MANAGEMENT AND POPULATION STRUCTURE OF *PHYTOPHTHORA CAPSICI* IN NEW YORK STATE

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Amara Ruth Camp

May 2009

ABSTRACT

The oomycete *Phytophthora capsici* causes Phytophthora blight on many vegetable hosts, resulting in devastating losses for growers in New York and around the world. Management of this pathogen requires an integrated approach, and the goal of the research presented here is to contribute to continued improvements in management recommendations. With this goal in mind, the efficacy of the potential biological control fungus *Muscodor albus* to control Phytophthora blight on five sweet pepper cultivars and one butternut squash cultivar via biofumigation was tested in the greenhouse. Three different rates of *M. albus* grown on rye grain (0.55 g/L, 1.9 g/L, and 3.75 g/L), mefenoxam (Ridomil Gold EC, Syngenta Crop Protection, Inc.), or nothing, were added to *P. capsici*-infested potting mix, and sweet pepper or butternut squash seedlings were transplanted into the potting mix one week later. Plants were rated for disease severity on a scale of zero (healthy) to five (dead) one week after transplanting. Although application of the highest rate of *M. albus* slightly reduced disease severity on the intermediately tolerant sweet pepper cultivars (Alliance, Aristotle, and Revolution), commercially-acceptable control was only achieved with the highly tolerant cultivar Paladin. Even Paladin peppers which received no curative treatment had low disease severity ratings, so the levels of control achieved on this cultivar may not be due to application of *M. albus*. None of the applied rates of *M. albus* controlled Phytophthora blight on butternut squash, or on the highly susceptible pepper cultivar Red Knight.

An improved understanding of the *P. capsici* population in New York will also help researchers to make better and more specific recommendations to local vegetable producers in the state. Therefore, in 2006 and 2007, 262 isolates of *P. capsici* were collected from 28 fields in New York and characterized for mating type and

mefenoxam sensitivity. No mefenoxam-resistant isolates were recovered from farms in western and central New York, while resistant isolates were frequently recovered in the Capital District and on Long Island. Both A1 and A2 mating types were recovered from many fields across the state. Isolates from three fields in western New York (field WNY), the Capital District (field CD) and Long Island (field LI) were selected for further characterization using five microsatellite loci. Based on mating type and alleles observed at these loci, 12, 20 and 6 genotypes were identified in each field, respectively. Both mating types were recovered from all three fields, and in fields CD and LI, ratios of A1 to A2 isolates were not significantly different from 1:1, while the ratio in field WNY did deviate significantly from 1:1. Fields WNY and LI were not in Hardy-Weinberg equilibrium, but field CD was. All three fields were highly differentiated from each other, with pairwise fixation indices (F_{ST}) ranging from 0.224 to 0.586. Overall, nearly 46% of the variation across all three fields could be attributed to variation among fields, and *P. capsici* populations in these three fields had different levels of diversity.

BIOGRAPHICAL SKETCH

Amara grew up in Rhode Island, where she developed an early interest in plants and science while gardening at home and visiting local parks, zoos and museums with her family. She attended Juniata College in Huntingdon, PA, where she continued to explore her interests in plants and research through a summer at Educational Concern for Hunger Organization (ECHO) in North Fort Myers, Florida, and an internship in the Plant Pathology department at The Ohio Agricultural Research and Development Center of the Ohio State University. After graduating from Juniata College in 2005, she spent a year living in the East End of Pittsburgh and working for Grow Pittsburgh on a small organic vegetable farm located within the city limits in the Stanton Heights neighborhood. Here she indulged her passion for growing plants, and also had the opportunity to observe plant pathogens in their "natural environment." Eager to learn more about these microbes and how they cause disease in plants, Amara began her graduate studies in the fall of 2006 in the (then) Department of Plant Pathology (now the Department of Plant Pathology and Plant-Microbe Biology) at Cornell University. She did her thesis research with Drs. Helene Dillard and Christine Smart on the vegetable pathogen *Phytophthora capsici*.

To my parents
who taught me to be curious and introduced me to the joy of growing plants

and

To Kevin and Susan
who continue to encourage me to pursue that joy

ACKNOWLEDGMENTS

I am very grateful to my advisors Drs. Helene Dillard and Christine Smart for their advice, direction, and encouragement. They taught me by example how to communicate effectively with various audiences, always made time for me, and enthusiastically volunteered to help with my project, whether I was transplanting peppers or tossing rotten cucumbers into the field. I would also like to thank my other committee members, Drs. William Fry and Anu Rangarajan for their advice and many valuable contributions, and for Dr. Fry's willingness to make space for me in his lab during my time in Ithaca.

The past and present members of the Smart lab, Holly Lange, Maryann Herman, Ning Zhang, Tanya Taylor, Jun Gu, Balaji Vasudevan, Kevin Conley, Sarah Davidson, Kathleen Kohl, Christopher Peeck, Robert Lee, Samantha Tandle, Angela McKinney, Leslie Hopke, and Rachel Bailey, have assisted me with my research in many and varied ways, and have made the lab an enjoyable and supportive place to work. I would also like to thank members of the Dillard and Reiners labs for their help in the field, members of the Fry lab for technical assistance, Denis Shah for statistical assistance, Dr. Michael Milgroom for advice on the statistical analysis and interpretation of the results of the population study, and Dr. Margaret McGrath for collecting symptomatic plants on Long Island. I am very grateful to Drs. Julia Meitz and Adele McLeod at the University of Stellenbosch in South Africa for allowing me to use the microsatellite primers they designed, and for providing advice and assistance as I learned this technique.

This work was funded in part by a grant from the New York State Department of Agriculture and Markets, and additional support was provided by AgraQuest, Inc., Harris Moran Seed Company, and a Cornell Travel Grant.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Phytophthora capsici (Leonian) is an important vegetable pathogen because of its rapid spread through fields during the growing season, its ability to persist in a field for many years in spite of rotation to non-host crops, and the limited availability of effective control strategies. First identified in 1918 on Chile peppers in New Mexico (Leonian 1922), *P. capsici* is the causative agent of Phytophthora blight and is found world-wide (Erwin and Ribeiro 1996). It is a soil-borne oomycete, and thrives in warm, wet weather, causing devastating losses on host crops (Erwin and Ribeiro 1996; Hausbeck and Lamour 2004). More than one third of the vegetable acreage (including fresh market and processing acreage) in New York is susceptible to Phytophthora blight, and many growers have experienced severe losses as a result of epidemics, particularly in wet growing seasons.

Sexual reproduction

Phytophthora capsici reproduces sexually by means of oospores, which are formed when antheridia and oogonia fuse (Figure 1.1 A). Because *P. capsici* is heterothallic, production of oogonia and antheridia is stimulated by the presence of two different mating types, or compatibility types (A1 and A2), in close proximity (Ristaino and Johnston 1999). A few *P. capsici* isolates do not produce oospores in the presence of either mating type (Bowers and Mitchell 1991), or they produce a few oospores in the presence of both mating types (Ristaino 1990; Bowers and Mitchell 1991; Islam et al. 2005). Once oogonia and antheridia have been produced, both outcrossing and self-fertilization can occur in other *Phytophthora* species (Shattock et

al. 1986; Ko 1988), so it is likely that the same is true of *P. capsici*. However, in at least one study, all oospore offspring from a cross appeared to be products of outcrossing, and not self-fertilization (Lamour and Hausbeck 2001b).

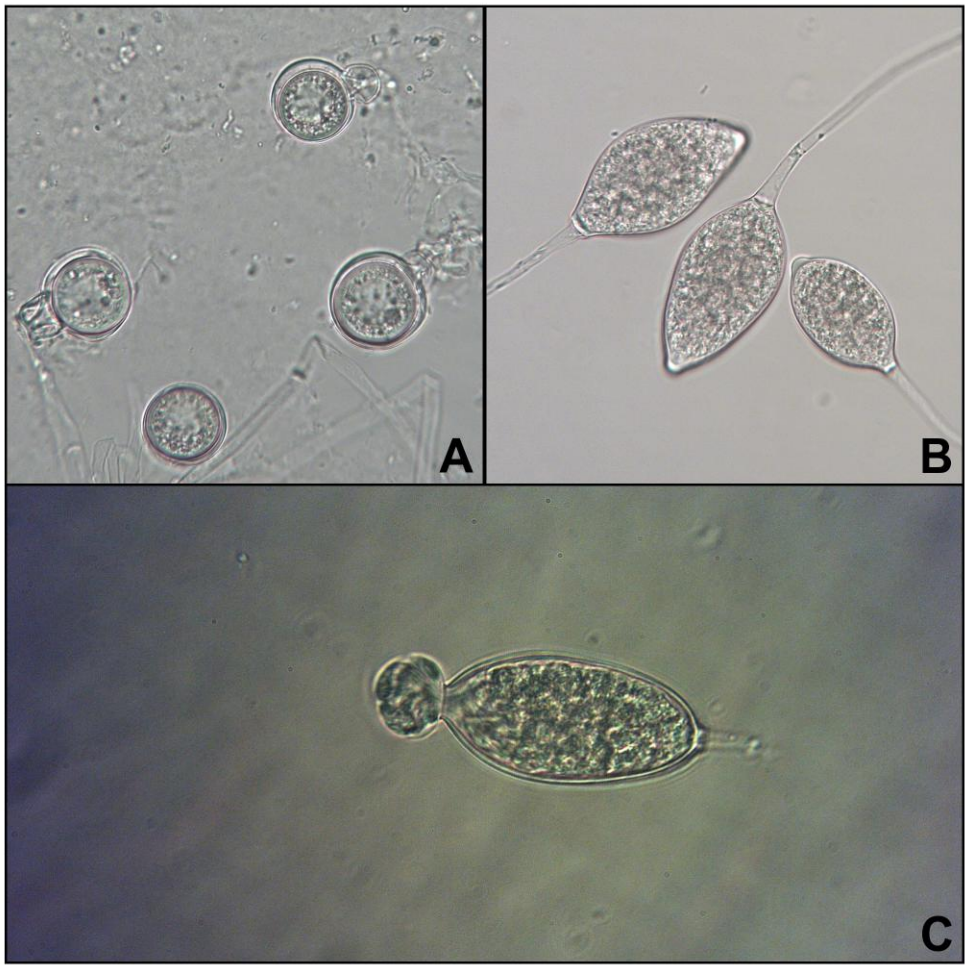

Figure 1.1 Sexual and asexual reproductive structures of *Phytophthora capsici*. Thick-walled sexual oospores of *P. capsici* are produced after the fusion of oogonia and antheridia (A). Asexual papillate sporangia of *P. capsici* are produced on long pedicels (B), and release motile zoospores (C).

Oospores develop within infected stems or fruits of host plants, and the oospores remain in the soil after the plant tissue rots, germinating when conditions are favorable. Cycles of low and high soil moisture (but not constant saturation) stimulate germination, but oospores do not all germinate simultaneously (Hord and Ristaino 1992; Ristaino and Johnston 1999). A dormancy period of a month, or more increases

oospore germination rates (Satour and Butler 1968; Zentmyer and Erwin 1970), and either hyphae or sporangia are produced upon germination (Zentmyer and Erwin 1970; Hord and Ristaino 1991). Oospore germination rates as high as 51% have been achieved in the lab, and germination can occur between 16°C and 32°C, although the optimal temperature for germination is around 24°C. *In vitro* germination rates increase when oospores are placed in soil extracts, as opposed to distilled water, and light is not required for germination, although germination is improved when oospores are formed in the dark. When oospores were incubated in water, root extract or soil extract for up to 12 days, germination rate increased with incubation time (Hord and Ristaino 1991).

Sexual reproduction in *P. capsici* is common, with both the A1 and A2 mating types being found in the same fields in many states, including Connecticut, Pennsylvania, California, Ohio, New York, North Carolina, and Michigan (Hausbeck and Lamour 2004). Each oospore produces offspring of a single genotype, and a cross between two parental isolates can produce many oospore offspring, each with a different genotype, and with potentially differential virulence on vegetable hosts (Satour and Butler 1968; Bowers and Mitchell 1991), different mating types, and a range of sensitivities to the fungicide mefenoxam (Lamour and Hausbeck 2000). This can include the production of oospore offspring which are completely resistant to mefenoxam, even if the parents were only partially resistant (Lamour and Hausbeck 2002). Significantly, oospore offspring can also be more virulent than either of the parental isolates (Satour and Butler 1968), and they can differ from the parental isolates in their pathogenicity on various host differentials (Polach and Webster 1972).

Thus, oospores supply primary inoculum and a source of genetic diversity at the beginning of each growing season and are therefore an important part of the life cycle of *P. capsici* (Bowers and Mitchell 1991; Ristaino and Johnston 1999; Lamour

and Hausbeck 2000). As few as one oospore per gram of soil can start an epidemic in a field (Bowers and Mitchell 1991), and in multiple Michigan studies, no identical isolates (as defined by amplified fragment length polymorphism, or AFLP, fingerprints) were collected in successive years in a single field. This suggests that only the sexual oospores survived the crop-free winter in Michigan (Lamour and Hausbeck 2001b; Lamour and Hausbeck 2003).

Asexual reproduction

Asexual sporangia are produced on the surface of host tissue, especially when relative humidity is high (Weber 1932; Crossan et al. 1954; Zentmyer and Erwin 1970; Anderson and Garton 2000). Each sporangium can germinate directly to produce hyphae, or, in the presence of adequate moisture, each one can produce and release 20-40 motile zoospores within a few hours (Zentmyer and Erwin 1970; Bernhardt and Grogan 1982; Hausbeck and Lamour 2004) (Figure 1.1, B-C). Like oospores, production of sporangia and production and release of zoospores is also sensitive to soil water potential (Bernhardt and Grogan 1982; Ristaino and Johnston 1999).

Chemotactic zoospores respond to plant root exudates and electrical fields (Hickman 1970), as well as to gravity (Erwin and Ribeiro 1996), and can be splashed to new plant tissue, or moved in surface water (Ristaino et al. 1994; Café-Filho and Duniway 1995; Ristaino et al. 1997; Roberts et al. 2005; Gevens et al. 2007). Zoospores of *Phytophthora spp.* tend to congregate and encyst just behind plant root tips, where elongation of roots is occurring and where the concentration of root exudates is high (Hickman 1970). Zoospores also attach to crowns or fruits, encyst, and then germinate. Hyphae produced either from direct germination of sporangia or germination of zoospores enter new host tissue through stomata or via direct

penetration (Crossan et al. 1954). In Michigan, the asexual reproductive structures of *P. capsici* rarely overwinter (Lamour and Hausbeck 2002), and in New York, field studies suggest that the sporangia and hyphae cannot survive the winter (*unpublished*, Camp, Dillard, Smart). In a Florida study conducted in a controlled environment, asexual propagules (mycelia and sporangia) survived for up to 44 days in sandy soil, but only at high soil moisture levels (Roberts et al. 2005). Zoospores and sporangia tend to be short-lived propagules, while oospores can survive longer in the soil, although survival of all propagules of *P. capsici* is influenced by soil temperature and water matric potential (Bowers et al. 1990). This is consistent with observations of other *Phytophthora* spp. (Duniway 1979).

Role of sexual and asexual reproduction in disease cycle

Asexual reproduction is very important within a single field, in a single growing season, allowing for the rapid spread of Phytophthora blight in a susceptible crop (Zentmyer and Erwin 1970; Lamour and Hausbeck 2002). One study in North Carolina found no correlation between initial density of *P. capsici* inoculum and final incidence of disease on pepper, confirming the importance of secondary inoculum in this polycyclic disease (Ristaino 1991). Sexual reproduction is important in maintaining the population of *P. capsici* in a field from year to year. It ensures survival of *P. capsici* in crop-free periods (via oospore production), and also supplies a means of outcrossing and increased genetic diversity in the population (Lamour and Hausbeck 2002). In general, the ability to reproduce sexually is considered to be an advantage for many *Phytophthora* species (especially heterothallic ones), because it allows the formation of oospores which can survive in the absence of a host plant, and also provides a mechanism for the removal of deleterious mutations which might otherwise accumulate (Goodwin 1997).

Host range

Phytophthora capsici infects a broad range of vegetable crops (Figure 1.2), including all cucurbits, peppers, tomatoes and eggplants, but not potatoes (Erwin and Ribeiro 1996; Hausbeck and Lamour 2004). Recently, *P. capsici* was also isolated from snap beans in Michigan (Gevens et al. 2008) and Long Island (*personal communication*, M. T. McGrath), and lima beans in Delaware, Maryland and New Jersey (Davidson et al. 2002). Symptoms on beans appear as water-soaked foliar lesions, stem and pod lesions, and general wilting (Davidson et al. 2002; Gevens et al. 2008). In artificially-inoculated field trials in New York, we have observed primarily pod lesions and some foliar lesions. In a Michigan study, Frasier firs were also susceptible to *P. capsici*, resulting in bronzing of needles and root rot (Quesada-Ocampo et al. 2009).

While *P. capsici* attacks many crop plants, symptoms are not identical on all hosts (Café-Filho and Duniway 1995; Erwin and Ribeiro 1996; Ristaino and Johnston 1999). Under favorable conditions (high soil moisture and 25°-30°C), infection of the crown or roots of the plant can lead to rapid wilting and plant death, especially of peppers and cucurbits (Café-Filho and Duniway 1995). Fruit infection results in rotting and "melting" of cucurbits, and lesions on pepper, tomato, and eggplant fruit (Weber 1932; Erwin and Ribeiro 1996). Fruit infections may be accompanied by characteristic powdery white sporulation on the fruit surface when humidity is high (Weber 1932; Erwin and Ribeiro 1996; Ristaino and Johnston 1999). In Michigan, fruit rot is a serious problem on cucumbers, but not on peppers, while other parts of the country report more problems with pepper fruit rot (Hausbeck and Lamour 2004). Pepper plants seem to become less-susceptible to at least the crown rot phase of Phytophthora blight as they age (Reifschneider et al. 1986; Kim, Y. J. et al. 1989).

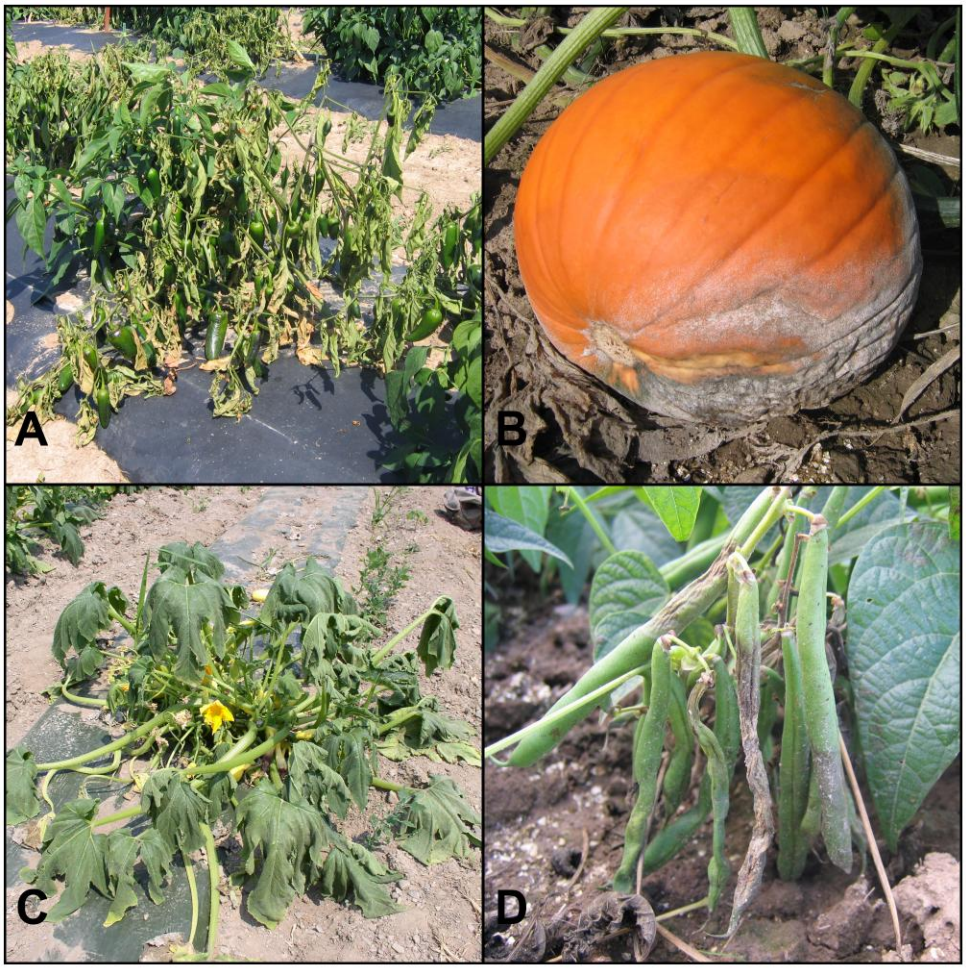

Figure 1.2 Host range and symptoms of *Phytophthora capsici*. *P. capsici* infects a variety of vegetable hosts, causing fruit rot, wilting, and plant death. Hosts include (but are not limited to) peppers (A), pumpkins (B), cucurbits (C), and beans (D).

Overall the fact that cucurbit fruits are frequently in direct contact with potentially-infested soil makes these fruit especially vulnerable to the fruit rot phase of the disease, although plant wilting and death is also common, sometimes accompanied by crown rot (Erwin and Ribeiro 1996; Hausbeck and Lamour 2004). In general, squash tend to be highly susceptible to Phytophthora blight. While the entire pepper, eggplant or tomato plant may be killed by a root or crown infection, the fruit are not in direct contact with soil or irrigation water as frequently as cucurbit fruits, and infection of tomato or pepper fruit requires this contact with infested soil or water (Café-Filho and Duniway 1995). In Michigan, tomato and cucumber plants may

remain relatively asymptomatic after infection (although fruit may develop symptoms), but symptoms sometimes appear following heavy rain. In addition, fruit of all host crops may be latently infected for several days, so that apparently healthy fruit is harvested from the field, but later rots during transportation or storage (Hausbeck and Lamour 2004).

