogen of an organic potting mix amended with 20% (v/v) vermicompost (Jack et al., 2011).

Table A1.2. Elemental analysis of Southern Yellow Pine (SYP) biochar compared to biochars produced from wood substrates by Rajokovish et. al (2011), and to a vermicompost-amended organic transplant media.

Element	SYP Biochar (mg/kg)	Pine[z] (mg/kg):	Mixed Woodchips[y] (mg/kg):	Vermicompost Mix[x] (mg/kg):
Be	0			
Se	4			
Sb	2			
Ba	44			
Cd	0			
Na	273	93-351	311	
Mg	1783	143-796	1267	
K	1404	196-996	1573	2645
As	0			
V	0			
B	24			
Cr	0			
Ca	6022	1480-2927	5427	
Zn	3	21-66	93	
Sr	26			
Li	0			
Al	164			
P	546	1-255	270	842.5
S	185	48-1692	193	
Ti	5			
Mn	292	28-349	270	
Fe	825	40-1166	4208	
Co	0			
Ni	0			
Cu	7			
Mo	0			
Pb	0			

[z]Range of elemental composition (mg/kg) of biochars made from a pine feedstock and pyrolized at varying temperatures (Rajkovich et al., 2011). [y]Elemental composition of biochar produced from mixed woodchips (Rajkovich et al., 2011). [x]Elemental composition of an organic potting mix amended with 20% (v/v) vermicompost (Jack et al., 2011).

Greenhouse Study

The experiment was conducted in a greenhouse at the New York State Agricultural Experiment Station in Geneva, NY where plants were grown under natural light and a maximum temperature of 24 °C. A randomized complete blocks design was used with each treatment replicated three times. Biochar was sieved to 2mm and was mixed with Cornell Mix potting soil—a mixture of peat, perlite, and vermiculite at a ratio of 4:1:1—using dry weight equivalents at the rates given in Tables A1.3 and A1.4. Similar rates have been used in previous studies and have shown improved plant health and yield as well as reduced disease severity (Elad et al., 2010; Jack et al., 2011; Rajkovich et al., 2011). Cabbage seedlings (var. Amtrak, Bejo Seeds, Geneva, NY) at the two-leaf stage were transferred from seedling trays to 10 cm experimental pots containing the biochar-amended soils. Plants were maintained in the greenhouse for 28 days and were fertilized once on 22 June with Miracle Grow All Purpose Plant Food (24-8-16) at 3.95 g/L (Scotts Company, LLC, Marysville, OH, USA).

Plants were moved into a moist chamber (relative humidity of 100%, temperature of 21 °C, and 12 hour light/dark periods) where they were inoculated with a suspension of *A. brassicicola* spores or a mock inoculum—deionized water—in order to determine the effect of biochar on cabbage growth. The fungal inoculum was produced using *A. brassicicola* isolate #1153 which was grown in 100mm by 15 mm Petri dishes containing Difco Bacto Potato Dextrose Agar (BD Biosciences, Franklin Lakes, NJ, USA). A conidial suspension was made by flooding each culture plate with sterilized de-ionized water, gently scraping the agar surface with a rubber policeman, and straining the suspension through cheesecloth to remove mycelial fragments. Using a haemocytometer, the concentration of conidia in the inoculum was determined to be 1.67×10^6 spores/ml. The inocula were sprayed on cabbage foliage on 06 July

using handheld aerosol spray bottles (Thermo Fisher Scientific, Waltham, MA, USA) and plants were kept in the moist chamber for 48 hours after the inoculation in order to encourage successful infection. Plants were then returned to the greenhouse where they were monitored for nine days. Disease severity was measured every three days by estimating the percent leaf area covered in ALS lesions. Plant growth was also recorded at two and eight days post inoculation and was measured as centimeters from the soil surface to the base of the shoot apical meristem.

Disease severity data was used to calculate area under the disease progress curve (AUDPC), a method for quantifying disease development over time. AUDPC was calculated for each set of time-points, and was then summed over all time-points to yield an overall AUDPC. Effect of biochar amendments on disease severity was evaluated using a mixed linear model, with AUDPC as the dependent variable, and treatment and random block as the independent variables. Plant height data was evaluated for each time-point using a mixed linear model, with height as the dependent variable, and treatment and random block as the independent variables. Tukey's honestly significant difference was used to separate treatment means. All analyses were performed using JMP Pro version 10.0.0 (SAS Institute Inc, Cary, NC, USA).

Application of biochar to potting soil of cabbage plants had no significant effect on the severity of ALS symptoms for any of the rates tested (Table A1.3). At the first two time-points there was an observable increase in mean disease severity of plants that were grown in 20% biochar but this difference was not significant. Similarly, there were no significant differences between height of plants grown in biochar-amended soil compared to the unamended control, between biochar rates (Table A1.4). Cabbage plants grown in 20% (w/w) biochar amended soil were observably smaller (on average 1.53 cm shorter) than control plants, but this difference was not significant.

Table A1.3. Effect of biochar rate on ALS disease severity on cabbage plants.

% Biochar (w/w)	Disease Severity				AUDPC
	7/10/2012	7/13/2012	7/16/2012	7/19/2012	
0.0	0.33	0.42	0.57	0.62	4.42
0.2	0.34	0.44	0.63	0.66	4.72
2.0	0.31	0.46	0.63	0.68	4.77
20	0.53	0.56	0.64	0.67	5.42
	p = 0.0933	p = 0.1751	p = 0.5533	p = 0.6134	p = 0.2425

Table A1.4. Effect of biochar rate on cabbage growth (cm).

% Biochar (w/w)	Plant Height (cm)	
	7/12/2012	7/18/2012
0.0	9.23	9.77
0.2	8.5	9
2.0	9.3	10.3
20	7.7	8.1
	p = 0.5183	p = 0.2729

REFERENCES

Elad Y, David DR, Harel YM, *et al.*, 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. *Phytopathology* **100**, 913-21.

Elmer WH, Pignatello JJ, 2011. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. *Plant Disease* **95**, 960-6.

Graber ER, Harel YM, Kolton M, *et al.*, 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant and Soil* **337**, 481-96.

Harel YM, Elad Y, Rav-David D, *et al.*, 2012. Biochar mediates systemic response of strawberry to foliar fungal pathogens. *Plant and Soil* **357**, 245-57.

Hoitink HaJ, Boehm MJ, 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology* **37**, 427-46.

Hoitink HaJ, Fahy PC, 1986. Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**, 93-114.

Jack ALH, Rangarajan A, Culman SW, Sooksa-Nguan T, Thies JE, 2011. Choice of organic amendments in tomato transplants has lasting effects on bacterial rhizosphere communities and crop performance in the field. *Applied Soil Ecology* **48**, 94-101.

Kolton M, Harel YM, Pasternak Z, Graber ER, Elad Y, Cytryn E, 2011. Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants. *Applied and Environmental Microbiology* **77**, 4924-30.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D, 2011. Biochar effects on soil biota - A review. *Soil Biology & Biochemistry* **43**, 1812-36.

Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J, 2011. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils* **48**, 271-84.

Whipps JM, 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52**, 487-511.