Isolates of *P. capsici* vary in their virulence on different host crops (Palloix et al. 1988; Ristaino 1990; Lee, B. K. et al. 2001; Islam et al. 2005; French-Monar et al. 2006b), and on different cultivars of the same host crop (Reifschneider et al. 1986; Kim, F. S. and Hwang 1992; Lee, B. K. et al. 2001; Islam et al. 2005; Silvar et al. 2006). Similarly, certain *P. infestans* isolates are better adapted, or even exclusively adapted, to infect either tomato or potato, but not necessarily both (Fry et al. 1992). While *P. capsici* isolates generally infect both cucurbitaceous and solanaceous hosts, regardless of the isolate's origin, some isolates from cucurbits tend to cause less severe disease on solanaceous hosts than on cucurbit hosts, and vice versa (Ristaino 1990; Lee, B. K. et al. 2001). Ristaino compared the susceptibility of peppers to *P. capsici* isolates collected from cucurbits, sweet peppers, and hot peppers. In that study, isolates from cucurbits differed in their virulence on peppers and in their morphology. However, morphological variability was not sufficient to separate isolates based on the host of origin (Ristaino 1990). Islam et al. also observed some variability in optimal growth temperatures and in colony morphology of 30 isolates collected from pumpkin in Illinois. The clustering of these isolates into virulence groups also corresponded to clustering based on random amplified polymorphic DNA, or RAPD analysis (Islam et al. 2005). In a Brazilian study utilizing RAPD analysis, 22 isolates of *P. capsici* clustered mostly by host plant from which the isolate was obtained (Luz et al. 2003). Mchau and Coffey (1995) also reported extensive diversity of morphological and physiological traits of *P. capsici* isolates collected from around the world.

Spread of **P. capsici** *within and between fields*

Evidence collected to date indicates that sporangia are not wind-dispersed but that they are spread by splashing or wind-driven water, overhead irrigation, and in-row water movement (Ristaino 1991; Ristaino et al. 1994; Café-Filho and Duniway 1995; Ristaino et al. 1997; Ristaino and Johnston 1999; Lamour and Hausbeck 2002; Hausbeck and Lamour 2004). This is in contrast to *P. infestans*, which is readily wind-dispersed (Fry et al. 1992). Ristaino et al. reported that disease spread primarily along rows, rather than between rows, except when water drained across rows in a field (Ristaino et al. 1994). In the soil, movement of inoculum to plant roots is more important in causing symptom development than is movement of roots to inoculum, or direct contact between roots of plants (Sujkowski et al. 2000). However, movement of zoospores through soil may be limited, depending on the soil type (Café-Filho and Duniway 1995). Planting solanaceous and bushing cucurbit crops on raised beds covered with plastic mulch can provide a physical barrier between *P. capsici* in the soil and the susceptible host, reducing contact between infested soil and aerial host tissues. However, plastic mulch does not prevent the spread of *P. capsici* within a row through the soil, or in surface water on top of the mulch. In fact, surface inoculum of *P. capsici* can spread rapidly on plastic mulch (Springer and Johnston 1982; Ristaino et al. 1997). Even on bare soil, *P. capsici* inoculum can also be spread in surface water up to 70 m downstream from inoculum sources with regular furrow irrigation (Café-Filho and Duniway 1995). Upstream spread is minimal. In New York, we have observed that movement of infected fruits or farm equipment between fields, as well as cultivation of a field can also spread *P. capsici* (either oospores or sporangia) between and within fields, respectively.

Management – cultural practices

Based on the way in which *P. capsici* spreads within and between fields, a number of cultural practices may be used to prevent the introduction of *P. capsici* into a field, limit spread and disease development during the growing season, or reduce inoculum survival from year to year. Exclusion is the first line of defense for a grower. Because oospores may be present in either infected fruit or soil, preventing the movement of both soil and fruit (even symptomless fruit, which may be latently infected) between fields can delay the introduction of *P. capsici* into a new field.

Controlling water in the field (by not over-irrigating and by promoting good drainage) is perhaps the most important cultural control strategy for Phytophthora blight, as it minimizes favorable conditions for the pathogen and limits spread within the field (Springer and Johnston 1982; Ristaino 1991; Biles et al. 1992; Café-Filho et al. 1995; Café-Filho and Duniway 1996; Xie et al. 1999). In Chile pepper fields of New Mexico, disease incidence was higher with furrow irrigation than with drip irrigation (Sanogo and Carpenter 2006). Ristaino reported that less-frequent irrigation and less rain during a season (especially rainfall events exceeding 2 cm) were correlated with later disease onset and lower disease incidence (Ristaino 1991). Because delayed onset of disease can be correlated with increased yields (Ristaino 1991), properly managing water is still an important management strategy even in fields with a history of Phytophthora blight. However, in fields and years with high rainfall, population densities of *P. capsici* are not affected by the level of irrigation (Ristaino et al. 1992). Thus, where wet growing seasons are relatively common, or initial soil populations of *P. capsici* are extremely high, reducing soil moisture may be either not possible, or not helpful in controlling disease (Hausbeck and Lamour 2004).

Because *P. capsici* can infest irrigation water, water sources for irrigation of susceptible crops should also be chosen with care (Roberts et al. 2005; Gevens et al.

2007). Viable *P. capsici* inoculum has been recovered from irrigation ponds, at very low levels, up to 63 days after zoospores were placed in the ponds (Roberts et al. 2005). In New York, we have observed that infested irrigation water can be a means of spreading *P. capsici* from a single infested field to additional fields on a farm. In addition, preventing soil from splashing onto fruit by planting into a mowed cover crop (Ristaino et al. 1997), or using trellises to limit contact between cucurbit fruits and the ground can be effective, although not always practical, depending on the cucurbit cultivar, the scale of production, and the value of the crop (Ristaino and Johnston 1999; Hausbeck and Lamour 2004). Although cultivating peppers on plastic mulch is common because it increases yields (Ristaino and Johnston 1999), this practice can also hasten the spread of Phytophthora blight in a field (Springer and Johnston 1982; Ristaino et al. 1997).

Crop rotation can reduce the amount of inoculum which survives in a field from year to year (Ristaino and Johnston 1999), but because sexual reproduction and the production of oospores is common in *P. capsici*, even long rotations (5 years) do not completely eliminate *P. capsici* inoculum from a field (Lamour and Hausbeck 2001b). The effectiveness of crop rotations can also be reduced by the presence of susceptible (but often asymptomatic) weeds in a field during rotation to a non-host crop. Susceptible weed species include common purselane, *Portulaca oleracea* (Ploetz et al. 2002; French-Monar et al. 2006a), Carolina geranium, *Geranium carolinianum*, American black nightshade, *Solanumn americanum*, and *S. nigram* (French-Monar et al. 2006a).

Soil solarization has the potential to be an effective management tool in climates where high summertime temperatures are achieved and maintained for long periods of time, but complete control has not been achieved. In a Florida trial, oospores, sporangia, and mycelia were buried in soil, but although temperatures of 40-

45°C were reached at inoculum depths, viable *P. capsici* cultures were recovered from the soil, more than 300 days later. However, inoculum levels (measured in colony forming units (cfu) per g soil) were generally lower in solarized soil, compared to untreated soil (French-Monar et al. 2007). In another study, solarization reduced oospore inoculum of *P. capsici* to similar levels as those achieved through use of methyl bromide only in the upper soil layer (to a depth of 10 cm). At a depth of 25 cm, solarization did not reduce inoculum levels (Coelho et al. 1999).

Management - chemical

In the past, *P. capsici* had been controlled with the fumigant methyl bromide, but as of 2007, a critical use exemption has been required for continued use of this chemical to control *P. capsici* (French-Monar et al. 2007). Clearly, methyl bromide is not a long-term sustainable management tool (Hausbeck and Lamour 2004). The systemic phenylamide fungicide, mefenoxam inhibits RNA synthesis in *P. capsici* (Davidse et al. 1988) and has been very effective in susceptible populations of *P. capsici*, but resistance to this fungicide has already developed in many populations around the United States and internationally (Biles et al. 1992; Ristaino et al. 1997; Pennisi et al. 1998; Ristaino and Johnston 1999; Matheron and Porchas 2000a; Lamour and Hausbeck 2001a; Hausbeck and Lamour 2004; French-Monar et al. 2007; Café-Filho and Ristaino 2008; Davey et al. 2008). Resistance to mefenoxam in *P. capsici* is likely conveyed by a single, incompletely dominant locus, which is unlinked to mating type (Lamour and Hausbeck 2000; Lamour and Hausbeck 2002), similar to the situation seen in other *Phytophthora* species. For example, mefenoxam sensitivity is controlled primarily by a single dominant gene in *P. infestans* (Lee, T. Y. et al. 1999). There is little or no cost to *P. capsici* in maintaining resistance to mefenoxam, in the absence of fungicide application (Lamour and Hausbeck 2001a; Lamour and

Hausbeck 2002; Café-Filho and Ristaino 2008), and resistance to the fungicide mefenoxam can be induced by exposing *P. capsici* to ultra violet radiation (Bruin and Edgington 1982). Such exposure is common in the field, since sporangia are produced on the surface of fruits. It is likely that resistance to mefenoxam arose multiple times within a population of *P. infestans* in the Netherlands (Fry et al. 1991), suggesting that mefenoxam could readily occur in *P. capsici* populations, as well.

The importance of sexual reproduction in the life cycle of *P. capsici* exacerbates the problem of mefenoxam resistance, by putting the resistance gene into a variety of genetic backgrounds in *P. capsici*. This increases the likelihood that resistance to mefenoxam will be present in otherwise well-adapted and competitive genotypes and that there will be no cost to maintaining mefenoxam resistance (Lamour and Hausbeck 2000). In addition, because oospores may survive for years in the soil before germination (Goodwin 1997; Lamour and Hausbeck 2003; Hausbeck and Lamour 2004; Babadoost and Pavon 2007; French-Monar et al. 2007), a mefenoxam-resistant oospore could escape selective pressure against fungicide resistance if it does not germinate during a rotation away from mefenoxam use.

These studies all indicate that resistance to mefenoxam is likely to develop rapidly and to persist in fields where it is not already present, necessitating alternative management options. Other fungicide chemistries are available and can reduce losses (Matheron and Porchas 2000b; Matheron and Porchas 2007; Matheron and Porchas 2008), but fungicides do not provide complete protection from *P. capsici* under extremely conducive conditions or high inoculum levels (Matheron and Porchas 2000a). Therefore, while fungicides can be an important component of an integrated management approach, they will not provide a complete and exclusive solution to the problem of Phytophthora blight on vegetable crops.

Management – host tolerance and resistance

Host tolerance or resistance would be a highly desirable way to control Phytophthora blight. Several sweet pepper cultivars that are tolerant to *P. capsici* are available, but in New York field trials, these tolerant cultivars did succumb under high disease pressure. Previous studies have also reported that lengthy exposures to inoculum and high inoculum levels can overcome host resistance in peppers (Smith et al. 1967; Barksdale et al. 1984; Kim, Y. J. et al. 1989). The sweet pepper cultivar Paladin has consistently shown high levels of tolerance to crown rot caused by *P. capsici* (Ristaino and Johnston 1999; Babadoost and Islam 2002; Johnston et al. 2002; Miller 2002; Babadoost 2006; Stieg et al. 2006), and is becoming more popular among vegetable growers in New York for that reason. However, it is not completely immune from infection by *P. capsici*, and is not resistant to all isolates of *P. capsici*, including those collected from diverse regions of the United States, like New Jersey and New Mexico (Oelke et al. 2003). The fruit are also prone to silvering and sometimes develop spicy flavors as they ripen (Wyenandt and Kline 2006). Red Knight has traditionally been a very popular sweet pepper cultivar in New York, but is highly susceptible to *P. capsici* (McGrath and Davey 2007).

An early study divided 23 isolates of *P. capsici* into 14 "strains," based on their ability to infect various hosts (tomatoes, eggplants, squash and watermelons) and different pepper lines (Polach and Webster 1972). Since then, various studies have reported at least 9 (Oelke et al. 2003), 13 (Sy et al. 2008), or 14 (Glosier et al. 2008) different races of *P. capsici* based on susceptibility of different pepper cultivars to root rot, and four (Oelke et al. 2003) different races based on susceptibility of peppers to foliar blight. Based on ten differential pepper lines, Oelke et al. concluded that *P. capsici* isolates from New Mexico and Turkey are able to overcome more host plant race-specific resistance genes (R-genes) than are isolates from New Jersey (Oelke et

al. 2003). Glosier et al. reported that races of *P. capsici* were not geographically limited, either internationally or even to regions within the same state (Glosier et al. 2008). These studies suggest that pepper cultivars may have differential resistance to isolates of *P. capsici*. However, in each study, the number of races reported was only slightly smaller than the total number of isolates tested, and further work may be needed to define these races more clearly.

The genetic basis of this resistance is not well understood and may be more complex than dominant R-genes. One study suggested that two dominant genes without additive effects provided high levels of tolerance to the pepper root rot phase of *P. capsici* in the pepper lines PI129469, PI201232 and PI201234 (Smith et al. 1967). A single dominant gene conferring resistance to fruit rot has been reported in the cultivar Waxy Globe (Saini and Sharma 1978). Another study reported that a single dominant gene (with some modification) was responsible for resistance to both foliar and root rot phases of Phytophthora blight in the pepper lines Fyuco and P51 (Barksdale et al. 1984). Ortega et al. (1991) proposed that resistance to Phytophthora blight crown rot in the pepper line Criollo de Morelos-334 (CM-334) is controlled by genes at three loci, with additive effects among loci. The same authors also suggested that there were three resistance genes each in the lines PI201232, PI201234, and Line 29, and that these three lines, plus CM-334 share one gene in common (Ortega et al. 1992). According to Reifschneider et al. (1992), two genes in line CNPH 148 (derived from CM-334) are responsible for resistance to root and crown rot caused by a Brazilian isolate of *P. capsici*. Sy et al. (2005) reported that single dominant genes are responsible for resistance of CM-334 to root rot, foliar blight and stem blight caused by a New Mexican isolate of *P. capsici*, and that the gene for stem blight resistance is different from both the gene conferring foliar blight resistance and the gene conferring root rot resistance. Recently, Monroy-Barbosa et al. (2008) proposed that there were at

least five R-genes in the resistant Chile pepper line CM-334 which confer resistance to the root rot phase of Phytophthora blight. Although some genes might be linked, each gene appeared to be at a single locus, indicating that pyramiding of more than two R-genes in a pepper cultivar might be possible (Monroy-Barbosa and Bosland 2008). Considering these studies, it is possible that resistance to different phases of Phytophthora blight (eg, root rot versus fruit rot) or different races or isolates of *P. capsici* are under the control of different genes which may be inherited independently (Reifschneider et al. 1992). This adds to the confusion about the genetics of host resistance to *P. capsici* in pepper, and also suggests potential obstacles in developing resistant varieties in other hosts (eg, cucurbits).

No resistance or tolerance is currently available in commercial Chile pepper (Sanogo and Carpenter 2006), eggplant, tomato or cucurbit cultivars. Gevens et al. (2006) screened more than 300 commercial cucumber cultivars and plant introductions for resistance to the fruit infection stage of *P. capsici*, and while sporangial production was reduced on some cultivars, none had complete resistance to the fruit infection stage of the disease. *Cucurbita pepo* accessions and the wild cucurbit *C. lundelliana* are being screened for possible sources of resistance (Kabelka et al. 2007; Padley et al. 2007). In a controlled environment, the Korean pumpkin cultivar Danmatmaetdol was slightly to highly resistant to infection by *P. capsici* when a soil drench or wounding inoculation technique was used, but showed no resistance when zoospores were applied to the foliage. Additionally, resistance was dependent on the *P. capsici* isolate used in inoculation (Lee, B. K. et al. 2001).

Management – biological

Because of the phase-out of methyl bromide (Hausbeck and Lamour 2004; French-Monar et al. 2007), alternative biological fumigants would be useful tools for

managing Phytophthora blight in fields with a history of *P. capsici* infestation. *Brassica* species produce various sulfur-containing glucosinolates, which break down to produce some antimicrobial products (Mayton et al. 1996). Therefore, the incorporation of *Brassica* tissue into agricultural soil has been proposed as a way to destroy plant pathogens prior to planting susceptible host crops, and reductions in inoculum levels have been observed with many pathogens (Mayton et al. 1996; Ochiai et al. 2007). In one study, adding chopped or shredded cabbage to soil prior to solarization did not significantly reduce the amount of *P. capsici* inoculum compared to solarization, alone (Coelho et al. 1999). Kim, K. D. et al. (1997) reported no reduction in disease severity on bell pepper seedlings in the greenhouse when mustard residue was incorporated into soil prior to inoculation with *P. capsici* zoospores. In addition, some phytotoxicity of tomato seedlings has been observed after the incorporation of cabbage residue into soil (Ramirez-Villapudua and Munnecke 1988).

The endophytic fungus *Muscodor albus* has also been considered for use as a biofumigant to control *P. capsici*. This fungus produces a variety of volatile organic compounds and inhibits many fungi, oomycetes and bacteria, *in vitro* (Strobel 2006). Because *M. albus* was first isolated from a cinnamon tree in Honduras, grows slowly, and does not produce spores or other survival structures, it is unlikely that it could successfully colonize temperate soils, posing a low threat of becoming invasive. However, live cultures added to soil could release volatile compounds, killing or inhibiting *P. capsici* inoculum and preventing infection of susceptible host crops (Mercier and Manker 2005). Several studies have demonstrated the efficacy of *M. albus* in controlling *P. capsici* on the susceptible sweet pepper cultivar California Wonder (Mercier and Manker 2005), and *Rhizoctonia solani* on broccoli (Mercier and Manker 2005; Mercier and Jimenez 2007) and radish (Baysal et al. 2007).

Population structure of **P. capsici**

Worldwide, *P. capsici* is very diverse, and genetically complex (Forster et al. 1990; Oudemans and Coffey 1991; Mchau and Coffey 1995; Erwin and Ribeiro 1996), especially those isolates collected from vegetable crops and classified as either subgroup CAP1 (Oudemans and Coffey 1991) or subgroup CapA (Mchau and Coffey 1995). Reports vary as to whether populations are structured based on host plant or geographic distance. Fifteen isolates collected from around the world did not group by either geographic location or host plant when nuclear DNA was analyzed through restriction fragment length polymorphism, or RFLP (Forster et al. 1990) and similar results were obtained from an isozyme study of 84 isolates (Oudemans and Coffey 1991). In a Spanish study, 16 isolates collected from a relatively small geographic area (multiple farms within 7 km of each other) separated into three groups by RAPD analysis, but these groups were not related to variations in virulence on four pepper cultivars, or to the specific origin of the isolates. All three groups were closely related to each other, but distantly related to isolates from other countries. While the degree of similarity between pairs of isolates from different countries could sometimes be explained by geographic distance between countries, it could also sometimes be explained by host plant (Silvar et al. 2006). In another study, twenty-four isolates were collected from processing pumpkins in six locations in Illinois (approximately within a 30 km radius), and these isolates clustered into six RAPD groups. These groups corresponded to differences in disease severity on pumpkin seedlings, but not to the geographic origin of the isolates, although this may have been a consequence of the close geographic location of the sampling sites (Islam et al. 2005). Bowers et al. (2007) used AFLP, RFLP, and sequencing of two ITS regions (regions 1 and 2) and several genes to study populations of *P. capsici*. Using any of these methods, *P. capsici* isolates collected around the United States from diverse vegetable crops did

not group by either state or host plant. There was also substantial heterozygosity within the two sequenced ITS regions of *P. capsici*.

Extensive work has been done in Michigan on *P. capsici*, including studies of the structure of that state's population. Lamour and Hausbeck (2000) established that sexual reproduction was occurring in Michigan's vegetable fields, because oospores were found in naturally-infected fruit and because all six combinations of mating type (A1 and A2) with mefenoxam sensitivity (sensitive, intermediately sensitive, and resistant) were represented in 498 isolates recovered from 11 farms. In addition, eight of the sampled farms had approximately 1:1 ratios of A1 to A2 mating types, suggesting random mating.

In a single field sampled in two consecutive years, abundant genotypic diversity was found, with more than half of the 262 isolates collected having unique AFLP genotypes. There were no genotypes in common between the two years. AFLP fingerprinting resulted in 37 polymorphic loci, and a single genotype represented an increasing proportion of the collected isolates on three sequential sampling dates during one season, indicating that this genotype was well-adapted for the particular field, and was out-competing other genotypes in the field that year (Lamour and Hausbeck 2001a). This is in contrast to what has been observed for *P. infestans* in sexually-reproducing populations. In the Toluca Valley of central Mexico, genotypic diversity did not decrease during an epidemic, but at least 50% of the genotypes recovered at each sampling were unique (Fry et al. 1992).

In addition to providing evidence for selective pressure on oospore progeny as *P. capsici* reproduces asexually throughout the growing season, this study also suggests (i) that primary inoculum at the beginning of the second growing season came from oospores, and not from asexual propagules that survived the Michigan winter; and (ii) that the population structure and gene pool were not substantially

affected by the failure of all asexual propagules to survive the winter (Lamour and Hausbeck 2001a). This is noteworthy, since genetic drift can occur when genotype survival is limited by a crop-free period, or by a winter which kills all inoculum except oospores (Fry et al. 1992). Failure to recover the same genotype two years in a row can be an indication that genetic drift is occurring (Goodwin 1997). Thus, the fact that there is little differentiation between populations from different years indicates that genetic drift is not occurring in this Michigan population (Lamour and Hausbeck 2001a).

Of 57 isolates collected from a Michigan cucumber field in 1998 and 47 isolates collected from the same field in 2001 (cropped to tomatoes), 89% of the isolates collected had unique genotypes, based on an AFLP analysis, and, again, the same genotype was not detected in both years. There were approximately equal proportions of A1 to A2 isolates, and 57 unique genotypes were identified. In addition, isolates from each year were not grouped together in a cluster analysis, further illustrating the lack of differentiation among isolates in the same field over different years. These isolates were more similar to each other than to isolates collected from other growing regions in Michigan, and are reproductively isolated from populations which are as little as 8 km away (Lamour and Hausbeck 2003). Similar results were reported in 2002, indicating that, in Michigan, clonal lineages are limited to a single field during a single season, and that populations from different geographic regions are genetically isolated (Lamour and Hausbeck 2002). A similar situation has been observed in the Mexican population of *P. infestans*, where populations from northeastern, northwestern, and central Mexico were significantly different from each other (Goodwin et al. 1992), and even within central Mexico, populations from different valleys were differentiated (Fry et al. 1992). In contrast, *P. infestans* appears to be panmictic within the Toluca Valley of central Mexico (Fry et al. 1992).

While it is well-established that *P. capsici* in Michigan is highly diverse and that sexual reproduction is important in the Michigan population (Lamour and Hausbeck 2000; Lamour and Hausbeck 2001b; Lamour and Hausbeck 2002; Lamour and Hausbeck 2003), this may not be true of all populations of *P. capsici*. In southern Italy, 60 isolates recovered from two greenhouse production operations over six years were all consistently of the A2 mating type (Pennisi et al. 1998). In coastal Peru, the *P. capsici* population appears to be clonal, with only A2 mating type isolates recovered, and nearly identical genotypes reported by AFLP analysis, probably as a result of continuous pepper cropping, movement of infested water, or survival of asexual propagules during crop-free periods (Hurtado-Gonzáles et al. 2008). *P. capsici* isolates collected from cacao in Brazil were monomorphic at all tested isozyme loci, suggesting that this population may also be clonal (Oudemans and Coffey 1991; Mchau and Coffey 1995).

It may be hypothesized that the *P. capsici* population in New York State is similar to the population in Michigan. However, as described above, not all populations of *P. capsici* around the world have similar structures (Oudemans and Coffey 1991; Mchau and Coffey 1995; Hurtado-Gonzáles et al. 2008). Similarly, populations of *P. infestans* from different parts of the world also vary in their structure (Fry et al. 1992). Therefore, it is important to investigate the nature of the *P. capsici* population in New York, as this will have important implications for local disease management and will help researchers make better recommendations to New York's growers about managing Phytophthora blight.

REFERENCES

Anderson, T. R. and Garton, R. 2000. First report of blight of field peppers caused by *Phytophthora capsici* in Ontario. Plant Disease 84(6): 705-705.

Babadoost, M. 2006. Performance of bell pepper cultivars in a commercial field infested with *Phytophthora capsici*. HortScience 41(4): 981-981.

Babadoost, M. and Islam, S. Z. 2002. Bell peppers resistant to Phytophthora blight. Phytopathology 92:S5.

Babadoost, M. and Pavon, C. 2007. Survival of *Phytophthora capsici* oospores in soil. Phytopathology 97(7): S6-S6.

Barksdale, T. H., Papavizas, G. C. and Johnston, S. A. 1984. Resistance to foliar blight and crown rot of pepper caused by *Phytophthora capsici*. Plant Disease 68: 506-509.

Baysal, F., Mera, J. R. and Miller, S. A. 2007. Effects of *Muscodor*, serenade and conventional fungicides on Rhizoctonia root and hypocotyl rot and clubroot of radish. (abstr.). Phytopathology 97:S9.

Bernhardt, E. A. and Grogan, R. G. 1982. Effect of soil matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica*, *Phytophthora capsici*, and *Phytophthora cryptogea*. Phytopathology 72(5): 507-511.

Biles, C. L., Lindsey, D. L. and Liddell, C. M. 1992. Control of Phytophthora root-rot of Chile peppers by irrigation practices and fungicides. Crop Protection 11(3): 225-228.

Bowers, J. H., Martin, F. N., Tooley, P. W. and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology 97(4): 492-503.

Bowers, J. H. and Mitchell, D. J. 1991. Relationship between inoculum level of *Phytophthora capsici* and mortality of pepper. Phytopathology 81(2): 178-184.

Bowers, J. H., Papavizas, G. C. and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Disease 74(10): 771.

Bruin, G. C. A. and Edgington, L. V. 1982. Induction of fungal resistance to metalaxyl by ultraviolet-irradiation. Phytopathology 72(5): 476-480.

Café-Filho, A. C. and Duniway, J. M. 1995. Dispersal of *Phytophthora capsici* and *P. parasitica* in furrow-irrigated rows of bell pepper, tomato and squash. Plant Pathology 44(6): 1025-1032.

Café-Filho, A. C., Duniway, J. M. and Davis, R. M. 1995. Effects of the frequency of furrow irrigation on root and fruit rots of squash caused by *Phytophthora capsici*. Plant Disease 79(1): 44-48.

Café-Filho, A. C. and Ristaino, J. B. 2008. Fitness of isolates of *Phytophthora capsici* resistant to mefenoxam from squash and pepper fields in North Carolina. Plant Disease 92(10): 1439-1443.

Café-Filho, A. C. and Duniway, J. M. 1996. Effect of location of drip irrigation emitters and position of *Phytophthora capsici* infections in roots on Phytophthora root rot of pepper. Phytopathology 86(12): 1364-1369.

Coelho, L., Chellemi, D. O. and Mitchell, D. J. 1999. Efficacy of solarization and cabbage amendment for the control of *Phytophthora* spp. In north Florida. Plant Disease 83(3): 293-299.

Crossan, D. F., Haasis, F. A. and Ellis, D. E. 1954. Phytophthora blight of summer squash. Plant Disease Reporter 38(8): 557-559.

Davey, J. F., Gregory, N. F., Mulrooney, R. P., Evans, T. A. and Carroll, R. B. 2008. First report of mefenoxam-resistant isolates of *Phytophthora capsici* from lima bean pods in the mid-Atlantic region. Plant Disease 92(4): 656.

Davidse, L. C., Gerritsma, O. C. M., Ideler, J., Pie, K. and Velthuis, G. C. M. 1988. Antifungal modes of action of metalaxyl, cyprofuram, benalaxyl and oxadixyl in phenylamide-sensitive and phenylamide-resistant strains of *Phytophthora megasperma* f. sp. *medicaginis* and *Phytophthora infestans*. Crop Protection 7(6): 347-355.

Davidson, C. R., Carroll, R. B., Evan, T. A. and Mulrooney, R. P. 2002. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatis*) in the mid-Atlantic region. Plant Disease 86: 1049.

Duniway, J. M. 1979. Water relations of water molds. Annual Review of Phytopathology 17: 431-460.

Erwin, D. C. and Ribeiro, O. K. 1996. Phytophthora diseases worldwide. St. Paul, Minnesota, The American Phytopathological Society.

Forster, H., Oudemans, P. and Coffey, M. D. 1990. Mitochondrial and nuclear-DNA diversity within 6 species of *Phytophthora*. Experimental Mycology 14(1): 18-31.

French-Monar, R. D., Jones, J. B., Ozores-Hampton, M. and Roberts, P. D. 2007. Survival of inoculum of *Phytophthora capsici* in soil through time under different soil treatments. Plant Disease 91(5): 593-598.

French-Monar, R. D., Jones, J. B. and Roberts, P. D. 2006a. Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. Plant Disease 90(3): 345-350.

French-Monar, R. D., Schultz, D. C. and Roberts, P. D. 2006b. Pathogenicity of a diverse group of isolates of *Phytophthora capsici* from Florida on four main vegetable crops. Phytopathology 96(6): S185-S185.

Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C. and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81(10): 1330-1336.

Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G. and Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. Annual Review of Phytopathology 30: 107-129.

Gevens, A. J., Ando, K., Lamour, K. H., Grumet, R. and Hausbeck, M. K. 2006. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. Plant Disease 90(10): 1276-1282.

Gevens, A. J., Donahoo, R. S., Lamour, K. H. and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology 97(4): 421-428.

Gevens, A. J., Donahoo, R. S., Lamour, K. H. and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. Plant Disease 92: 201-209.

Glosier, B. R., Ogundiwin, E. A., Sidhu, G. S., Sischo, D. R. and Prince, J. P. 2008. A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. Euphytica 162(1): 23-30.

Goodwin, S. B. 1997. The population genetics of *Phytophthora*. Phytopathology 87(4): 462-473.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N. and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82(9): 955-961.

Hausbeck, M. K. and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Disease 88(12): 1292-1303.

Hickman, C. J. 1970. Biology of *Phytophthora* zoospores. Phytopathology 60: 1128-1135.

Hord, M. J. and Ristaino, J. B. 1991. Effects of physical and chemical factors on the germination of oospores of *Phytophthora capsici* in vitro. Phytopathology 81(12): 1541-1546.

Hord, M. J. and Ristaino, J. B. 1992. Effect of the matric component of soil water potential on infection of pepper seedlings in soil infested with oospores of *Phytophthora capsici*. Phytopathology 82(7): 792.

Hurtado-Gonzáles, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R. and Lamour, K. 2008. Survival and spread of *Phytophthora capsici* in coastal Peru. Phytopathology 98(6): 688-694.

Islam, S. Z., Babadoost, M., Lambert, K. N., Ndeme, A. and Fouly, H. M. 2005. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. Plant Disease 89(2): 191-197.

Johnston, S. A., Kline, W. L., Fogg, M. L. and Zimmerman, M. D. 2002. Varietal resistance evaluation for control of Phytophthora blight of pepper. Phytopathology 92: S40.

Kabelka, E., Padley, L., Roberts, P., Ramos, L., Martinez, M. and Klassen, W. 2007. Resistance to *Phytophthora capsici* within winter squash (*Cucurbita moschata*) derived from a wild *Cucurbita* species. HortScience 42(4): 1014.

Kim, F. S. and Hwang, B. K. 1992. Virulence to Korean pepper cultivars of isolates of *Phytophthora capsici* from different geographic areas. Plant Disease 76(5): 486-489.

Kim, K. D., Nemec, S. and Musson, G. 1997. Control of Phytophthora root and crown rot of bell pepper with compost and soil amendments in the greenhouse. Applied Soil Ecology 5: 169-179.

Kim, Y. J., Hwang, B. K. and Park, K. W. 1989. Expression of age-related resistance in pepper plants infected with *Phytophthora capsici*. Plant Disease 73: 745-747.

Ko, W. H. 1988. Hormonal heterothallism and homothallism in *Phytophthora*. Annual Review of Phytopathology 26: 57-73.

Lamour, K. H. and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90(4): 396-400.

Lamour, K. H. and Hausbeck, M. K. 2001a. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91(6): 553-557.

Lamour, K. H. and Hausbeck, M. K. 2001b. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91(10): 973-980.

Lamour, K. H. and Hausbeck, M. K. 2002. The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. Phytopathology 92(6): 681-684.

Lamour, K. H. and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Disease 87(7): 841-845.

Lee, B. K., Kim, B. S., Chang, S. W. and Hwang, B. K. 2001. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. Plant Disease 85(5): 497-500.

Lee, T. Y., Mizubuti, E. and Fry, W. E. 1999. Genetics of metalaxyl resistance in *Phytophthora infestans*. Fungal Genetics and Biology 26(2): 118-130.

Leonian, L. H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopathology 12: 401-408.

Luz, E. D. M. N., Cerqueira, A. O., Faleiro, F. G., Dantas Neto, A., Matsuoka, K. and Marques, J. R. B. 2003. Genetic diversity of *Phytophthora capsici* isolates from different hosts based on RAPD markers, pathogenicity and morphology. Fitopatologia Brasileira 28(5): 559-564.

Matheron, M. E. and Porchas, M. 2000a. Comparison of five fungicides on development of root, crown, and fruit rot of Chile pepper and recovery of *Phytophthora capsici* from soil. Plant Disease 84(9): 1038-1043.

Matheron, M. E. and Porchas, M. 2000b. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. Plant Disease 84(4): 454-458.

Matheron, M. E. and Porchas, M. 2007. Comparison of fungicides for management of the soil phase of Phytophthora blight, 2005. Plant Disease Management Reports (online). Report 1:V137. DOI:10.1094/PDMR01, The American Phytopathological Society, St. Paul, MN.

Matheron, M. E. and Porchas, M. 2008. Efficacy of fungicides for management of the soil phase of Phytophthora blight on pepper plants, 2006. Plant Disease Management Reports (online). Report 2:V083. DOI:10.1094/PDMR02, The American Phytopathological Society, St. Paul, MN

Mayton, H. S., Olivier, C., Vaughn, S. F. and Loria, R. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86(3): 267-271.

McGrath, M. T. and Davey, J. F. 2007. Efficacy of fungicides for control of Phytophthora blight in pepper on crown rot tolerant and susceptible cultivars, 2006. Plant Disease Management Reports (online). Report 1:V131. DOI:10.1094/PDMR01, The American Phytopathological Society, St. Paul, MN.

Mchau, G. R. A. and Coffey, M. D. 1995. Evidence for the existence of 2 subpopulations in *Phytophthora capsici* and a redescription of the species. Mycological Research 99: 89-102.

Mercier, J. and Jimenez, J. I. 2007. Potential of the volatile-producing fungus *Muscodor albus* for control of building molds. Canadian Journal of Microbiology 53(3): 404-410.

Mercier, J. and Manker, D. C. 2005. Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile-producing fungus *Muscodor albus*. Crop Protection 24(4): 355-362.

Miller, S. A., Lewis Ivey, M.L., and Mera, J. 2002. Response of pepper cultivars and experimental breeding lines to Phytophthora blight, 2001. Biological and cultural tests for control of plant disease (Online). Report 17:V16. DOI: 1094/BC17. American Phytopathological Society, St. Paul, MN.

Monroy-Barbosa, A. and Bosland, P. W. 2008. Genetic analysis of Phytophthora root rot race-specific resistance in Chile pepper. Journal of the American Society for Horticultural Science 133(6): 825-829.

Ochiai, N., Powelson, M. L., Dick, R. P. and Crowe, F. J. 2007. Effects of green manure type and amendment rate on Verticillium wilt severity and yield loss in Russet Burbank potato. Plant Disease 91: 400-406.

Oelke, L. M., Bosland, P. W. and Steiner, R. 2003. Differentiation of race specific resistance to Phytophthora root rot and foliar blight in *Capsicum annuum*. Journal of the American Society for Horticultural Science 128(2): 213-218.

Ortega, R. G., Espanol, C. P. and Zueco, J. C. 1991. Genetics of resistance to *Phytophthora capsici* in the pepper line scm-334. Plant Breeding 107(1): 50-55.

Ortega, R. G., Espanol, C. P. and Zueco, J. C. 1992. Genetic relationships among 4 pepper genotypes resistant to *Phytophthora capsici*. Plant Breeding 108(2): 118-125.

Oudemans, P. and Coffey, M. D. 1991. A revised systematics of 12 papillate *Phytophthora* species based on isozyme analysis. Mycological Research 95: 1025-1046.

Padley, L., Roberts, P. and Kabelka, E. 2007. Screening *Cucurbita pepo* for resistance to *Phytophthora capsici*. HortScience 42(4): 1014.

Palloix, A., Daubeze, A. M. and Pochard, E. 1988. Phytophthora root-rot of pepper influence of host genotype and pathogen strain on the inoculum density-disease severity relationships. Journal of Phytopathology-Phytopathologische Zeitschrift 123(1): 25-33.

Pennisi, A. M., Agosteo, G. E., Cacciola, S. O., Pane, A. and Faedda, R. 1998. Insensitivity to metalaxyl among isolates of *Phytophthora capsici* causing root and crown rot of pepper in southern Italy. Plant Disease 82(11): 1283-1283.

Ploetz, R., Heine, G., Haynes, J. and Watson, M. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. Annals of Applied Biology 140(1): 61-67.

Polach, F. J. and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. Phytopathology 62(1): 20-26.

Quesada-Ocampo, L. M., Fulbright, D. W. and Hausbeck, M. K. 2009. Susceptibility of Fraser fir to *Phytophthora capsici*. Plant Disease 93(2): 135-141.

Ramirez-Villapudua, J. and Munnecke, D. E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. Phytopathology 78(3): 289-295.

Reifschneider, F. J. B., Boiteux, L. S., Dellavecchia, P. T., Poulos, J. M. and Kuroda, N. 1992. Inheritance of adult-plant resistance to *Phytophthora capsici* in pepper. Euphytica 62(1): 45-49.

Reifschneider, F. J. B., Café-Filho, A. C. and Rego, A. M. 1986. Factors affecting expression of resistance in pepper (*Capsicum annuum*) to blight caused by *Phytophthora capsici* in screening trials. Plant Pathology 35(4): 451-456.

Ristaino, J. B. 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. Phytopathology 80(11): 1253-1259.

Ristaino, J. B. 1991. Influence of rainfall, drip irrigation, and inoculum density on the development of Phytophthora root and crown rot epidemics and yield in bell pepper. Phytopathology 81(8): 922-929.

Ristaino, J. B., Hord, M. J. and Gumpertz, M. L. 1992. Population densities of *Phytophthora capsici* in field soils in relation to drip irrigation, rainfall, and disease incidence. Plant Disease 76(10): 1017-1024.

Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. Plant Disease 83(12): 1080-1089.

Ristaino, J. B., Larkin, R. P. and Campbell, C. L. 1994. Spatial dynamics of disease symptom expression during Phytophthora epidemics in bell pepper. Phytopathology 84(10): 1015-1024.

Ristaino, J. B., Parra, G. and Campbell, C. L. 1997. Suppression of Phytophthora blight in bell pepper by a no-till wheat cover crop. Phytopathology 87(3): 242-249.

Roberts, P. D., Urs, R. R., French-Monar, R. D., Hoffine, M. S., Seijo, T. E. and McGovern, R. J. 2005. Survival and recovery of *Phytophthora capsici* and oomycetes in tailwater and soil from vegetable fields in Florida. Annals of Applied Biology 146(3): 351.

Saini, S. S. and Sharma, P. P. 1978. Inheritance of resistance to fruit rot (*Phytophthora capsici* Leon) and induction of resistance in bell pepper (*Capsicum annuum* l.). Euphytica 27(3): 721-723.

Sanogo, S. and Carpenter, J. 2006. Incidence of Phytophthora blight and Verticillium wilt within Chile pepper fields in New Mexico. Plant Disease 90(3): 291-296.

Satour, M. M. and Butler, E. E. 1968. Comparative morphological and physiological studies of the progenies from intraspecific matings of *Phytophthora capsici*. Phytopathology 58: 183-192.

Shattock, R. C., Tooley, P. W. and Fry, W. E. 1986. Genetics of *Phytophthora infestans*: Characterization of single-oospore cultures from a1 isolates induced to self by intraspecific stimulation. Phytopathology 76(4): 407-410.

Silvar, C., Merino, F. and Díaz, J. 2006. Diversity of *Phytophthora capsici* in northwest Spain: Analysis of virulence, metalaxyl response, and molecular characterization. Plant Disease 90(9): 1135-1142.

Smith, P. G., Kimble, K. A., Grogan, R. G. and Millet, A. H. 1967. Inheritance of resistance in peppers to Phytophthora root rot. Phytopathology 57(4): 377-&.

Springer, J. K. and Johnston, S. A. 1982. Black polyethylene mulch and Phytophthora blight of pepper. Plant Disease 66(4): 281-281.

Stieg, J. R., Walters, S. A., Bond, J. P. and Babadoost, M. 2006. Effects of fungicides and cultivar resistance for *Phytophthora capsici* control in bell pepper production. HortScience 41(4): 30.

Strobel, G. 2006. *Muscodor albus* and its biological promise. Journal of Industrial Microbiology & Biotechnology 33(7): 514-522.

Sujkowski, L. S., Parra, G. R., Gumpertz, M. L. and Ristaino, J. B. 2000. Temporal dynamics of Phytophthora blight on bell pepper in relation to the mechanisms of dispersal of primary inoculum of *Phytophthora capsici* in soil. Phytopathology 90(2): 148-156.

Sy, O., Bosland, P. W. and Steiner, R. 2005. Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and Phytophthora foliar blight resistance in *Capsicum annuum* l. Journal of the American Society for Horticultural Science 130(1): 75-78.

Sy, O., Steiner, R. and Bosland, P. W. 2008. Recombinant inbred line differential identifies race-specific resistance to Phytophthora root rot in *Capsicum annuum*. Phytopathology 98(8): 867-870.

Weber, G. F. 1932. Blight of peppers in Florida caused by *Phytophthora capsici*. Phytopathology 22(9): 775-780.

Wyenandt, A. and Kline, W. L. 2006. Effects or cultivar and production system on the development of skin separation (silvering) in bell pepper fruit in New Jersey. Phytopathology 96(6): S125-S125.

Xie, J. H., Cardenas, E. S., Sammis, T. W., Wall, M. M., Lindsey, D. L. and Murray, L. W. 1999. Effects of irrigation method on Chile pepper yield and Phytophthora root rot incidence. Agricultural Water Management 42(2): 127-142.

Zentmyer, G. A. and Erwin, D. C. 1970. Development and reproduction of *Phytophthora*. Phytopathology 60(7): 1120-1127.

CHAPTER 2

[*]EFFICACY OF *MUSCODOR ALBUS* FOR THE CONTROL OF PHYTOPHTHORA BLIGHT OF SWEET PEPPER AND BUTTERNUT SQUASH

Abstract

The efficacy of *Muscodor albus*, a potential soil biofumigant, to control root and stem rot by *Phytophthora capsici*, was examined in a greenhouse study. *Phytophthora capsici*-infested potting mix was treated with three rates of *M. albus*, mefenoxam (Ridomil Gold EC, Syngenta Crop Protection, Inc.) or nothing. Seedlings of five sweet pepper cultivars and one butternut squash cultivar were transplanted into the treated potting mix. After 7 days, the plants were rated on a scale of 0 (healthy) to 5 (dead). The experiment was conducted three times and there was a significant interaction between pepper cultivar and soil treatment. Treatment with the highest rate of *M. albus* resulted in a slight but significant reduction in disease severity on Alliance, Aristotle, Paladin and Revolution peppers, compared to the pathogen-only control, while no significant decreases in disease severity were observed with butternut squash or the highly susceptible pepper cultivar Red Knight. Of the four less-susceptible pepper cultivars, *M. albus*, as applied in this study, reduced disease severity to commercially-acceptable levels only on the most tolerant cultivar, Paladin.

[*] Camp, A. R., Dillard, H. R., and Smart, C. D. 2008. Efficacy of *Muscodor albus* for the control of Phytophthora blight of sweet pepper and butternut squash. Plant Dis. 92:1488-1492.

Introduction

Phytophthora capsici (Leonian) was first isolated from Chile peppers (*Capsicum annuum*) in New Mexico in 1918 (Leonian 1922), and since then it has also been reported on sweet peppers, tomatoes, eggplants and cucurbits (Hausbeck and Lamour 2004), as well as snap beans (Gevens et al. 2008) and lima beans (Davidson et al. 2002). Cucurbit hosts are susceptible to root, crown and fruit rots that result in either plant death or rotting of fruit before or after harvest, causing significant yield losses (Hausbeck and Lamour 2004). Current control recommendations include cultural practices to reduce standing water in the field (Hausbeck and Lamour 2004), tolerant cultivars (Johnston et al. 2002; Driver and Louws 2003; Hausbeck and Lamour 2004) and chemical fungicides and fumigants (Hausbeck and Lamour 2004). In production regions around the country, isolates of *P. capsici* that are insensitive to the fungicide mefenoxam are becoming an increasing problem (Hausbeck and Lamour 2004). Areas of Michigan have received exemptions to continue use of the fumigant methyl bromide in order to continue production of susceptible hosts in the presence of mefenoxam-insensitive isolates of *P. capsici* (Hausbeck and Lamour 2004), but this is not a long-term sustainable solution to the problem. Some sweet pepper cultivars that are tolerant to *P. capsici* are available, but no resistance or tolerance is currently available for hot peppers, eggplants, tomatoes or cucurbits (Hausbeck and Lamour 2004).

The tropical endophytic fungus *Muscodor albus* was first isolated from a cinnamon tree (*Cinnamomum zeylanicum*) in Honduras (Strobel 2006). It produces a variety of volatile organic compounds that inhibit *in vitro* a number of fungal, oomycete and bacterial species, including plant pathogens (Strobel 2006). Thus, it has been proposed that *M. albus* could have agricultural applications as a soil biofumigant to kill soil borne plant pathogens (including *P. capsici*) (Strobel 2006). Since *P.*

capsici is not aerially dispersed (Hausbeck and Lamour 2004), intentional cultural practices could be employed to prevent or at least delay the re-introduction of *P. capsici* into a field that had been fumigated with *M. albus*. Furthermore, because the production of long-lived oospores by *P. capsici* limits the effectiveness of crop rotation to control Phytophthora blight (Hausbeck and Lamour 2004), the potential destruction of oospores by biofumigation with *M. albus* (although not yet demonstrated) would be especially useful to growers.

There have been a number of reports of the successful use of *M. albus* as a biofumigant. Several studies successfully used *M. albus* to control post-harvest diseases of fruits, including gray mold (*Botrytis cinerea*) on grapes (Gabler et al. 2006), brown rot (*Monilinia fructicola*) on peaches (Schnabel and Mercier 2006), blue mold (*Penicillium expansum*), gray mold (*B. cinerea*) and brown rot (*M. fructicola*) on apples (Mercier and Jimenez 2004), and green mold (*Penicillium digitatum*) and sour rot (*Geotrichum citri-aurantii*) on lemons (Mercier and Smilanick 2005) . Stinson et al. (2003) reported that a rate of 2 g *M. albus* inoculum in 425 g autoclaved and pathogen-infested soil significantly reduced disease severity on sugar beet caused by *Rhizoctonia solani*, *Pythium ultimum* and *Aphanomyces cochliodes*, and on eggplant caused by *Verticillium dahliae*, compared to pathogen-only controls. In a field experiment, *M. albus* applied at 3.75 g/L soil or 1.9 g/L soil controlled root and hypocotyl rots on radishes caused by *R. solani* (Baysal et al. 2007).

In addition, in a greenhouse study, Mercier and Manker (2005) demonstrated that *M. albus* provided complete control of both damping-off of broccoli seedlings caused by *Rhizoctonia solani* and Phytophthora blight on a susceptible sweet pepper cultivar (California Wonder). There are no published studies using *M. albus* to control Phytophthora blight on cucurbits. Because *P. capsici* continues to be a significant problem for vegetable growers in New York State, and since previous studies have

indicated the potential for the successful control of *P. capsici* with *M. albus*, this study was initiated in order to test the efficacy of *M. albus* on additional cultivars and crops. The goals of this study were to (i) determine whether *M. albus* is effective as a biofumigant against Phytophthora blight on five sweet pepper cultivars and one butternut squash cultivar and (ii) determine whether efficacy of *M. albus* varies based on host tolerance of pepper cultivars.

Materials and Methods

Plant materials. Five sweet pepper cultivars were used in this experiment: Alliance (Harris, Rochester, NY), Aristotle (Seminis Inc., Saint Louis, MO), Paladin (Syngenta Crop Protection, Inc., Greensboro, NC), Red Knight (Seminis Inc.) and Revolution (Harris). All cultivars were seeded into Cornell potting mix (composed of peat, perlite and vermiculite in a 4:1:1 ratio) and were germinated and grown in the greenhouse in 128-cell flats under natural light for 24-38 days before being transplanted into treated soil (described below). Additionally, Butternut squash (cv. 'Waltham,' Stokes Seeds, Inc., Buffalo, NY) were seeded into Cornell potting mix in 50-cell flats and were germinated and grown under natural light in the greenhouse for 11-14 days prior to transplanting.

Preparation of *P. capsici* inoculum. The *P. capsici* isolate (NY 0664-01) used in this experiment was isolated from a pepper plant in New York in 2006 and is sensitive to mefenoxam. The isolate was cultured on 100 mm x 15 mm Petri dishes of 15% V8 agar for 5-7 days (Lamour and Hausbeck 2000). Equal areas of each agar plate colonized by *P. capsici* were cut into small cubes about 0.5 cm in diameter and the contents of one plate was used to inoculate 1 L of V8-vermiculite substrate (0.5 L 20% V8 broth and 1 L vermiculite) which had been mixed and sterilized in a 2-L Erlenmeyer flask (Ristaino et al. 1988). The agar plugs of *P. capsici* were mixed into

the substrate by gentle shaking and the inoculated flasks were incubated in the dark at room temperature for 10 to 12 days and shaken three times per week. Before inoculating soil, approximately 1 g of vermiculite was removed from each of the flasks and incubated on 15% V8 agar to confirm that *P. capsici* had colonized the substrate. Substrate without *P. capsici* was also made for use in non-inoculated controls.

Soil inoculation and treatments. To make infested potting mix, 12 L of *P. capsici*-inoculated V8-vermiculite substrate was thoroughly mixed with 36 L of moistened Cornell potting mix to achieve a 1:4 ratio of vermiculite inoculum to potting mix, similar to the protocol used by Mercier and Manker (2005). This *P. capsici*-inoculated potting mix was then divided into five portions (each containing about 9.5 L) for treatment with different rates of *M. albus*. The *M. albus* used in this trial was obtained from AgraQuest, Inc. (Davis, CA) and had been grown on rye grain before being dried for storage. Three of the *P. capsici*-inoculated soil portions were treated with *M. albus* formulated on rye grain at a rate of 3.75, 1.9, or 0.55 g/L of soil. The remaining two portions were left untreated for the *P. capsici*-only control and the mefenoxam + *P. capsici* control. After treatment, the potting mix in each portion was mixed thoroughly and used to fill 30 square plastic pots (10.16 cm) with approximately 300 cc of potting mix.

To produce potting mix for the no-pathogen controls, uninoculated V8-vermiculite substrate was mixed with Cornell potting mix in a 1:4 ratio, as above. The potting mix was divided into 4 portions for treatment with *M. albus* inoculum at the three rates described above, and one portion of soil was left untreated. From each container, 30 pots were filled with approximately 300 cc potting mix, as for the inoculated soil.

In total there were nine soil treatments for each of the five pepper cultivars and one squash cultivar: *P. capsici* only, *P. capsici* + mefenoxam, *P. capsici* + *M. albus* at 3.75 g/L, *P. capsici* + *M. albus* at 1.9 g/L, *P. capsici* + *M. albus* at 0.55 g/L, no *M. albus* + no *P. capsici*, only *M. albus* at 3.75 g/L, only *M. albus* at 1.9 g/L and only *M. albus* at 0.55 g/L. All pots from all treatments were covered with plastic and stored in the dark at 20-22°C for seven days, to enable the *M. albus* to grow and fumigate the soil (Stinson et al. 2003).

Efficacy of *M. albus* to control *P. capsici* on sweet peppers. One week after the soil was inoculated, two pepper seedlings were transplanted into each pot. All seedlings were thoroughly watered prior to transplanting. Five replications of each treatment and cultivar combination were arranged in a randomized complete block design on greenhouse benches. The greenhouse was kept at approximately 24°C during the day and 20°C at night, with about 15 h of natural light. Approximately 100 ml of water was added to each pot (from the top), except for those pots which were to be treated with mefenoxam. Pots treated with mefenoxam received 100 ml each of a Ridomil Gold EC solution (Syngenta Crop Protection, Inc.) at a rate of 1.5 L/ha. All pots were watered 2-3 days after transplanting with 150 ml of water, gently poured onto the top of the pot to avoid splashing.

Each pot (containing two plants) was rated as a unit using a scale adapted from Silvar et al. (2006): 0 = both plants healthy; 1 = less than or equal to 50% of total stem area with lesions and/or less than or equal to 50% of all leaves wilted or missing; 2 = more than 50% of total stem area with lesions or more than 50% of all leaves wilted or missing; 3 = less than or equal to 50% of total stem area having lesions and more than 50% of all leaves wilted or missing, or vice versa; 4 = more than 50% of total stem area having lesions and more than 50% of leaves wilted or missing, but growing tip still upright and green; 5 = both plants dead. Plants were rated when the *P. capsici*-

only control plants were dead (7 days after transplanting). The entire experiment was repeated three times, thus with five replicates per experiment there were 15 ratings for each treatment-cultivar combination.

Efficacy of *M. albus* to control *P. capsici* on butternut squash. In the butternut squash experiment there were a total of nine soil treatments (as in the pepper experiment), but only one butternut squash cultivar. One week after the soil was inoculated, two squash seedlings were transplanted into each pot, and pots were watered with either 100 ml of water or 100 ml of mefenoxam (Ridomil Gold EC at a rate of 1.5 L/ha). Five replications of each treatment were arranged in a randomized complete block design on a greenhouse bench. The greenhouse was kept at approximately 24°C during the day and 20°C at night, with approximately 15 h of natural light. All pots were watered 2 to 3 days after transplanting with 150 ml of water gently poured onto the top of the pot to avoid splashing. Each pot (containing two plants) was rated as a unit using the same scale described above. Plants were rated when the *P. capsici*-only control plants were dead (7 days after transplanting). The entire experiment was repeated three times, thus with five replicates per experiment there were 15 ratings for each treatment.

Statistical Analyses. For both the pepper experiment and the butternut squash experiment, results were pooled for statistical analysis across all three repetitions of the respective experiment. Control treatments which did not receive *P. capsici* (ie, no *M. albus* + no *P. capsici*, only *M. albus* at 3.75 g/L, only *M. albus* at 1.9 g/L and only *M. albus* at 0.55 g/L) were not included in the statistical analysis, so that there were a total of five treatments and either five pepper cultivars or a single butternut squash cultivar in each analysis. All data were analyzed using SAS version 9.1.3 (Cary, NC).

Data from the pepper experiment was analyzed using a nonparametric test for two-way factorial experiments, described by Shah and Madden (2004). It was

followed by calculation of the relative treatment effects and their 95% confidence intervals using the LD_CI macro written by Brunner et al. (Brunner et al. 2002). The relative treatment effects are estimated using the ranks of the observations and are directly related to the values of the observations, so that smaller relative treatment effects for a treatment indicate smaller values for the observations (disease severity ratings) in that treatment (Brunner et al. 2002). Relative treatment effects always have values between 0 and 1, and the disease severities on two treatment-cultivar combinations can be said to be significantly different from each other if the 95% confidence intervals of the relative treatment effects do not overlap.

Data from the butternut squash experiment was analyzed using a Kruskal-Wallis test performed with the program 'npar1way.' Rank sums calculated with this program were then used to perform a Bonferroni-Dunn test (Sheskin 1996).

Results

Disease development on pepper and butternut squash. Disease developed rapidly in pathogen-inoculated controls. After 3 or 4 days, leaves began to wilt and water soaked lesions were observed on the stems just above the soil line of the butternut squash and Red Knight peppers that had been treated with only *P. capsici*. Seven days after transplanting, all of these butternut squash and Red Knight peppers were rated either 4 or 5. No phytotoxic effects of *M. albus* were observed on any pepper cultivar or on Waltham butternut squash.

Susceptibility of sweet pepper cultivars. As expected, there were differences in susceptibility among pepper cultivars. Paladin was the most tolerant pepper cultivar and Red Knight was the most susceptible pepper cultivar (Table 2.1, Figure 2.1). The susceptibility of Alliance, Aristotle and Revolution peppers was intermediate to that of Paladin and Red Knight, with Revolution being the most tolerant of the three

intermediately-tolerant cultivars (Figure 2.1, Table 2.1).

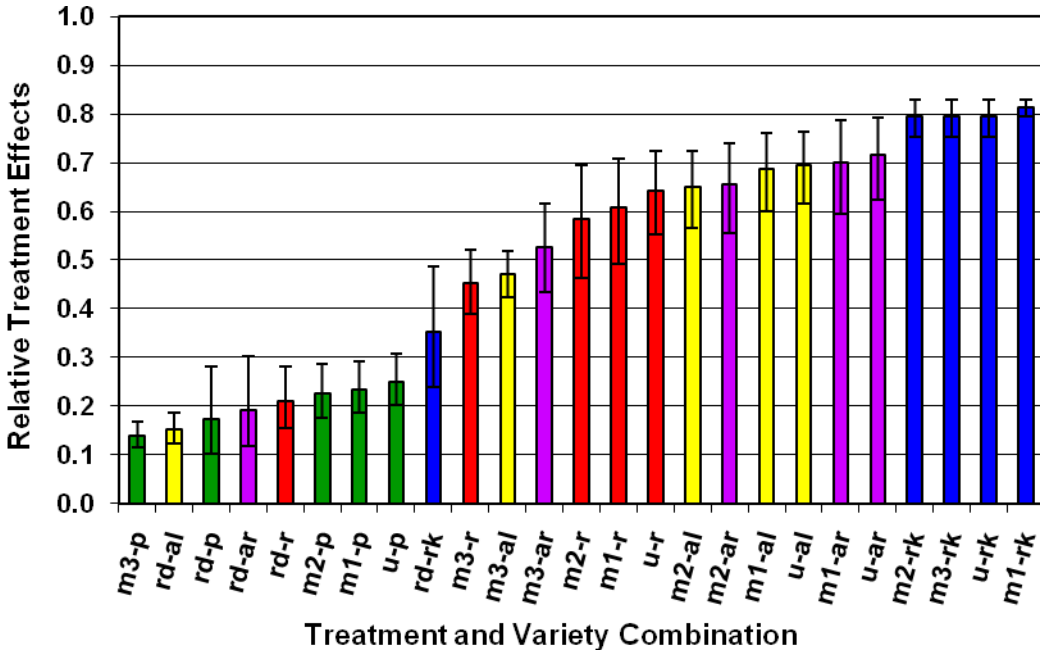

Figure 2.1 Disease severity on sweet peppers treated with *Muscodor albus*. Graphical representation of the relative treatment effects for each combination of treatment applied to potting mix and pepper cultivar and its effect on disease severity of Phytophthora blight on sweet peppers. Disease severity was rated on an ordinal scale from 0 (healthy plants) to 5 (dead plants), 7 days after transplanting, and data was combined from the three experiments. Error bars indicate the 95% confidence intervals of the relative treatment effects. The first two letters and numbers indicate the soil treatment (m1 = 0.55 g/L *Muscodor albus*, m2 = 1.9 g/L *Muscodor albus*, m3 = 3.75 g/L *Muscodor albus*, u = *Phytophthora capsici* only, and rd = mefenoxam). The second one or two letters indicate the pepper cultivar (p = Paladin, al = Alliance, r = Revolution, ar = Aristotle, and rk = Red Knight).

Table 2.1 Disease severity on five sweet pepper cultivars treated with *Muscodor albus*. Median disease rating, mean rank, relative treatment effect ($\hat{p}_{ij}$) and 95% confidence interval of the relative treatment effect for combinations of soil treatment and sweet pepper cultivar.[a]

Treatment-cultivar combination	Median[b]	Mean rank[c]	$\hat{p}_{ij}$	95% CI for $\hat{p}_{ij}$
Alliance				
M. albus 3.75 g/L	4.0	202.5	0.471	(0.425, 0.519)
M. albus 1.9 g/L	4.0	202.5	0.649	(0.566, 0.724)
M. albus 0.55 g/L	5.0	305.0	0.686	(0.600, 0.760)
P. capsici only	5.0	305.0	0.694	(0.615, 0.763)
Mefenoxam	0.0	48.0	0.151	(0.122, 0.187)
Aristotle				
M. albus 3.75 g/L	4.0	202.5	0.525	(0.434, 0.615)
M. albus 1.9 g/L	5.0	305.0	0.654	(0.556, 0.739)
M. albus 0.55 g/L	5.0	305.0	0.700	(0.594, 0.787)
P. capsici only	5.0	305.0	0.715	(0.623, 0.792)
Mefenoxam	0.0	48.0	0.191	(0.118, 0.303)
Paladin				
M. albus 3.75 g/L	0.0	48.0	0.139	(0.116, 0.167)
M. albus 1.9 g/L	0.0	48.0	0.226	(0.176, 0.286)
M. albus 0.55 g/L	1.0	116.5	0.235	(0.187, 0.292)
P. capsici only	1.0	116.5	0.250	(0.201, 0.307)
Mefenoxam	0.0	48.0	0.172	(0.103, 0.282)
Red Knight				
M. albus 3.75 g/L	5.0	305.0	0.794	(0.753, 0.829)
M. albus 1.9 g/L	5.0	305.0	0.794	(0.753, 0.829)
M. albus 0.55 g/L	5.0	305.0	0.812	(0.794, 0.829)
P. capsici only	5.0	305.0	0.794	(0.753, 0.829)
Mefenoxam	1.0	116.5	0.352	(0.240, 0.485)
Revolution				
M. albus 3.75 g/L	3.0	155.0	0.454	(0.388, 0.521)
M. albus 1.9 g/L	4.0	202.5	0.583	(0.462, 0.693)
M. albus 0.55 g/L	4.0	202.5	0.607	(0.493, 0.709)
P. capsici only	4.0	202.5	0.642	(0.553, 0.722)
Mefenoxam	0.0	48.0	0.210	(0.154, 0.282)

[a] Rating data taken 7 days after transplanting was combined from the three experiments.
[b] Disease severity was rated on an ordinal scale from 0 (healthy plants) to 5 (dead plants).
[c] Mean rank is the average of the rank scores assigned to all replicates of each treatment-cultivar combination. Thus, treatment-cultivar combinations with smaller mean ranks received lower disease severity ratings.

Efficacy of *M. albus* to control *P. capsici* on sweet peppers. Results of the pepper experiment are presented as median disease severity rating, relative treatment effect and 95% confidence interval of the relative treatment effect (Table 2.1). Although median ratings between two treatment-cultivar combinations may be the same, whether or not there is a significant difference is determined by the 95% confidence interval of the relative treatment effect. To more easily visualize significant differences, relative treatment effects and their 95% confidence intervals are graphed in Figure 2.1.

Mefenoxam and the highest rate of *M. albus*, but not the other rates, significantly decreased disease severity on Alliance, Aristotle, Paladin and Revolution peppers. Although both mefenoxam and the highest rate of *M. albus* significantly reduced disease severity compared to the pathogen-only control, mefenoxam provided better control than *M. albus*. There were no significant differences in disease severity between Revolution peppers and either Alliance or Aristotle peppers when these cultivars were treated with the highest rate of *M. albus*. Only treatment with mefenoxam significantly reduced disease severity on Red Knight peppers (Figure 2.1, Table 2.1). Overall, only the use of the cultivar Paladin (in combination with any treatment applied to the soil), or other sweet pepper cultivars treated with mefenoxam resulted in median disease ratings of 0 or 1 (Table 2.1).

Interaction between pepper cultivar and treatment of soil on disease severity. Both sweet pepper cultivar and soil treatment had significant effects on disease severity ratings ($P < 0.0001$; Table 2.2). The interaction between soil treatment and cultivar was also significant ($P < 0.0001$; Figure 2.2, Table 2.2). Thus, disease severity on each pepper cultivar varied with the treatment applied to the soil, but disease severity did not vary in the same way across all cultivars.

Table 2.2 Interaction between treatment of potting mix and sweet pepper cultivar. Results of nonparametric analysis of variance for the effects of treatment and cultivar on disease severity caused by *P. capsici* on sweet peppers in the greenhouse.[a]

	Analysis of variance-type statistics[b]			
Effect	**df_N**	**df_D**	**F**	***P* value**
Treatment	3.81	226	90.3	<0.0001
Cultivar	3.57	226	107.25	<0.0001
Treatment x Cultivar	11.8	226	4.97	<0.0001

[a] Treatments were 0.55 g/L *M. albus*, 1.9 g/L *M. albus*, 3.75 g/L *M. albus*, *P. capsici* only, and mefenoxam. Cultivars were Paladin, Alliance, Revolution, Aristotle, and Red Knight. Disease severity was rated on an ordinal scale from 0 (healthy plants) to 5 (dead plants), 7 days after transplanting. Data was combined from the three experiments.
[b] df_N = numerator degrees of freedom, df_D = denominator degrees of freedom.

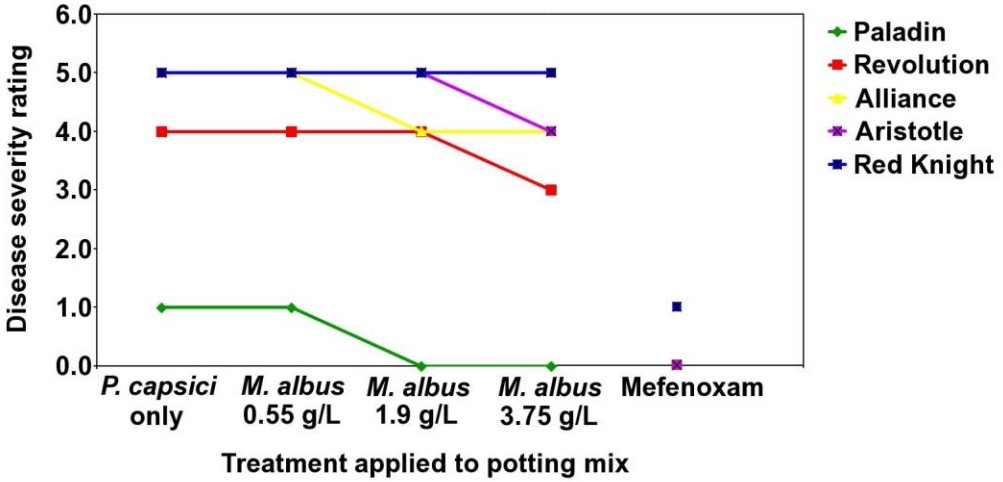

Figure 2.2 Visualization of the interaction between pepper cultivar and treatment of potting mix. Median disease severity caused by Phytophthora blight on five pepper cultivars across five treatments applied to potting mix. All potting mix was infested with *Phytophthora capsici* and treated with mefenoxam, three rates of *Muscodor albus*, or no additional treatment. Disease severity was rated on an ordinal scale from 0 (healthy plants) to 5 (dead plants), 7 days after transplanting, and data was combined from the three experiments. The fact that the lines representing the different cultivars do not remain equidistant across the soil treatments indicates that an interaction has likely occurred, and this is confirmed by the analysis shown in Table 2.2 (Sheskin 1996).

Efficacy of *M. albus* to control *P. capsici* on butternut squash. The butternut squash cultivar 'Waltham' was highly susceptible to *P. capsici* (Table 2.3). Treatment with mefenoxam significantly reduced disease severity on butternut squash compared to treatment with either *P. capsici* alone or *P. capsici* with any rate of *M. albus* (Table 2.3). Mefenoxam was the only treatment that prevented rapid plant death (Table 2.3).

Table 2.3 Disease severity on butternut squash treated with *Muscodor albus*. Effect of soil treatments on median disease severity caused by *P. capsici* on butternut squash in the greenhouse.[a]

Treatment	Disease Severity[b]
M. albus 3.75g/L	4.5a
M. albus 1.9g/L	4.0a
M. albus 0.55g/L	5.0a
P. capsici only	5.0a
Mefenoxam	0.0b

[a] The cultivar Waltham was used and disease severity was rated on an ordinal scale from 0 (healthy plants) to 5 (dead plants), 7 days after transplanting. Rating data was combined from the three experiments.
[b] Medians followed by the same number are not significantly different from each other by a Kruskal-Wallis test followed by a Dunn's test at $p = 0.05$.

Discussion

P. capsici continues to be a serious disease of peppers and cucurbits in temperate climates, in spite of the use of crop rotation and fungicides (Hausbeck and Lamour 2004). Previous studies have documented the successful control of soil-borne (Mercier and Manker 2005; Baysal et al. 2007) and post-harvest (Mercier and Jimenez 2004; Mercier and Smilanick 2005; Gabler et al. 2006; Schnabel and Mercier 2006) diseases using *M. albus*. Mefenoxam currently provides good control of Phytophthora blight in fields where *P. capsici* is still sensitive to the fungicide, but mefenoxam-insensitive isolates are prevalent in many fields (Hausbeck and Lamour 2004), including both Long Island and some areas of upstate New York (Hausbeck and

Lamour 2004); *unpublished data*). There are, however, still some locations in upstate New York that have mefenoxam-sensitive populations (*unpublished data*). Because insensitivity to mefenoxam can develop in populations of *P. capsici* that are repeatedly exposed to the fungicide, alternative control methods for Phytophthora blight are needed, whether chemical, biological, or cultural (Hausbeck and Lamour 2004).

Under the conditions of this study, the previously-described complete biofumigation effects of *M. albus* on *P. capsici* were not observed. Addition of the highest rate of *M. albus* did reduce disease severity in 4 of the 5 pepper cultivars tested, but the level of disease was still very high in all cultivars except Paladin. The potting mix in the Mercier and Manker (2005) study was inoculated with *P. capsici* at a much higher rate (inoculum to potting mix ratio of 1:1, compared to 1:4 in this study); thus the level of inoculum does not account for the difference observed between these two studies. A likely explanation for the results obtained by Mercier and Manker is the rate of *M. albus* used to fumigate the potting mix. Soil was inoculated with 25g of *M. albus* (grown on rye grain, as in this experiment) per liter of potting mix, which is a significantly higher rate than was used in this study (the rate suggested by the manufacturer). Also, different strains of the pathogen were used in each study and these strains may differ in aggressiveness on pepper.

It has been reported that the volatile compounds produced by *M. albus* do not diffuse long distances through the soil (Mercier and Manker 2005). Thus, perhaps not all *P. capsici* inoculum in the pots was exposed to *M. albus* volatiles when *M. albus* was added to the potting mix at low rates, regardless of how thoroughly it was mixed into the soil. This could explain why *M. albus* effectively controlled *P. capsici* (Strobel 2006) and *P. erythroseptica* (Schotsmans et al. 2008) in *in vitro* experiments (where the volatiles do not have to diffuse through soil). Volatiles produced by *M. albus* may also diffuse over longer distances through air in storage containers than

through soil, accounting for the successful use of *M. albus* to control post-harvest diseases. In addition, Stinson et al. (2003) reported that soil pathogens were differentially inhibited depending on the formulation of *M. albus*, so it is also possible that *M. albus* grown on a different substrate (other than rye grain) might result in better control of *P. capsici*.

The pepper cultivar 'Paladin' is widely reported to be tolerant to *P. capsici* (Babadoost and Islam 2002; Johnston et al. 2002; Driver and Louws 2003; Hausbeck and Lamour 2004) and was the most tolerant of the cultivars included in this study. However, it has been noted that this tolerance can be overcome in the field under conducive environmental conditions (Hausbeck and Lamour 2004). Because the highest rate of *M. albus* used in this study significantly reduced disease severity on all pepper cultivars except Red Knight, it is possible that a high rate of *M. albus* could effectively limit losses of tolerant pepper cultivars under high disease pressure. In this study, Revolution was more susceptible than Paladin, but not as susceptible as Red Knight peppers, which is consistent with a previous report of some tolerance of this cultivar to *P. capsici* in the field (40% incidence after artificial inoculation; Louws and Driver 2007).

There have been mixed reports of the tolerance level of the pepper cultivar Aristotle to Phytophthora blight, ranging from 20% mortality (Driver and Louws 2003) to 91% mortality (McGrath and Davey 2007) in field trials. One study reported Alliance peppers to be fairly susceptible to Phytophthora blight (more than 60% incidence after artificial field inoculation; Louws and Driver 2007). Under the conditions of this study, both Aristotle and Alliance peppers responded similarly to the applied soil treatments and together with Revolution peppers were classified as 'less tolerant,' since they were slightly less susceptible than Red Knight peppers. The differences in reported levels of tolerance on each pepper cultivar over multiple

studies may be due to different experimental conditions and the use of different isolates of *P. capsici*.

Red Knight is a highly susceptible pepper cultivar (McGrath and Davey 2007) and similar results were seen in this study, where Red Knight was the most susceptible cultivar tested. Application of *M. albus* did not reduce disease severity on Red Knight, and, in sharp contrast to Paladin, treatment with mefenoxam produced a substantial reduction in disease severity on Red Knight.

As applied in this study, *M. albus* did not control *P. capsici* on butternut squash. All plants exposed to *P. capsici* (except those treated with mefenoxam) died rapidly. Although no Phytophthora resistance is currently available in butternut squash, research is in progress to identify sources of resistance to *P. capsici* (Kabelka et al. 2007; Padley et al. 2007). The results of the present study indicate that *M. albus* does not control *P. capsici* on highly susceptible cucurbit hosts.

As applied in this study, *M. albus* did not provide complete biofumigation of *P. capsici*-infested soil; however, at 3.75 g/L it did significantly reduce disease severity on partially-tolerant pepper cultivars. Therefore, while *M. albus* will likely not protect highly susceptible host crops from infection with *P. capsici*, it is possible that *M. albus* could be used in combination with host plant tolerance to reduce severity of Phytophthora blight. Additional studies are needed to determine if the use of *M. albus* in conjunction with resistant cultivars could be part of an effective integrated pest management program.

REFERENCES

Babadoost, M. and Islam, S. Z. 2002. Bell peppers resistant to Phytophthora blight. Phytopathology 92:S5.

Baysal, F., Mera, J. R. and Miller, S. A. 2007. Effects of *Muscodor*, Serenade and conventional fungicides on Rhizoctonia root and hypocotyl rot and clubroot of radish. (abstr.). Phytopathology 97:S9.

Brunner, E., Domhof, S. and Langer, F. 2002. Nonparametric analysis of longitudinal data in factorial experiments. New York, John Wiley & Sons.

Davidson, C. R., Carroll, R. B., Evan, T. A. and Mulrooney, R. P. 2002. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatis*) in the mid-Atlantic region. Plant Disease 86: 1049.

Driver, J. G. and Louws, F. J. 2003. Management of Phytophthora crown and root rot in peppers. Phytopathology 93: S22.

Gabler, F. M., Fassel, R., Mercier, J. and Smilanick, J. L. 2006. Influence of temperature, inoculation interval, and dosage on biofumigation with *Muscodor albus* to control postharvest gray mold on grapes. Plant Disease 90(8): 1019-1025.

Gevens, A. J., Donahoo, R. S., Lamour, K. H. and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. Plant Disease 92: 201-209.

Hausbeck, M. K. and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Disease 88(12): 1292-1303.

Johnston, S. A., Kline, W. L., Fogg, M. L. and Zimmerman, M. D. 2002. Varietal resistance evaluation for control of Phytophthora blight of pepper. Phytopathology 92: S40.

Kabelka, E., Padley, L., Roberts, P., Ramos, L., Martinez, M. and Klassen, W. 2007. Resistance to *Phytophthora capsici* within winter squash (C*ucurbita moschata*) derived from a wild *Cucurbita* species. HortScience 42(4): 1014.

Lamour, K. H. and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90(4): 396-400.

Leonian, L. H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopathology 12: 401-408.

Louws, F. J. and Driver, J. G. 2007. Evaluation of pepper lines for resistance to Phytophthora crown and root rot, 2006. Plant Disease Management Reports (online). Report 1:V118. DOI:10.1094/PDMR01, The American Phytopathological Society, St. Paul, MN.

McGrath, M. T. and Davey, J. F. 2007. Efficacy of fungicides for control of Phytophthora blight in pepper on crown rot tolerant and susceptible cultivars, 2006. Plant Disease Management Reports (online). Report 1:V131. DOI:10.1094/PDMR01, The American Phytopathological Society, St. Paul, MN.

Mercier, J. and Jimenez, J. I. 2004. Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus*. Postharvest Biology and Technology 31(1): 1-8.

Mercier, J. and Manker, D. C. 2005. Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile-producing fungus *Muscodor albus*. Crop Protection 24(4): 355-362.

Mercier, J. and Smilanick, J. L. 2005. Control of green mold and sour rot of stored lemon by biofumigation with *Muscodor albus*. Biological Control 32(3): 401-407.

Padley, L., Roberts, P. and Kabelka, E. 2007. Screening *Cucurbita pepo* for resistance to *Phytophthora capsici*. HortScience 42(4): 1014.

Ristaino, J. B., Duniway, J. M. and Marois, J. J. 1988. Influence of frequency and duration of furrow irrigation on the development of Phytophthora root rot and yield in processing tomatoes. Phytopathology 78: 1701-1706.

Schnabel, G. and Mercier, J. 2006. Use of a *Muscodor albus* pad delivery system for the management of brown rot of peach in shipping cartons. Postharvest Biology and Technology 42(1): 121-123.

Schotsmans, W. C., Braun, G., DeLong, J. M. and Prange, R. K. 2008. Temperature and controlled atmosphere effects on efficacy of *Muscodor albus* as a biofumigant. Biological Control 44(1): 101-110.

Shah, D. A. and Madden, L. V. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. Phytopathology 94(1): 33-43.

Sheskin, D. J. 1996. Handbook of parametric and nonparametric statistical procedures. Boca Raton, CRC Press.

Silvar, C., Merino, F. and Díaz, J. 2006. Diversity of *Phytophthora capsici* in northwest Spain: Analysis of virulence, metalaxyl response, and molecular characterization. Plant Disease 90(9): 1135-1142.

Stinson, A. M., Zidack, N. K., Strobel, G. A. and Jacobsen, B. J. 2003. Mycofumigation with *Muscodor albus* and *Muscodor roseus* for control of seedling diseases of sugar beet and Verticillium wilt of eggplant. Plant Disease 87(11): 1349-1354.

Strobel, G. 2006. *Muscodor albus* and its biological promise. Journal of industrial microbiology & biotechnology 33(7): 514-522.

CHAPTER 3

SENSITIVITY TO MEFENOXAM AND POPULATION STRUCTURE OF *PHYTOPHTHORA CAPSICI* IN NEW YORK

Introduction

The heterothallic oomycete *Phytophthora capsici* causes Phytophthora blight on many hosts, including peppers, cucurbits, eggplants, tomatoes (Erwin and Ribeiro 1996; Hausbeck and Lamour 2004), and beans (Davidson et al. 2002; Gevens et al. 2008), and results in devastating crop losses around the world (Erwin and Ribeiro 1996). Recently, Fraser fir has also been identified as a host (Quesada-Ocampo et al. 2009). *P. capsici* reproduces asexually by means of sporangia and zoospores, and sexually, by means of oospores (Erwin and Ribeiro 1996). Production of sporangia and release of zoospores is especially rapid under wet conditions, such as those caused by heavy rain, over-irrigation, or poorly-drained field soil (Zentmyer and Erwin 1970; Duniway 1979; Bernhardt and Grogan 1982). Movement of water (via drainage, windblown rain, splashing from soil onto aerial plant tissue, etc.) is an important dispersal mechanism for this pathogen within a field (Ristaino et al. 1994; Café-Filho and Duniway 1995; Ristaino et al. 1997), and because spores of *P. capsici* are common in surface water used for irrigation, they can also be spread between fields by water movement (Roberts et al. 2005; Gevens et al. 2007). In New York, we have observed that movement of infected fruits, infested soil, or farm equipment can spread *P. capsici* within and between fields, and similar observations have been made in Michigan (Hausbeck and Lamour 2004). Wind dispersal does not appear to be important in the long-distance spread of *P. capsici* (Lamour and Hausbeck 2002; Hausbeck and Lamour 2004). Because *P. capsici* is heterothallic, both the A1 and the

A2 mating types must be present in close proximity for the long-lived, overwintering oospores to be produced (Ristaino and Johnston 1999). Thus, in cold climates where the more tender asexual propagules (hyphae, sporangia, and zoospores) cannot survive the winter, sexual reproduction is required for survival from one growing season to another (Duniway 1979; Bowers et al. 1990; Lamour and Hausbeck 2003; Babadoost 2007).

Cultural control methods include avoiding or limiting exposure of hosts to excess moisture in the field (Springer and Johnston 1982; Ristaino 1991; Biles et al. 1992; Café-Filho et al. 1995; Café-Filho and Duniway 1996; Xie et al. 1999), rotating to non-susceptible host crops (Hausbeck and Lamour 2004), removal of susceptible weed hosts from the field (Ploetz et al. 2002; French-Monar et al. 2006), and planting tolerant sweet pepper varieties (Ristaino and Johnston 1999; Johnston et al. 2002; Miller, S. A. et al. 2002; Stieg et al. 2006). In addition, potentially-infested soil or plant material should not be discarded in vegetable production fields, because of the potential to move *P. capsici* spores to new locations in this way (Hausbeck and Lamour 2004). The phenylamide fungicide mefenoxam is very effective against susceptible isolates of *P. capsici*, but resistance has already developed in many populations (Biles et al. 1992; Ristaino et al. 1997; Pennisi et al. 1998; Ristaino and Johnston 1999; Matheron and Porchas 2000; Lamour and Hausbeck 2001a; Hausbeck and Lamour 2004; French-Monar et al. 2007; Café-Filho and Ristaino 2008; Davey et al. 2008).

Numerous genetic methodologies can be used to study the population structure of diploid organisms, like *P. capsici*. Microsatellite, or simple sequence repeat (SSR) markers have been used to study a variety of plant pathogens, including *Phytophthora infestans* (Lees et al. 2006; Widmark et al. 2007), *Phytophthora ramorum* (Ivors et al. 2006; Prospero et al. 2007), *Phytophthora cinnamomi* (Dobrowolski et al. 2003),

Phaeosphaeria nodorum (Blixt et al. 2008), and *Cryphonectria parasitica* (Kubisiak et al. 2007). These genomic regions are composed of a string of one to six base pairs repeated some variable number of times, and the size of each microsatellite locus is directly related to the number of repeats at that locus. The number of repeats may differ between individuals as a result of unequal crossing over during recombination or slippage during replication of DNA. Thus, different alleles at each microsatellite locus can be distinguished by amplifying the locus with a polymerase chain reaction (PCR) and separating the PCR products based on size using electrophoresis. Microsatellites are very useful molecular markers because they are co-dominant and can be neutral (if chosen from non-coding regions of the genome). In addition, many alleles may be present at a single locus, and the presence of microsatellite alleles of different sizes can be easily scored and compared between different studies (Tautz and Renz 1984; Queller et al. 1993).

Many studies have undertaken the characterization of *P. capsici* populations by various molecular techniques, and in various parts of the world (Oudemans and Coffey 1991; Mchau and Coffey 1995; Lamour and Hausbeck 2000; Lamour and Hausbeck 2001a; Lamour and Hausbeck 2002; Lamour and Hausbeck 2003; Islam et al. 2005; Silvar et al. 2006; Bowers et al. 2007; Hurtado-Gonzáles et al. 2008). Several of these studies have indicated little or no population substructure based on the geographic origin of the samples, either on local (isolates collected from fields within a region) or global (isolates collected from different countries) levels (Forster et al. 1990; Mchau and Coffey 1995; Islam et al. 2005; Silvar et al. 2006; Bowers et al. 2007). In Michigan, where Phytophthora blight has been devastating to the pickling cucumber industry (Hausbeck and Lamour 2004), such characterization has indicated that mefenoxam resistance is widespread (Lamour and Hausbeck 2000), sexual reproduction is common (Lamour and Hausbeck 2000; Lamour and Hausbeck 2003),

populations within fields are highly diverse (Lamour and Hausbeck 2001a), and little or no gene flow occurs between populations in fields separated by at least 1 km (Lamour and Hausbeck 2001b; Lamour and Hausbeck 2002).

Although not all populations of *P. capsici* have the same characteristics as those found in Michigan (Mchau and Coffey 1995; Hurtado-Gonzáles et al. 2008), preliminary reports of *P. capsici* in New York are consistent with what has been observed in Michigan, including the recovery of both A1 and A2 mating types from the same field on Long Island and in upstate New York, as well as the recovery of mefenoxam-resistant isolates from a single field in upstate New York (Hausbeck and Lamour 2004). Because the population structure of plant pathogens can have important implications for the biology of the pathogen and disease management (Fry et al. 1992; McDermott and McDonald 1993; Milgroom 1995; Milgroom 1996; Goodwin 1997), it was important to conduct a survey of *P. capsici* in New York, using biological characteristics like mating type and mefenoxam sensitivity, as well as microsatellite markers to characterize the population. Knowledge of the specific *P. capsici* populations affecting New York growers will help in the development of appropriate management recommendations that are specific for New York. The objectives of this study were to determine: (i) to what extent isolates of *P. capsici* in New York are resistant to mefenoxam; (ii) whether *P. capsici* in New York is highly diverse; (iii) whether populations in New York are structured based on geographic location.

Materials and Methods

Collection of symptomatic plants. In 2006 and 2007, plants showing characteristic symptoms of Phytophthora blight were collected from growers' fields in four vegetable-production regions of New York: western, central, the Capital District

(near Albany, New York), and Long Island. In the single field sampled in western New York in 2007, some symptomatic plants were collected from recently-rogued plants, and others were collected from nine sites in the same field with two to seven plants sampled at each site (Figure 3.1). From all other fields, symptomatic plants were sampled at random (statistically arbitrarily). In 2006, fields in western and central New York were sampled in August and September. In 2007, samples from western New York were collected in late June and samples from central New York were collected in late July. Various fields on Long Island were sampled in late August through early September 2007, and in early November 2007. Fields in the Capital District were sampled in late September 2007.

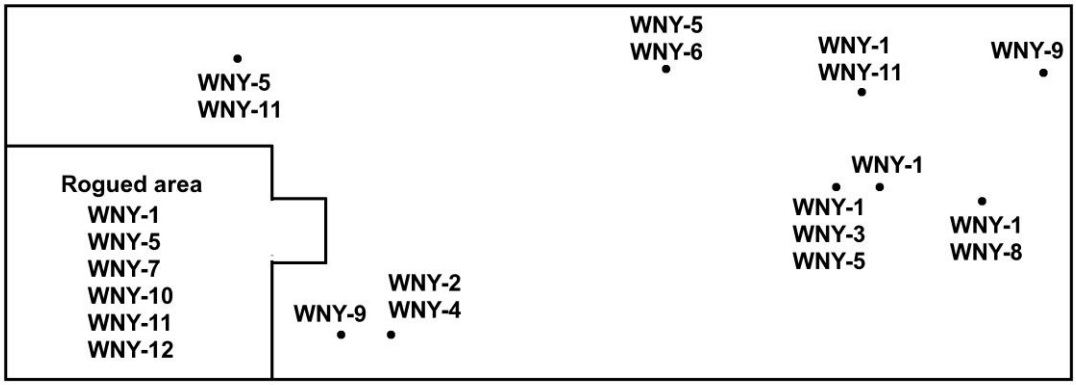

Figure 3.1 Relative location of sampling sites in an approximately 1.5 ha field located in western New York (field WNY) from which plants showing symptoms of Phytophthora blight were sampled in June 2007. Each dot indicates a location where two to seven plants were collected. The genotypes which were recovered from each site in the field are also indicated.

Isolation and confirmation of *P. capsici*. Small pieces of symptomatic plant tissue were surface disinfested in 10% bleach for two to three minutes, rinsed in sterile distilled water and plated on PARP (25 mg pentachloronitrobenzene (Sigma-Aldrich Co., St. Louis, MO), 250 mg ampicillin sodium salt (Fisher Scientific, Fair Lawn, NJ), 10 mg rifampicin (Fisher Scientific) dissolved in 1 ml 95% ethanol, 0.4 ml pimaricin (2.5% aqueous solution; MP Biomedicals, LLC, Solon, OH), 17 g cornmeal agar

(Becton, Dickinson and Company, Sparks, MD), and 1 L distilled water), or PARPH (PARP, plus 20 mg/L hymexazol; Sigma-Aldrich); recipe adapted from Schmitthenner, A.F. and Bhat (1994). Plates were incubated at room temperature until colonies began to grow from the tissue, at which point edges of these colonies were transferred to new PARP or PARPH plates. Isolates were identified as *P. capsici* by isolation on selective media (PARP or PARPH), production of ovoid, papillate sporangia on long pedicels (Leonian 1922) when grown on 15% unclarified V8 (UCV8) agar and exposed to light, and species-specific polymerase chain reaction (described below). To obtain single zoospore cultures of each isolate, colonies were transferred to UCV8 agar and incubated at room temperature under lab lighting to induce sporulation, as previously described (Lamour and Hausbeck 2000). Only single zoospore cultures were used in the following experiments. Both prior to and after single zoosporing, cultures were maintained on UCV8, PARP, or potato dextrose agar (PDA; 39 g/L, Becton, Dickinson and Company) and transferred every 1-2 months until stored. To store isolates, plugs taken from the expanding edge of a single zoospore culture on PDA were transferred to sterile distilled water and maintained in the dark at room temperature (Schmitthenner, A.F. and Bhat 1994). Stored isolates were taken out of storage and restored approximately every 12 months.

Mating type and mefenoxam sensitivity. To determine the mating type of each isolate, a 5-10 mm plug taken from a 1-3 week old PARP culture was transferred to the center of a UCV8 plate, approximately 3 cm away from two plugs of a *P. capsici* isolate of known A1 mating type (ATCC number MYA-2338). This procedure was repeated with a known A2 isolate of *P. capsici* (ATCC number MYA-2290), and both plates were incubated in the dark for at least a week before being inspected under a light microscope for the presence of oospores. Isolates which formed oospores with the A1 mating type standard, but not with the A2 mating type standard were

determined to be mating type A2, and vice versa (Tooley et al. 1985). Plates containing both the A1 and A2 test strains were included as positive controls.

To determine whether isolates were sensitive to mefenoxam, a circular disk was cut from the edge of a 1-3 week old PARP culture of each isolate using a sterile 10 mm cork borer. Disks were transferred to the center of 100 mm-wide UCV8 plates amended with no mefenoxam, 5 µg/ml mefenoxam, and 100 µg/ml mefenoxam (Goodwin et al. 1996). Media was amended with Ridomil Gold EC (Syngenta Crop Protection, Inc., Greensboro, NC) to give the appropriate amount of active ingredient. Plates were incubated in the dark for 3-7 days, until mycelia on the 0 µg/ml mefenoxam control had covered at least half of the plate. The entire diameter of each colony was measured at its widest point, and the diameter of the plug was subtracted from the diameter of each colony before reporting colony growth on mefenoxam-amended media as a percentage of the no-mefenoxam control. An isolate was scored as sensitive, if growth on 5 µg/ml mefenoxam-amended UCV8 was less than 40% of growth on the unamended UCV8; intermediately resistant, if growth on 5 µg/ml mefenoxam-amended UCV8 was greater than 40% of growth on the unamended UCV8, but growth on 100 µg/ml mefenoxam-amended UCV8 was less than 40% of growth on the unamended UCV8; or resistant if growth on both 5 µg/ml and 100 µg/ml mefenoxam-amended UCV8 was more than 40% of growth on the unamended UCV8 (Silvar et al. 2006). All mefenoxam sensitivity tests were conducted at least twice.

Harvesting mycelia and extracting DNA. Single zoospore cultures of each isolate were grown for one to three weeks on PARP before three to four plugs (approximately 1 cm in diameter) were transferred to approximately 12 ml of sterile potato dextrose broth (24 g/L, MP Biomedicals, Solon, OH) in 15 mm x 100 mm Petri dishes (Fisher Scientific). Plates were sealed with Parafilm and incubated in the dark

for 5 days to 3 weeks, depending on how quickly the mycelia grew. Mycelia were harvested by vacuum filtration, separated from agar plugs, and wrapped in aluminum foil or stored in microcentrifuge tubes and frozen at -20°C until DNA was extracted.

DNA was extracted using the Qiagen DNeasy Plant Mini kit (Valencia, CA) or the MoBio UltraClean™ Soil DNA kit (Carlsbad, CA), following the manufacturers' instructions, with slight modifications to the Qiagen protocol, as follows. To disrupt the tissue in the first step of the Qiagen extraction protocol, 40-80 mg of thawed mycelia (not lyophilized) were placed in a round-bottom 2 ml microcentrifuge tube with a sterile ball bearing and shaken in a Qiagen TissueLyser at 30 cycles per second for 1 minute and 30 seconds. The process was repeated if the tissue was not completely ground. In the final step of the kit protocol, the incubation with Buffer AE was extended to 15 minutes and Buffer AE was added in 2 aliquots of 50 µl each (with centrifugation following each incubation), to improve yield. With the MoBio kit, 30-70 mg of thawed mycelia was used for extraction and the spin filter was incubated for 15 minutes at room temperature with Solution S5 before the final elution of DNA.

Confirmation of isolates as *P. capsici* using species-specific primers. Extracted DNA was amplified with *P. capsici*-specific primers (PC-3, 5'-GTGTTGTCCTTCGGGTCGACTG-3' and PC-6R, 5'-GGAAAAGCATTCAATAAGCGCCTG; Zhang et al. 2008). For this reaction, the total volume was 25 µl, containing 2.5 µl extracted genomic DNA, 0.2 µM primers, 0.2 mM total dNTPs, 2.5 µl 1x ThermoPol reaction buffer with 2 mM $MgSO_4$, and 0.5 U *Taq* DNA polymerase (New England Biolabs, Ipswich, MA). The reaction took place in either an Eppendorf Mastercycler® gradient (Eppendorf, Westbury, NY) or a PTC-100™ Thermo Cycler (MJ Research, Boston, MA) PCR machine with an initial incubation of 5 minutes at 94°C, followed by 35 cycles of 94°C for 45 seconds, 62°C for 45 seconds, and 72°C for 45 seconds, and a final incubation step at 72°C for 10

minutes. The expected product was 219 base pairs in length and products were visualized on a 1% agarose gel. Negative controls containing no genomic DNA were run with all reactions to check for contaminated reagents, and DNA from known isolates of *P. capsici* were included as positive controls.

Amplification and analysis of microsatellite fragments. Of the isolates collected in 2007, confirmed as *P. capsici*, and characterized for mating type and mefenoxam sensitivity, isolates from three fields were selected for molecular analysis of population structure using five microsatellite markers. One field each in western New York, the Capital District, and Long Island (subsequently referred to as fields WNY, CD, and LI, respectively) was chosen. Microsatellite regions were amplified from genomic DNA using primers obtained from Dr. Adele McLeod (University of Stellenbosch, South Africa) and are not significantly similar to known proteins or expressed genes (*personal communication*). Forward primers AC11-F, TTC5-F, CTT16-F, AAG16-F and TTC18-F were labeled at the 5' end with fluorescent dyes (Applied Biosystems, Foster City, CA), and reverse primers were unlabeled (Table 3.1). Each PCR reaction occurred in a total volume of 25 µl, containing 25 ng of genomic DNA, 0.25 µM primers, 0.16 mM total dNTPs, 2.5 µl GeneAmp® 10x PCR Buffer with 15 mM $MgCl_2$, and 0.63 U AmpliTaq® Gold (Applied Biosystems, Inc.). For amplification of locus TTC18, 2.5 µl Standard *Taq* buffer with 1.5 mM $MgCl_2$ and 0.63 U *Taq* DNA polymerase (New England Biolabs) was used, instead of the Applied Biosystems Taq and buffer. All reactions took place in either an Eppendorf Mastercycler® gradient or a PTC-100™ Thermo Cycler PCR machine and used a touchdown program: 20 cycles of 94°C for 40 s, 60°C for 40 s (with a reduction of 0.5°C per cycle), and 72°C for 20 s, followed by 20 cycles of 94°C for 40 s, 50°C for 40 s, and 72°C for 20 s, ending with a 10 minute incubation at 72°C. Exposure of primers and PCR products to light was minimized to avoid degradation of the

fluorescent dyes.

Table 3.1 Primer sequences for amplification of five microsatellite loci from *Phytophthora capsici*. Each forward primer was labeled at the 5' end with the indicated fluorescent dye, while reverse primers were not labeled.

Locus	Dye	Forward primer (5'-3')	Reverse primer (5'-3')
AC11	PET	GCTACAAGCTGCCACAGGTGTC	CACCGCAGATTCGCTAAGAGCC
TTC5	VIC	GTCGAGATCTGGTCGGTTCTAG	CTGTTTACTGCGCCAATCACCTG
CTT16	6-FAM	TGAGCTGCATTGTGAACGG	TTACCACCTCGATGGTGC
AAG16	NED	GCTTGCATCAATTTATCGCAG	ATTGTGAACGGTCATCACTG
TTC18	6-FAM	CTTGATCAATGGCGACACAG	GCGCCTCCTTCTACTTATCG

PCR products for loci TTC5, CTT16 and AAG16 were mixed in a 1:1:1 ratio, while products for loci AC11 and TTC18 were analyzed individually. For fragment analysis, 1 µl of either mixed or unmixed PCR product was added to a 0.2 ml well of a 96-well plate (Fisher Scientific, Fair Lawn, NJ) with 10 µl hi-di formamide (Sigma-Aldrich) and 0.2 µl GeneScan™-500 LIZ® Size Standard (Applied Biosystems, Inc.). Plates were sealed with Microseal 'B' Film (BioRad, Hercules, CA) to prevent evaporation, spun down briefly (approximately 1000 rpm for 1 minute), and wrapped in aluminum foil to prevent degradation of fluorescent dyes. Fragment size was determined on a 3730xl DNA Analyzer (Applied Biosystems, Inc.), and resulting electropherograms were visualized and peaks were scored in Peak Scanner (Applied Biosystems, Inc.). If peaks were not clear, larger volumes (2 or 3 µl) of PCR products were re-analyzed, or were analyzed separately (in the case of loci TTC5, CTT16 and AAG16). Amplification of the TTC18 locus did not always produce clear peaks with the New England Biolabs *Taq* and Standard *Taq* buffer. In these cases, reactions were repeated using AmpliTaq® Gold with GeneAmp® 10x PCR Buffer, or New England Biolabs *Taq* with ThermoPol.

Statistical analysis. A chi-square goodness-of-fit test was used to determine whether mating types of isolates recovered from fields WNY, CD and LI were in a 1:1

ratio (Sheskin 1996). The probability that isolates recovered from the same field with the same five-locus (not including mating type) genotype were the products of sexual reproduction, instead of being clones (P_{sex}), was calculated using GenClone software, without considering departures from Hardy-Weinberg equilibrium in each field (Arnaud-Haond et al. 2005; Arnaud-Haond and Belkhir 2007). All other analyses were done with Arlequin (Excoffier et al. 2005). To test the level of differentiation between *P. capsici* populations in the three fields (WNY, CD and LI), and between isolates of different mating types within each field, pairwise F_{ST} values were calculated (1000 permutations) using Nei's method, which considers the number of identical DNA fragments (or alleles) shared between or among populations (Nei and Li 1979). An analysis of molecular variance (AMOVA) was used to estimate the variation across all three fields which resulted from differences among fields (F_{ST}), and to estimate fixation indices (F_{IS}) in each field, using 5000 permutations, according to the methods of Weir and Cockerham (1984). To test the hypothesis of random mating in each field, an exact test of Hardy-Weinberg equilibrium was performed on each locus in each field using a Markov chain with 1,000,000 steps and 5,000 de-memorization steps.

Both F_{IS} and F_{ST} estimate population differentiation or deviation. F_{IS} estimates the deviation of a population from random mating, thereby estimating the amount of inbreeding in the population (Goodwin 1997; Balloux et al. 2003). F_{ST} estimates the deviation of populations (or subpopulations) from each other, and may be used to describe either a pairwise comparison (eg, between two fields, or between two mating types within a field), or it may be reported for a single population. In the former case, F_{ST} estimates the differentiation between the two populations (or subpopulations). In the latter case, it can be interpreted as the proportion of the total variation in a population which is accounted for by variation among different subpopulations (Hartl and Clark 1997). In this study, the three fields are subpopulations of *P. capsici* within

the New York population.

Results

Isolate collection, mating type, and sensitivity to mefenoxam in New York. In 2006 and 2007, 262 isolates of *P. capsici* were recovered from symptomatic summer squash, pumpkin, pepper, tomato, winter squash, and eggplant, confirmed with *P. capsici*-specific PCR primers, and characterized with respect to mating type and mefenoxam sensitivity. The number of isolates recovered per growing region ranged from 19 to 136, and the number of fields from which isolates were recovered in each region ranged from two to 13 (Table 3.2). Of the 28 fields sampled, 10 or more isolates were recovered from 10 fields, and both mating types (A1 and A2) were found in five of these fields. Isolates of both mating types were recovered from each growing region. All of the isolates recovered from central and western New York were sensitive to mefenoxam, while mefenoxam resistance was widespread in the Capital District and on Long Island (Table 3.2). In the Capital District, mefenoxam-resistant isolates were recovered from all but two fields, and 63% of the isolates collected in this region were resistant to mefenoxam. In Long Island, only six of the 13 fields sampled contained mefenoxam-resistant isolates, resulting in 25% of the recovered isolates being either intermediately or fully resistant to mefenoxam. Detailed information about all fields sampled in 2006 and 2007 and the mating type and mefenoxam sensitivity of isolates collected in these fields is presented in Appendix 4.

Table 3.2 Summary of mating type and mefenoxam sensitivity of isolates of *Phytophthora capsici* collected from different growing regions of New York in 2006 and 2007.

Region[a]	Total Isolates	Fields[b]	Mating Type		Mefenoxam Sensitivity[c]		
			A1	A2	S	I	R
Western NY	37	2	9	28	37	0	0
Central NY	19	4	3	16	19	0	0
Capital District	70	9	38	32	26	0	44
Long Island	136	13	81	55	102	2	32
All of NY	262	28	131	131	184	2	76

[a] Growing region in New York from which the isolate was collected.
[b] Number of fields from which isolates were recovered.
[c] S = sensitive, I = intermediately sensitive, and R = resistant to the fungicide mefenoxam.

The three fields selected for analysis with microsatellite markers (WNY, CD, and LI) were representative of the growing regions in which they were located, in that both mating types were recovered from each field and only mefenoxam sensitive isolates were recovered from field WNY. In fields LI and CD, 39% and 43%, respectively, of the isolates collected were either intermediately or fully resistant to mefenoxam (Table 3.3). In fields CD and LI, ratios of A1 to A2 isolates were not significantly different from a 1:1 ratio, but the ratio of A1 to A2 isolates collected in field WNY did differ significantly from 1:1 at $P = 0.001$ ($\chi^2 = 11.11$, df = 1). When data were clone-corrected, the ratio of A1 to A2 isolates did not differ significantly from 1:1 in any of the three fields, but this difference may be due to the reduction in sample size caused by clone correction.

Table 3.3 Mating type and sensitivity to mefenoxam for *Phytophthora capsici* isolates collected in 2007 from three fields in New York. Isolates from these three fields were further characterized using five microsatellite markers.

Field[a]	Total Isolates	Mating Type		Mefenoxam Sensitivity[b]		
		A1	A2	S	I	R
WNY	36	8	28	36	0	0
CD	23	9	14	13	0	10
LI	44	20	24	27	1	16

[a] Field in New York from which the isolate was collected; field WNY was located in western New York, field CD was located in the Capital District, and field LI was located on Long Island.
[b] S = sensitive, I = intermediately sensitive, and R = resistant to the fungicide mefenoxam.

Microsatellite analysis of population structure in three fields. One or two alleles were amplified at each of the five microsatellite loci in all 103 isolates studied. Across all fields, three to eight alleles were observed at each microsatellite locus, but one or two alleles tended to predominate at each locus in each field. Twelve alleles were observed in only one field, and only five alleles were observed in all three fields. In fields WNY, CD and LI, one to five, three to five, or two to four alleles, respectively, were observed at each locus. Except for loci TTC5, CTT16 and AAG16 in field WNY, all loci were polymorphic in all fields (Table 3.4).

Table 3.4 Allele sizes and frequencies at five microsatellite loci in *Phytophthora capsici* isolates collected from three fields in New York in 2007.

		Allele frequencies[b]		
Locus	Size[a]	WNY	CD	LI
AC11	232	0.00	0.02	0.00
	239	0.00	0.09	0.57
	241	0.68	0.89	0.36
	243	0.26	0.00	0.00
	259	0.06	0.00	0.07
TTC5	434	1.00	0.72	0.83
	440	0.00	0.22	0.17
	446	0.00	0.07	0.00
CTT16	307	0.00	0.70	0.86
	310	0.00	0.00	0.07
	326	1.00	0.24	0.07
	335	0.00	0.04	0.00
	338	0.00	0.02	0.00
AAG16	274	0.00	0.70	0.88
	277	0.00	0.00	0.07
	291	1.00	0.24	0.06
	299	0.00	0.04	0.00
	302	0.00	0.02	0.00
TTC18	359	0.03	0.00	0.52
	371	0.00	0.00	0.07
	374	0.01	0.02	0.00
	377	0.44	0.41	0.00
	380	0.14	0.15	0.34
	383	0.38	0.26	0.00
	386	0.00	0.15	0.00
	389	0.00	0.00	0.07

[a] Allele sizes in base pairs.

[b] Allele frequencies among isolates collected in each of three fields in New York; field WNY was located in western New York, field CD was located in the Capital District, and field LI was located on Long Island.

Genotypes were distinguished by mating type of each isolate and the alleles observed at the five microsatellite loci. In fields LI, CD and WNY 6, 20, and 12 genotypes, respectively, were identified, resulting in 14%, 87%, and 33%, respectively, of the recovered isolates in these fields having unique genotypes. Based only on the five microsatellite loci, 14%, 83%, and 28% of isolates in fields LI, CD, and WNY, respectively, had unique genotypes. Individual genotypes were designated by the field name (CD, LI or WNY) followed by a number. Some genotypes (CD-3 and LI-1; CD-8 and CD-9; WNY-8 and WNY-9) were only distinguishable by mating type (Table 3.5). In field CD, each genotype was represented by only one or two recovered isolates, while in fields WNY and LI, up to 11 and 15 isolates, respectively, represented a single genotype. Only one genotype (WNY-1) was found in more than a single field (fields WNY and CD). In field WNY, where the relative collection location of each isolate was noted, multiple genotypes were frequently recovered from the same sampling site in the field, and most genotypes were recovered from multiple sampling sites (Figure 3.1). In addition, on two occasions, two different genotypes were isolated from different lesions on the same plant (WNY-5 and WNY-6; and WNY-3 and WNY-5, respectively).

Table 3.5 Genotypes of *Phytophthora capsici* isolates collected from three fields in New York and distinguished by mating type and allele size (in base pairs) at five microsatellite loci.

	# Isolates[a]				Microsatellite locus[b]				
Genotype	WNY	CD	LI	MT[c]	AC11	TTC5	CTT16	AAG16	TTC18
CD-1	0	1	0	A2	232/241	434/434	307/326	274/291	377/377
CD-2	0	1	0	A2	232/241	434/434	326/326	291/291	377/383
CD-3	0	1	0	A1	239/239	434/434	307/307	274/274	380/380
LI-1	0	0	15	A2	239/239	434/434	307/307	274/274	380/380
CD-4	0	1	0	A2	239/239	434/446	307/307	274/274	383/383
LI-2	0	0	5	A1	239/241	434/434	307/307	274/274	359/359
LI-3	0	0	15	A1	239/241	434/440	307/307	274/274	359/359
CD-5	0	1	0	A1	239/241	434/440	307/307	274/274	380/380
CD-6	0	1	0	A1	241/241	434/434	307/307	274/274	383/383
CD-7	0	2	0	A1	241/241	434/434	307/307	274/274	386/386
CD-8	0	1	0	A1	241/241	434/434	307/326	274/291	377/377
CD-9	0	1	0	A2	241/241	434/434	307/326	274/291	377/377
CD-10	0	1	0	A2	241/241	434/434	307/326	274/291	383/383
LI-4	0	0	3	A2	241/241	434/434	310/310	277/277	371/371
WNY-1	8	1	0	A2	241/241	434/434	326/326	291/291	377/377
WNY-2	1	0	0	A1	241/241	434/434	326/326	291/291	377/377
WNY-3	1	0	0	A2	241/241	434/434	326/326	291/291	377/380
WNY-4	1	0	0	A2	241/241	434/434	326/326	291/291	377/383
WNY-5	11	0	0	A2	241/241	434/434	326/326	291/291	383/383
CD-11	0	1	0	A2	241/241	434/434	326/335	291/299	374/380
CD-12	0	2	0	A1	241/241	434/440	307/307	274/274	377/377
CD-13	0	1	0	A2	241/241	434/440	307/307	274/274	380/383
CD-14	0	1	0	A1	241/241	434/440	307/307	274/274	383/383
CD-15	0	2	0	A2	241/241	434/440	307/326	274/291	377/377
CD-16	0	1	0	A2	241/241	434/440	307/335	274/299	377/377
CD-17	0	1	0	A2	241/241	434/440	307/338	274/302	380/386
CD-18	0	1	0	A2	241/241	434/446	307/307	274/274	386/386
CD-19	0	1	0	A2	241/241	440/446	307/307	274/274	383/383
WNY-6	1	0	0	A2	241/243	434/434	326/326	291/291	374/380
LI-5	0	0	5	A2	241/259	434/434	307/326	274/291	359/389
WNY-7	1	0	0	A2	241/259	434/434	326/326	291/291	377/377
WNY-8	1	0	0	A1	241/259	434/434	326/326	291/291	380/380
WNY-9	2	0	0	A2	241/259	434/434	326/326	291/291	380/380
LI-6	0	0	1	A2	241/259	434/440	307/326	274/274	359/389
WNY-10	2	0	0	A1	243/243	434/434	326/326	291/291	359/380
WNY-11	3	0	0	A2	243/243	434/434	326/326	291/291	377/377
WNY-12	4	0	0	A1	243/243	434/434	326/326	291/291	377/383

[a] Number of isolates of each genotype collected from three fields in Western New York (WNY), the Capital District (CD), or Long Island (LI).
[b] Allele sizes in base pairs observed at each of five microsatellite loci.
[c] Mating type.

In field LI, observed levels of heterozygosity differed significantly from those expected under Hardy-Weinberg equilibrium at four of the five loci, while observed and expected heterozygosity were significantly different at only one locus in field CD. Because only a single allele was observed at three of the five loci in field WNY, observed and expected heterozygosity could only be calculated for two loci in this field, but observed heterozygosity differed significantly from expected heterozygosity at both of these polymorphic loci (Table 3.6). All reported P_{sex} probabilities were calculated for the first re-encounter of a genotype, and ranges were 0.02-0.97, 0.03-0.17, and 0.00-0.84 in fields WNY, CD, and LI, respectively. P_{sex} was less than 0.05 in only one of six multi-isolate genotypes in field WNY, and in only one of four, and two of five multi-isolate genotypes in fields CD and LI, respectively. When the same five-locus genotype was identified in more than one isolate, the number of isolates recovered per genotype ranged from 2 to 11 in field WNY, and from 3 to 15 in field LI. Only one or two isolates with the same genotype were recovered in field CD. For some genotypes in field LI (LI-1 and LI-3), P_{sex} values fell below 0.05 by the third re-encounter of an isolate, while this occurred only after five re-encounters for genotype WNY-5, and after seven re-encounters for WNY-1/WNY-2, which had identical microsatellite genotypes, but different mating types.

Table 3.6 Exact test of Hardy-Weinberg equilibrium for each of five microsatellite loci in *Phytophthora capsici* isolates collected from three fields in New York in 2007.

Field and locus	N[a]	H_o[b]	H_E[c]	*P*[d]	SD[e]
WNY					
AC11	36	0.14	0.47	0.00000	0.00000
TTC5[f]	36	NA	NA	NA	NA
CTT16[f]	36	NA	NA	NA	NA
AAG16[f]	36	NA	NA	NA	NA
TTC18	36	0.25	0.65	0.00000	0.00000
CD					
AC11	23	0.13	0.20	0.21277	0.00042
TTC5	23	0.52	0.44	0.62415	0.00052
CTT16	23	0.39	0.47	0.5926	0.00045
AAG16	23	0.39	0.47	0.59644	0.00054
TTC18	23	0.17	0.73	0.00000	0.00000
LI					
AC11	44	0.59	0.55	0.00815	0.00010
TTC5	44	0.34	0.29	0.32181	0.00045
CTT16	44	0.14	0.25	0.00001	0.00000
AAG16	44	0.11	0.23	0.00012	0.00001
TTC18	44	0.14	0.61	0.00000	0.00000

[a] Number of isolates.
[b] Observed heterozygosity at each locus.
[c] Expected heterozygosity at each locus under Hardy-Weinberg equilibrium.
[d] *P*-value for the exact test of Hardy-Weinberg equilibrium using a Markov chain with 1,000,000 steps.
[e] Standard deviation
[f] Because only a single allele was observed at each of these loci in field WNY, no calculation of observed and expected heterozygosity was performed.

According to Wright, values for fixation index (F_{ST}) less than 0.05 are consistent with low, but still potentially important, levels of genetic differentiation between populations, or among subpopulations within a larger population. Values between 0.15 and 0.25 suggest moderately great differentiation, and values exceeding 0.25 indicate very great levels of differentiation (Wright 1978; Balloux and Lugon-Moulin 2002). Based on these guidelines, pairwise comparisons between each of the three fields indicated high levels of differentiation between fields (Table 3.7). The state-wide population of *P. capsici* was also highly differentiated, with an F_{ST} value of

0.459. In fields WNY and LI, isolates of the A1 and A2 mating types were highly differentiated (F_{ST} = 0.285, *P* = 0.001 and F_{ST} = 0.329, *P* = 0.000, respectively), while A1 and A2 populations in field CD were less differentiated (F_{ST} = 0.088, *P* = 0.040). According to Brown (1979), a value for F_{IS} between 0 and 0.3 is characteristic of an outcrossing population, while F_{IS} > 0.9 is characteristic of an inbreeding population. Intermediate values for F_{IS} suggest that both outcrossing and inbreeding occur in a population (Goodwin 1997). In fields WNY, CD and LI, fixation indices (F_{IS}) calculated from an average of all loci were 0.656 (*P*<0.000), 0.308 (*P*=0.002) and 0.315 (*P*=0.001), respectively. All presented data was calculated without clone correction. Results calculated from clone-corrected data were similar, except in some cases for field LI, where the population size was drastically reduced by clone correction (from N=44 to N=6), rendering these differences suspect.

Table 3.7 Pairwise comparisons between populations of *Phytophthora capsici* collected in three different fields in New York. Differentiation is estimated by F_{ST} values calculated based on observed alleles at five microsatellite loci.

Pairs of Fields	F_{ST} values[a]
WNY v. LI	0.586
WNY v. CD	0.428
CD v. LI	0.224

[a] F_{ST} values calculated with 1000 permutations; p<0.00000.

Discussion

A successful integrated management strategy for Phytophthora blight in New York requires both knowledge of the effectiveness of various management strategies, and also a better understanding of the population of *P. capsici* affecting New York's growers. The prevalence of mefenoxam-resistant isolates in Long Island and the Capital District means that mefenoxam is no longer an effective management tool for *P. capsici* in these regions. This is a significant finding, because mefenoxam had

previously been an important component of Phytophthora blight management on some crops in the Capital District. The fact that no resistant isolates were recovered from central or western New York indicates that mefenoxam resistance, if present, is not as widespread in these regions as it is in the Capital District and Long Island. Hausbeck and Lamour (2004) previously reported the recovery of mefenoxam-resistant isolates of *P. capsici* from a single field in upstate New York, but not from a field on Long Island.

Based on observations in Michigan (Lamour and Hausbeck 2002), asexual propagules of *P. capsici* should not be able to survive the winter in New York, and our observations support this hypothesis (*unpublished*, Camp, Dillard, Smart). On many farms in New York, Phytophthora blight is a recurring problem from year to year (even after rotation to non-susceptible hosts), thus oospores are a likely source of inoculum. These oospores could not be produced without sexual reproduction and the presence of both mating types in close proximity (Ristaino and Johnston 1999). For this reason, the presence of both mating types in the same field has been interpreted as evidence for sexual reproduction in heterothallic oomycetes (Lamour and Hausbeck 2000; Widmark et al. 2007), although it does not prove that sexual reproduction is happening (Fry et al. 1992). Therefore, the frequent recovery of both A1 and A2 isolates of *P. capsici* from the same field in New York supports the hypothesis that sexual reproduction is occurring in these populations. In fields LI and CD, A1 and A2 isolates were recovered in a 1:1 ratio, providing additional evidence for both sexual reproduction and random mating in these fields (Fry et al. 1992; Liu et al. 1996; Milgroom 1996; Lamour and Hausbeck 2000; Lamour and Hausbeck 2003). The failure to recover both mating types from some fields in this study may be an artifact of small sample size, and does not rule out the possibility of sexual reproduction in those fields.

The history of field WNY is consistent with the hypothesis of sexual reproduction resulting in oospores as the primary inoculum at the beginning of each growing season in New York. In 2004, the grower reported problems with Phytophthora blight on eggplant planted in field WNY. A susceptible host crop was not planted in the field again until 2007, when symptomatic summer squash were collected for this study in late June. Assuming that asexual propagules did not survive over three winters, the 2007 epidemic in this field was probably started by germinating oospores. A study of the mapped recovery of *P. infestans* isolates from a single field in Sweden almost always recovered a particular genotype from only a single sampling site in the field, and this was interpreted as evidence for oospores as the primary inoculum in the epidemic (Widmark et al. 2007). If oospores were the primary inoculum in field WNY, and since *P. capsici* is not wind-dispersed, there are several potential explanations for the recovery of the same genotype from multiple sampling sites in field WNY. Movement of *P. capsici* inoculum in water across long distances within rows is well-documented (Ristaino et al. 1994; Café-Filho and Duniway 1995), while movement in water across rows may be somewhat less common (Café-Filho and Duniway 1995), depending on field-specific drainage patterns and other water movement in the field. Field WNY received 2.18 cm and 3.68 cm of rain nine and 11 days, respectively, prior to the sampling date, and the grower had also irrigated periodically prior to sampling. These water events could account for movement of asexual propagules within the field. In addition, harvesting had already begun in this field, and workers had been moving throughout the field as they "spot-rogued" infected plants. These activities could have spread inoculum in soil on shoes, or as spores clinging to clothing. Furthermore, movement of animals through the field (eg, deer) could have spread asexual propagules throughout the field. It is also noteworthy that only two of the five microsatellite loci used in this study were polymorphic in the

field WNY population, thereby decreasing our ability to differentiate clones. Interestingly, the genotypes which were recovered from the greatest number of sampling sites in this field (WNY-1/WNY-2 and WNY-5) were also the most likely to contain multiple clonal lineages, based on calculated P_{sex} values, and these values did not drop below 0.05 until the seventh and fifth re-encounter of the genotype, respectively.

The number of unique genotypes recovered in field CD and the fact that this population was in Hardy-Weinberg equilibrium even late in the season is consistent with high levels of sexual reproduction in this field (Burdon and Roelfs 1985; Tooley et al. 1985; Goodwin et al. 1992; Sujkowski et al. 1994; Ivors et al. 2006). However, relatively low proportions of unique genotypes were recovered in fields WNY and LI, and loci were generally not in Hardy-Weinberg equilibrium, suggesting clonality and rare outcrossing (Balloux et al. 2003; Ivors et al. 2006). The moderate to high differentiation between A1 and A2 isolates collected in all three fields is also more consistent with a non-randomly mating population where asexual reproduction is very important, and sexual reproduction is less important (Fry et al. 1991; Fry et al. 1992; Sujkowski et al. 1994; Milgroom 1996). Although clonal populations of *P. capsici* have been reported in Brazil (Oudemans and Coffey 1991; Mchau and Coffey 1995) and coastal Peru (Hurtado-Gonzáles et al. 2008), a strictly clonal population of *P. capsici* could not survive the winter in New York. Therefore, the occurrence of what appears to be rare outcrossing in fields WNY and LI probably has another explanation.

These observations may be explained, first, by the importance of asexual reproduction in the life cycle of *P. capsici*. After sexual oospores germinate to initiate an epidemic, secondary inoculum is produced asexually, allowing for the rapid spread of *P. capsici* throughout a field during an epidemic (Zentmyer and Erwin 1970; Ristaino 1991). Subsequent selection for a relatively small number of genotypes which

dominate a field population later in the epidemic has been seen in Michigan, giving the false impression of a less-diverse resident population when numerous isolates are collected with identical genotypes (Lamour and Hausbeck 2002). Clone correction (or including only one isolate of each genotype in the analysis) can remove the effects of clonal reproduction from consideration, but this decreases the population size, making it more difficult to reject the null hypothesis of random mating (Lenski 1993; Maynard Smith et al. 1993; Milgroom 1996). In some studies the choice to clone correct or not to clone correct data can greatly influence the results (Lenski 1993), but this was not the case in our study.

Because fields CD and LI were sampled relatively late in the growing season (September and November, respectively), selection and asexual propagation could have affected the populations in these fields. However, meaningful asexual reproduction and selection could also have occurred prior to the sampling of field WNY in late June. Ironically, more unique genotypes were recovered from field CD than from field WNY, even though the former was sampled later in the season. Perhaps the diversity and structure of the *P. capsici* population in field CD (or in the Capital District) differs from *P. capsici* populations in other parts of New York. Similar findings were reported in a study of the Mexican population of *P. infestans*, where isolates collected in the northwestern part of the country represented only a few genotypes, while almost all isolates collected in central Mexico had unique genotypes (Goodwin et al. 1992). However, it should be noted that field CD is located along the Mohawk River and floods nearly every year. The high level of diversity observed in this field could be a result of inoculum from upstream farms being deposited in the field during these flooding events, resulting in inflated estimates of genetic diversity.

Apparent deviations from a population structure consistent with frequent sexual reproduction could also be related to sampling strategy. In this study, all

samples were collected from infected plants, rather than from soil. Studies in Michigan have also sampled infected plants (Lamour and Hausbeck 2000; Lamour and Hausbeck 2001a; Lamour and Hausbeck 2001b; Lamour and Hausbeck 2002; Lamour and Hausbeck 2003), but surveys of *P. sojae* and *P. capsici* in Ohio either performed enzyme-linked immunosorbent assays (ELISA) directly on sampled soil, or baited the pathogen from the soil (Schmitthenner, A. F. et al. 1994; Miller, S. A., Madden, L. V., and Schmitthenner, A. F. 1997; Dorrance et al. 2003). Sampling soil instead of infected plants might be expected to result in recovery of a larger number of unique genotypes, since recovered isolates are most likely oospore offspring, and do not have to successfully infect the host plant in the field, a pre-condition which could exert pre-sampling selective pressure. In Michigan, highly diverse populations were consistently recovered, in spite of sampling only symptomatic plants, suggesting that this sampling method does not always lead to a severe underestimation of diversity (Lamour and Hausbeck 2000; Lamour and Hausbeck 2001a; Lamour and Hausbeck 2001b; Lamour and Hausbeck 2002; Lamour and Hausbeck 2003).

It is difficult to compare the levels of diversity observed in Michigan's population of *P. capsici* with those observed in this study, because Michigan studies have used a different method (AFLPs) to characterize populations of *P. capsici*. If anything, this study may have underestimated the genetic diversity in NY, since our technique allows two isolates to be definitively distinguished as different genotypes, while isolates with the same six-locus genotype are not necessarily genetically identical. The P_{sex} values calculated in each field support this conclusion, because for most multi-isolate genotypes they exceed 0.05 (Arnaud-Haond et al. 2005).

Nevertheless, the markers used in this study were sufficient to clearly characterize fields WNY, CD and LI as highly differentiated, based on the high F_{ST} values for pairwise comparisons between fields, as well as the high proportion of

variation accounted for by differences among fields (population-wide F_{ST}). This supports the hypothesis that populations of *P. capsici* in New York are regionally distinct and that gene flow between populations is minimal, as do the facts that (i) nearly half of observed alleles at the five microsatellite loci were found only in a single field, and (ii) the same six-locus genotype was only recovered from two different fields on one occasion. The departures from expected levels of heterozygosity under random mating (as indicated by higher F_{IS} values and deviation from Hardy-Weinberg equilibrium) in each field are also consistent with low gene flow between fields. Brown (1979) predicted F_{IS} values below 0.3 for outcrossing populations, and outcrossing (as opposed to self-fertilization) is expected to be common in *P. capsici*, both because it is heterothallic (Goodwin 1997), and also based on previous work (Lamour and Hausbeck 2001b). However, a population into which little or no new genetic material were moving would increasingly be characterized by matings between relatives, resulting in higher levels of inbreeding, even if self-fertilization were not occurring (Goodwin 1997). This is especially likely if subpopulations were founded by small numbers of individuals. If gene flow is restricted among fields in New York, then it is likely that a small number of genotypes founded each population of *P. capsici* and that distinct populations have been substantially influenced by the characteristics of the founding individuals. Obligate sexual reproduction could create new combinations of alleles present in the founding population (and alleles which arose through mutation), but different populations would grow less similar, over time. This is consistent with observations in Michigan of differentiation between populations of *P. capsici* collected from different fields, but a lack of differentiation between populations collected in the same field during different years (Lamour and Hausbeck 2001a; Lamour and Hausbeck 2001b; Lamour and Hausbeck 2003). The population in a field remains very similar over multiple years,

since gene flow among fields is very limited.

Very low levels of gene flow among fields separated by large distances are consistent with known dispersal mechanisms for *P. capsici*, which include movement of water, soil, and plant material, but not aerial dispersal (Ristaino et al. 1994; Café-Filho and Duniway 1995; Lamour and Hausbeck 2002; Hausbeck and Lamour 2004; Roberts et al. 2005). Human activity is the most likely explanation for long-distance spread of *P. capsici* via infected plants and infested soil, and long distance spread via water is probably limited to contiguous watersheds. The recovery of genotype WNY-1 from both western New York and the Capital District may be an example of the relatively rare movement of a genotype between distant fields, or the isolate recovered in field CD may not actually be a clone of those recovered in field WNY with the same six-locus genotype. The P_{sex} values calculated for both fields and discussed above support the latter hypothesis. Regardless, these results are consistent with the hypothesis that the movement of *P. capsici* inoculum among distant fields is not inevitable, and that modifications in human behavior could have an important impact on the spread of this destructive pathogen in New York. Therefore, culled fruit and potentially-infested soil should never be transported between vegetable fields. Steps should also be taken to prevent the contamination of irrigation sources, and to use only uninfested water to irrigate susceptible crops. If mefenoxam resistance is not yet present in central and western New York, then preventing the introduction of *P. capsici* inoculum from other parts of the state could be especially important as a means of prolonging the efficacy of this fungicide in central and western New York. Furthermore, we are not aware of any reports of Phytophthora blight in the Southern Tier (south central region) of New York, and preventing the introduction of this devastating pathogen onto farms in this region is certainly desirable.

Characterization of additional *P. capsici* isolates from around New York could

provide additional evidence for these hypotheses and improved management recommendations. For example, it is not known how geographically separated two New York fields must be, in order for their resident *P. capsici* populations to be different. In Michigan, even fields in close proximity to each other contained genetically isolated populations of *P. capsici* (Lamour and Hausbeck 2001b; Lamour and Hausbeck 2002). Watersheds and drainage patterns may also play an important role in determining which populations of *P. capsici* are distinct in New York. This study does not address temporal substructuring of *P. capsici* populations in the state, and knowledge of the similarity between populations from the same field in different years could provide additional evidence for the hypothesis that oospores are the source of primary inoculum for *P. capsici* epidemics in New York.

This study provides the first evidence for geographic sub-structuring of the *P. capsici* population in New York, supporting the hypothesis that long-distance spread of *P. capsici* between growing regions in the state is rare, and possibly preventable. These data confirmed somewhat less diversity and recombination in the New York *P. capsici* population than had been anticipated, although sample size and timing, the importance of asexual reproduction in the life cycle of *P. capsici*, and the markers used to characterize the population may have impacted these observations. The frequent recovery of both mating types from the same field provides additional evidence that sexual reproduction is occurring in New York. Also, the failure to recover mefenoxam-resistant isolates from western and central New York suggests that this fungicide may still be effective in some regions and fields in the state, at least for the time being. Mefenoxam is no longer an effective management tool for *P. capsici* in the Capital District region, or on Long Island, where mefenoxam-resistance is already widespread. The development of additional polymorphic microsatellite markers could help to distinguish additional multilocus genotypes in New York's *P.*

capsici population. However, based on the high levels of differentiation observed among the three fields studied here, these five markers may also be useful in future investigations of population structure in additional New York fields.

REFERENCES

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrão, E. A. and Duarte, C. M. 2005. Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting markers. Journal of Heredity 96(4): 434-440.

Arnaud-Haond, S. and Belkhir, K. 2007. GenClone: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. Molecular Ecology Notes 7: 15-17.

Babadoost, M. 2007. An IPM strategy for managing Phytophthora blight (*Phytophthora capsici*) in cucurbit fields. HortScience 42(4): 872-872.

Balloux, F., Lehmann, L. and de Meeus, T. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164(4): 1635-1644.

Balloux, F. and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11(2): 155-165.

Bernhardt, E. A. and Grogan, R. G. 1982. Effect of soil matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica*, *Phytophthora capsici*, and *Phytophthora cryptogea*. Phytopathology 72(5): 507-511.

Biles, C. L., Lindsey, D. L. and Liddell, C. M. 1992. Control of Phytophthora root-rot of Chile peppers by irrigation practices and fungicides. Crop Protection 11(3): 225-228.

Blixt, E., Olson, A., Hogberg, N., Djurle, A. and Yuen, J. 2008. Mating type distribution and genetic structure are consistent with sexual recombination in the Swedish population of *Phaeosphaeria nodorum*. Plant Pathology 57(4): 634-641.

Bowers, J. H., Martin, F. N., Tooley, P. W. and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology 97(4): 492-503.

Bowers, J. H., Papavizas, G. C. and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Disease 74(10): 771-777.

Brown, A. H. D. 1979. Enzyme polymorphism in plant populations. Theoretical Population Biology 15(1): 1-42.

Burdon, J. J. and Roelfs, A. P. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. Phytopathology 75(9): 1068-1073.

Café-Filho, A. C., Duniway, J. M. and Davis, R. M. 1995. Effects of the frequency of furrow irrigation on root and fruit rots of squash caused by *Phytophthora capsici*. Plant Disease 79(1): 44-48.

Café-Filho, A. C. and Ristaino, J. B. 2008. Fitness of isolates of *Phytophthora capsici* resistant to mefenoxam from squash and pepper fields in North Carolina. Plant Disease 92(10): 1439-1443.

Café-Filho, A. C. and Duniway, J. M. 1995. Dispersal of *Phytophthora capsici* and *P. parasitica* in furrow-irrigated rows of bell pepper, tomato and squash. Plant Pathology 44(6): 1025-1032.

Café-Filho, A. C. and Duniway, J. M. 1996. Effect of location of drip irrigation emitters and position of *Phytophthora capsici* infections in roots on Phytophthora root rot of pepper. Phytopathology 86(12): 1364-1369.

Davey, J. F., Gregory, N. F., Mulrooney, R. P., Evans, T. A. and Carroll, R. B. 2008. First report of mefenoxam-resistant isolates of *Phytophthora capsici* from lima bean pods in the Mid-Atlantic region. Plant Disease 92(4): 656.

Davidson, C. R., Carroll, R. B., Evan, T. A. and Mulrooney, R. P. 2002. First report of *Phytophthora capsici* infecting lima bean (*Phaseolus lunatis*) in the Mid-Atlantic region. Plant Disease 86: 1049.

Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L. and O'Brien, P. A. 2003. Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. Phytopathology 93(6): 695-704.

Dorrance, A. E., McClure, S. A. and deSilva, A. 2003. Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. Plant Disease 87(2): 139-146.

Duniway, J. M. 1979. Water relations of water molds. Annual Review of Phytopathology 17: 431-460.

Erwin, D. C. and Ribeiro, O. K. 1996. Phytophthora disease worldwide. St. Paul, Minnesota, The American Phytopathological Society.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47-50.

Forster, H., Oudemans, P. and Coffey, M. D. 1990. Mitochondrial and nuclear-DNA diversity within 6 species of *Phytophthora*. Experimental Mycology 14(1): 18-31.

French-Monar, R. D., Jones, J. B., Ozores-Hampton, M. and Roberts, P. D. 2007. Survival of inoculum of *Phytophthora capsici* in soil through time under different soil treatments. Plant Disease 91(5): 593-598.

French-Monar, R. D., Jones, J. B. and Roberts, P. D. 2006. Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. Plant Disease 90(3): 345-350.

Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C. and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81(10): 1330-1336.

Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G. and Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. Annual Review of Phytopathology 30: 107-129.

Gevens, A. J., Donahoo, R. S., Lamour, K. H. and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. Phytopathology 97(4): 421-428.

Gevens, A. J., Donahoo, R. S., Lamour, K. H. and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. Plant Disease 92: 201-209.

Goodwin, S. B. 1997. The population genetics of Phytophthora. Phytopathology 87(4): 462-473.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N. and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82(9): 955-961.

Goodwin, S. B., Sujkowski, L. S. and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. Phytopathology 86(7): 793-800.

Hartl, D. L. and Clark, A. G. 1997. Principles of population genetics. Sunderland, MA, Sinauer Associates, Inc.

Hausbeck, M. K. and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Disease 88(12): 1292-1303.

Hurtado-Gonzáles, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R. and Lamour, K. 2008. Survival and spread of *Phytophthora capsici* in coastal Peru. Phytopathology 98(6): 688-694.

Islam, S. Z., Babadoost, M., Lambert, K. N., Ndeme, A. and Fouly, H. M. 2005. Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. Plant Disease 89(2): 191-197.

Ivors, K., Garbelotto, M., Vries, I. D. E., Ruyter-Spira, C., Hekkert, B. T., Rosenzweig, N. and Bonants, P. 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. Molecular Ecology 15(6): 1493-1505.

Johnston, S. A., Kline, W. L., Fogg, M. L. and Zimmerman, M. D. 2002. Varietal resistance evaluation for control of Phytophthora blight of pepper. Phytopathology 92: S40.

Kubisiak, T. L., Dutech, C. and Milgroom, M. G. 2007. Fifty-three polymorphic microsatellite loci in the chestnut blight fungus, *Cryphonectria parasitica*. Molecular Ecology Notes 7(3): 428-432.

Lamour, K. H. and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. Phytopathology 90(4): 396-400.

Lamour, K. H. and Hausbeck, M. K. 2001a. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. Phytopathology 91(6): 553-557.

Lamour, K. H. and Hausbeck, M. K. 2001b. Investigating the spatiotemporal genetic structure of *Phytophthora capsici* in Michigan. Phytopathology 91(10): 973-980.

Lamour, K. H. and Hausbeck, M. K. 2002. The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. Phytopathology 92(6): 681-684.

Lamour, K. H. and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. Plant Disease 87(7): 841-845.

Lees, A. K., Wattier, R., Shaw, D. S., Sullivan, L., Williams, N. A. and Cooke, D. E. L. 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. Plant Pathology 55(3): 311-319.

Lenski, R. E. 1993. Assessing the genetic structure of microbial populations. Proceedings of the National Academy of Sciences of the United States of America 90(10): 4334-4336.

Leonian, L. H. 1922. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopathology 12: 401-408.

Liu, Y. C., Cortesi, P., Double, M. L., MacDonald, W. L. and Milgroom, M. G. 1996. Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*. Phytopathology 86(12): 1344-1351.

Matheron, M. E. and Porchas, M. 2000. Comparison of five fungicides on development of root, crown, and fruit rot of Chile pepper and recovery of *Phytophthora capsici* from soil. Plant Disease 84(9): 1038-1043.

Maynard Smith, J., Smith, N. H., Orourke, M. and Spratt, B. G. 1993. How clonal are bacteria. Proceedings of the National Academy of Sciences of the United States of America 90(10): 4384-4388.

McDermott, J. M. and McDonald, B. A. 1993. Gene flow in plant pathosystems. Annual Review of Phytopathology 31: 353-373.

Mchau, G. R. A. and Coffey, M. D. 1995. Evidence for the existence of two subpopulations in *Phytophthora capsici* and a redescription of the species. Mycological Research 99: 89-102.

Milgroom, M. G. 1995. Analysis of population structure in fungal plant pathogens. Disease analysis through genetics and biotechnology. Leslie, J. F. and Frederiksen, R. A., editors. Ames, IA, Iowa State University Press**:** 213-229.

Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Annual Review of Phytopathology 34: 457-477.

Miller, S. A., Lewis Ivey, M. L. and Mera, J. 2002. Response of pepper cultivars and experimental breeding lines to Phytophthora blight, 2001. Biological and cultural tests for control of plant disease (Online). Report 17:V16. DOI: 1094/BC17. American Phytopathological Society, St. Paul, MN.

Miller, S. A., Madden, L. V., and Schmitthenner, A. F. 1997. Distribution of *Phytophthora* spp. In field soils determined by immunoassay. Phytopathology 87(1): 101-107.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Oudemans, P. and Coffey, M. D. 1991. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. Mycological Research 95: 1025-1046.

Pennisi, A. M., Agosteo, G. E., Cacciola, S. O., Pane, A. and Faedda, R. 1998. Insensitivity to metalaxyl among isolates of *Phytophthora capsici* causing root and crown rot of pepper in southern Italy. Plant Disease 82(11): 1283-1283.

Ploetz, R., Heine, G., Haynes, J. and Watson, M. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. Annals of Applied Biology 140(1): 61-67.

Prospero, S., Hansen, E. M., Grunwald, N. J. and Winton, L. M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. Molecular Ecology 16(14): 2958-2973.

Queller, D. C., Strassmann, J. E. and Hughes, C. R. 1993. Microsatellites and kinship. Trends in Ecology & Evolution 8(8): 285-288.

Quesada-Ocampo, L. M., Fulbright, D. W. and Hausbeck, M. K. 2009. Susceptibility of Fraser fir to *Phytophthora capsici*. Plant Disease 93(2): 135-141.

Ristaino, J. B. 1991. Influence of rainfall, drip irrigation, and inoculum density on the development of Phytophthora root and crown rot epidemics and yield in bell pepper. Phytopathology 81(8): 922-929.

Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. Plant Disease 83(12): 1080-1089.

Ristaino, J. B., Larkin, R. P. and Campbell, C. L. 1994. Spatial dynamics of disease symptom expression during Phytophthora epidemics in bell pepper. Phytopathology 84(10): 1015-1024.

Ristaino, J. B., Parra, G. and Campbell, C. L. 1997. Suppression of Phytophthora blight in bell pepper by a no-till wheat cover crop. Phytopathology 87(3): 242-249.

Roberts, P. D., Urs, R. R., French-Monar, R. D., Hoffine, M. S., Seijo, T. E. and McGovern, R. J. 2005. Survival and recovery of *Phytophthora capsici* and oomycetes in tailwater and soil from vegetable fields in Florida. Annals of Applied Biology 146(3): 351.

Schmitthenner, A. F. and Bhat, R. G. 1994. Useful methods for studying *Phytophthora* in the laboratory. OARDC Special Circular. Wooster, OH, Department of Plant Pathology, Ohio Agricultural Research and Development Center Ohio State University.

Schmitthenner, A. F., Hobe, M. and Bhat, R. G. 1994. *Phytophthora sojae* races in Ohio over a 10-year interval. Plant Disease 78(3): 269-276.

Sheskin, D. J. 1996. Handbook of parametric and nonparametric statistical procedures. Boca Raton, CRC Press.

Silvar, C., Merino, F. and Díaz, J. 2006. Diversity of *Phytophthora capsici* in northwest Spain: Analysis of virulence, metalaxyl response, and molecular characterization. Plant Disease 90(9): 1135-1142.

Springer, J. K. and Johnston, S. A. 1982. Black polyethylene mulch and Phytophthora blight of pepper. Plant Disease 66(4): 281-281.

Stieg, J. R., Walters, S. A., Bond, J. P. and Babadoost, M. 2006. Performance of bell pepper cultivars in a commercial field infested with *Phytophthora capsici*. HortScience 41(4): 981-981.

Sujkowski, L. S., Goodwin, S. B., Dyer, A. T. and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84(2): 201-207.

Tautz, D. and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12(10): 4127-4138.

Tooley, P. W., Fry, W. E. and Gonzalez, M. J. V. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. Journal of Heredity 76(6): 431-435.

Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6): 1358-1370.

Widmark, A. K., Andersson, B., Cassel-Lundhagen, A., Sandstrom, M. and Yuen, J. E. 2007. *Phytophthora infestans* in a single field in southwest Sweden early in spring: Symptoms, spatial distribution and genotypic variation. Plant Pathology 56(4): 573-579.

Wright, S. 1978. Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. Chicago, University of Chicago Press.

Xie, J. H., Cardenas, E. S., Sammis, T. W., Wall, M. M., Lindsey, D. L. and Murray, L. W. 1999. Effects of irrigation method on Chile pepper yield and Phytophthora root rot incidence. Agricultural Water Management 42(2): 127-142.

Zentmyer, G. A. and Erwin, D. C. 1970. Development and reproduction of *Phytophthora*. Phytopathology 60(7): 1120-1127.

Zhang, N., McCarthy, M. L. and Smart, C. D. 2008. A macroarray system for the detection of fungal and oomycete pathogens of solanaceous crops. Plant Disease 92(6): 953-960.

APPENDIX ONE

[*]EFFICACY OF *MUSCODOR ALBUS* AND THE FUNGICIDE EF400 FOR CONTROL OF PHYTOPHTHORA BLIGHT ON WINTER SQUASH, 2007

On 29 Jun, a field at the Agricultural Experiment Station in Geneva, NY with no history of Phytophthora blight was infested with a mefenoxam-sensitive isolate of *P. capsici* at a rate of 1L of colonized substrate (vermiculite and 20% V8 broth in a 2:1 ratio) per 20 feet of row. One week later, ten 3.5-week-old butternut squash seedlings per treatment plot were transplanted into bare ground on a one foot spacing between plants. The six treatments included: the biofumigant fungus *Muscodor albus* (AgraQuest, Inc.) formulated on rye grain applied at a rate of 0.55, 1.9, or 3.75 g/L soil, the fungicide EF400 (USAgriTech, Inc.), Ridomil Gold EC (Syngenta Crop Protection, Inc.), or with no additional treatment (pathogen only). Both *P. capsici* and *M. albus* were incorporated into the soil on the same date to a depth of approximately four inches, using a rototiller. EF400 was applied as a foliar spray (23 fl oz/A) eleven days after transplanting, then applied weekly for the following 3 weeks for a total of four sprays using a CO_2 pressurized backpack sprayer at 40psi delivering 40 gal/A through two flat fan nozzles 19 in. apart. Ridomil Gold EC was applied at transplanting as a soil drench (1 pt/A) directed at the crown of the plant, using a CO_2 pressurized backpack sprayer at 40psi delivering 40 gal/A. Each treatment was replicated four times in a randomized complete block design. Starting two weeks after transplanting, each plant was rated weekly on a scale of 0 (healthy) to 5 (dead). All fruit were harvested 27 Sep.

Because of a dry growing season (total monthly rainfall (in.) of 2.62, 1.50 and 2.96 for Jul, Aug, and Sep, respectively), plots were irrigated approximately weekly using drip tape in order to produce relatively high disease pressure. None of the treatments resulted in yields or incidence that were significantly different from the pathogen-only control, although yields were highest and incidence was lowest for plots treated with Ridomil Gold EC. On 20 Jun, there were no significant differences in incidence of *P. capsici* between the six treatments. On 6 Aug, *M. albus* applied at 0.55 and 1.9 g/L soil and EF400 resulted in significantly higher disease incidence compared to Ridomil Gold EC. On 27 Aug, there were no significant differences in disease incidence, with the exception of *M. albus* applied at 1.9g/L soil, which was significantly higher than Ridomil Gold EC. No phytotoxicity was observed (Table A1.1).

[*] Camp, A.R., Lange, H.W., Dillard, H.R., and Smart, C.D. 2008. Efficacy of *Muscodor albus* and the fungicide EF400 for control of Phytophthora blight on winter squash, 2007. Plant Disease Management Reports (online). Report 2:V132. DOI: 10.1094/PDMR02. The American Phytopathological Society, St. Paul, MN.

Table A1.1 Yield and incidence of Phytophthora blight on butternut squash grown in soil infested with *Phytophthora capsici* and treated with *Muscodor albus* at three rates (3.75 g/L, 1.9 g/L, or 0.55 g/L), EF400, Ridomil Gold EC, or no curative treatment.

Treatment	Marketable Yield		Total Yield		Incidence[a]		
	Fruit wt (lb)[b]	Fruit no.[c]	Fruit wt (lb)[b]	Fruit no[c]	20 Jun	6 Aug	27 Aug
M. albus 3.75g/L	11.40ab[d]	3.75b	12.17ab	4.50b	1.50a	3.50abc	5.00ab
M. albus 1.9g/L	9.66ab	4.00ab	11.52ab	6.25ab	2.50a	5.50c	6.50b
M. albus 0.55g/L	8.00b	3.75b	11.71b	6.50b	3.25a	4.50c	4.75ab
EF400	17.60ab	6.50ab	22.14ab	8.75ab	1.75a	4.25c	4.75ab
Pathogen only	11.90ab	5.50ab	14.57ab	8.25ab	1.25a	3.25abc	3.75ab
Ridomil	24.10a	10.00a	25.68a	11.75a	0.50a	0.50ab	1.25a

[a] Mean number of plants (out of 10) rated 1 to 5 (on a scale of 0 to 5).
[b] Mean weight of fruit harvested per plot.
[c] Mean number of fruit harvested per plot.
[d] Column numbers followed by the same letter are not significantly different at P=0.05 determined by Kruskal-Wallis one-way analysis of variance and Bonferroni-Dunn test.

APPENDIX TWO

[*]TOLERANCE OF HOT AND SWEET PEPPER LINES TO PHYTOPHTHORA BLIGHT, 2008

Peppers were seeded in the greenhouse on 14 Apr and transplanted on 11 Jun into a field at the Agricultural Experiment Station in Geneva, NY on raised beds under black plastic at 13-in. spacing in a randomized complete block design. Red Knight, Revolution, Declaration, Paladin, and Aristotle were sweet peppers, while all other varieties were hot peppers. Each experimental unit consisted of one plot of five plants, and there were three replications. Prior to laying the plastic, 400 lbs/A fertilizer (10-10-10) was broadcast on the field and plants were fertilized at transplant with 40 lbs/A starter fertilizer (21-5-20). Plants were supplied with approximately 1 in. of water per week, as necessary, using drip tape under the plastic. Each plant was inoculated on three dates with a water suspension of zoospores from a New York isolate of *Phytophthora capsici*, applied with a hand-pump sprayer: on 6 Aug, 5,000 zoospores at the crown of each plant; on 8 Sep, 500,000 zoospores at the crown of each plant; and on 26 Sep, 500,000 zoospores at the crown, and 500,000 zoospores on the canopy. The number of wilted and dead plants was counted two to three times per week.

The growing season was wet, with 3.45, 4.99, 4.16, and 2.24 in. of rain in Jun, Jul, Aug, and Sep, respectively. Because of a wet spot in the field and heavy rains in mid to late Jul, some peppers drowned before inoculation. Only plants which were healthy on the first inoculation date were rated. Most symptoms developed within the first few weeks after the 6 Aug inoculation, with some additional symptom development after the second and third inoculations. Later infections may have been limited by the woody crown tissue of the older plants (hence, the canopy inoculation on 26 Sep). Although tolerance has been reported for the commercial varieties Revolution and Declaration, disease severity was high on these varieties, possibly because of excessive moisture. By the end of the experiment, all HMX7675, Red Knight, Revolution, and Declaration plants were either wilted or dead. The Harris Moran pepper lines HPX4488, HPX4571, HPX4555, CM334, PI201234, and HPX4465 had the lowest mean AUDPC's. The results indicate that some lines have the potential to be very tolerant to infection by *P. capsici*.

[*] Camp, A.R., Lange, H.W., Reiners, S., Dillard, H.R., and Smart, C.D. 2008. Tolerance of hot and sweet pepper lines to Phytophthora blight, 2008. Plant Disease Management Reports (online). Report 3:V018. DOI: 10.10994/PDMR03. The American Phytopathological Society, St. Paul, MN.

Table A2.1 Incidence of Phytophthora blight on sweet and hot pepper varieties reported as area under the disease progress curve (AUDPC).

Variety	Mean AUDPC[a]	Variety	Mean AUDPC[a]
HMX 7675	300.00 a[b]	HPX 4454	158.33 cdefghijk
Red Knight	300.00 a	HPX 4491	140.00 defghijkl
Revolution	290.00 ab	HPX 4447	133.33 defghijkl
Declaration	280.00 abc	HPX 4550	110.00 efghijklm
HPX 4565	280.00 abc	HPX 4540	106.67 efghijklm
HPX 4476	275.00 abc	HPX 4541	100.00 efghijklm
HPX 4452	270.00 abc	HPX 4458	100.00 efghijklm
HPX 4569	270.00 abc	HPX 4539	100.00 efghijklm
HPX 4419	262.50 abcd	HPX 4405	100.00 efghijklm
HPX 4400	255.00 abcd	HPX 4466	100.00 efghijklm
HPX 4403	250.00 abcd	HPX 4475	97.50 efghijklm
HPX 4453	245.00 abcd	HPX 4404	90.00 fghijklm
Autlan	240.00 abcd	HPX 4480	90.00 fghijklm
HPX 4473	240.00 abcd	HPX 4570	90.00 fghijklm
HPX 4412	225.00 abcde	HPX 4548	80.00 ghijklm
HPX 4446	220.00 abcde	Paladin	60.00 hijklm
HMX 7652	217.50 abcdef	HPX 4494	60.00 hijklm
HPX 4479	210.00 abcdef	HMX 7653	60.00 hijklm
HMX 6667	210.00 abcdef	HPX 4420	60.00 hijklm
HPX 4566	210.00 abcdef	HPX 4495	50.00 ijklm
HPX 4554	200.00 abcdefg	Aristotle	40.00 jklm
HPX 4414	195.00 abcdefg	HPX 4535	30.00 klm
HPX 4563	195.00 abcdefg	HMX 5658	20.00 lm
HPX 4461	180.00 abcdefgh	HPX 4556	20.00 lm
HPX 4442	180.00 abcdefgh	HPX 4488	0.00 m
HPX 4536	176.67 abcdefghi	HPX 4571	0.00 m
HPX 4415	173.33 abcdefghi	HPX 4555	0.00 m
HPX 4551	165.00 bcdefghij	CM334	0.00 m
HPX 4397	163.33 bcdefghij	PI201234	0.00 m
HPX 4418	160.00 cdefghij	HPX 4465	0.00 m
HPX 4481	160.00 cdefghij		

[a]The area under the disease progress curve (AUDPC) was calculated for each variety based on the proportion of plants in a plot with symptoms (wilted or dead). Plots containing fewer than 3 healthy plants at inoculation were not included. Means shown are the average among 2-3 replications, depending on how many plants of each variety were healthy at inoculation.
[b]Means followed by the same letter are not significantly different from each other based on an ANOVA of the AUDPC using a General Linear Model ($p<0.0001$), and separated by a Fisher's protected LSD test.

APPENDIX THREE

[*]TOLERANCE OF SUMMER AND WINTER SQUASH LINES TO PHYTOPHTHORA BLIGHT, 2008

Summer squash were direct-seeded on 11 Jun into a field at the Agricultural Experiment Station in Geneva, NY under black plastic with 26-in. spacing, and winter squash were direct-seeded on 19 Jun on bare soil with 24-in. spacing. Both trials were arranged in randomized complete block designs with three replications, and each experimental unit consisted of one plot of five or six plants. Fertilizer (400 lbs/A 10-10-10) was broadcast before seeding, and 40 lbs/A starter fertilizer (21-5-20) was applied to summer squash immediately prior to seeding. All plants were supplied with approximately 1 in. of water per week, as necessary, using drip tape. Because of germination problems and predation of young seedlings by birds, additional seed was germinated in the greenhouse in jiffy pots, and transplanted into the field, up to a month after the initial seeding date. Plants were inoculated on two dates (6 Aug and 3 Sep) with two rates of a water suspension of zoospores (for summer squash, 5,000 and 500,000 zoospores, respectively, and for winter squash, 5,000 and 54,000 zoospores, respectively) from a New York isolate of *Phytophthora capsici*, applied with a hand-pump sprayer at the crown of each plant. On 8 Sep, 5.5 mL/ft^2 of a mixture of V8 juice and vermiculite (with the same NY isolate of *P. capsici* growing in it) was evenly distributed on the soil surface throughout the winter squash field, especially around each developing fruit. Two to three times per week, the number of wilted and dead summer squash plants or the number of infected winter squash fruit was counted.

The growing season was wet, with 3.45, 4.99, 4.16, and 2.24 in. of rainfall in Jun, Jul, Aug, and Sep, respectively. Due to insect damage and germination failure, not all plants survived to inoculation. Symptom development in the summer squash was uneven, possibly caused by failure to accurately place the zoospores at the crown of the plants due to the size of the plants and density of the foliage at inoculation. There were few foliar symptoms (wilting) on the winter squash after the zoospore inoculations, but fruit infection was severe after inoculation with *P. capsici* in V8-vermiculite. Disease tended to be more severe on the yellow summer squash (eg, Sunray, Cougar, Superpik, Multipik), than on the green zucchini (eg, Leopard, Spineless Beauty). Leopard and Spineless Beauty showed symptoms on the fruit (not rated) before the plants began to wilt. The Harris Moran summer squash lines (SSXP4508, SSXP4442, SSXP4509, SSXP4441, and SSXP4443) varied in their susceptibility to Phytophthora blight, but tolerance in any of the varieties is minimal, if it exists, and even the least susceptible varieties still died. There were no significant differences in fruit infection among winter squash varieties.

[*] Camp, A.R., Lange, H.W., Reiners, S., Dillard, H.R., and Smart, C.D. 2008. Tolerance of summer and winter squash lines to Phytophthora blight, 2008. Plant Disease Management Reports (online). Report 3:V022. DOI: 10.10994/PDMR03. The American Phytopathological Society, St. Paul, MN.

Table A3.1 Incidence of Phytophthora blight on summer and winter squash varieties reported as area under the disease progress curve (AUDPC).

Squash type[a]	Variety	Mean AUDPC[b]
Summer	Sunray	200.00 a[c]
	Cougar	200.00 a
	SSXP4508	200.00 a
	Superpik	200.00 a
	SSXP4510	186.67 ab
	Jaguar	186.67 ab
	SSXP4442	186.67 ab
	Gold Rush	186.67 ab
	Multipik	183.33 ab
	Tigress	173.33 ab
	Lioness	173.33 ab
	Supersett	173.33 ab
	SSXP4509	170.00 ab
	Zucchini Elite	170.00 ab
	CMVZ13	166.67 ab
	Hurakan	160.00 ab
	SSXP4441	155.56 ab
	Linda	146.67 ab
	Spineless Beauty	143.33 ab
	SSXP4443	141.67 ab
	Leopard	126.67 b
Winter	WSXP1030	196.88 a[d]
	WSXP1031	183.33 a
	Waltham	162.30 a

[a] Summer squash were rated for incidence of plant symptoms (wilting or death), while winter squash were rated for incidence of fruit infection.

[b] The area under the disease progress curve (AUDPC) was calculated for each variety based on the proportion of summer squash plants in a plot with symptoms (wilted or dead), or the proportion of infected winter squash fruit. All plots contained at least three healthy plants at inoculation, and only these plants were rated for disease. Means represent the average among 3 replications, unless otherwise noted.

[c] Means followed by the same letter are not significantly different from each other based on an ANOVA of the AUDPC using a General Linear Model (P=0.0726 and P=0.68 for summer and winter squash, respectively), and separated by a Waller-Duncan test.

[d] Only two replications of variety WSXP1030 were included in the analysis because only one small fruit was produced in the third replicate (wet part of the field), and the fruit aborted shortly after inoculation.

APPENDIX FOUR

ISOLATES OF *PHYTOPHTHORA CAPSICI* COLLECTED FROM NEW YORK IN 2006 AND 2007

Table A4.1 Summary of *Phytophthora capsici* isolates collected from New York in 2006 and 2007[a]. The crops from which isolations were made, mating type, and mefenoxam sensitivity is summarized for each field.

			Mating Type		Mef. Sens.[b]		
# Isolates	Location	Crop	A1	A2	S	I	R
23	SC (Field CD)	pumpkin	9	14	13	0	10
1	GK	pumpkin	0	1	0	0	1
2	GP	pepper	1	1	0	0	2
4	GT	tomato	4	0	0	0	4
10	BE	eggplant	10	0	10	0	0
8	BS	s. squash	8	0	0	0	8
8	BWS	w. squash	0	8	0	0	8
1	BP	pepper	0	1	1	0	0
13	0752-	zucchini	6	7	2	0	11
70	**All of Capital District**		**38**	**32**	**26**	**0**	**44**
1	0664-1 (2006)	pepper	1	0	1	0	0
10	A (2006)	w. squash	0	10	10	0	0
6	B (2006)	w. squash	0	6	6	0	0
2	0759-	pumpkin	2	0	2	0	0
19	**All of central New York**		**3**	**16**	**19**	**0**	**0**
1	06120-1 (2006)	pepper	1	0	1	0	0
36	field WNY	s. squash	8	28	36	0	0
37	**All of western New York**		**9**	**28**	**37**	**0**	**0**
10	MMHA	pumpkin	10	0	10	0	0
5	MM1	pepper	3	2	5	0	0
6	MM2	pepper	4	2	6	0	0
6	MMS2	unknown	4	2	4	1	1
7	MMH	pumpkin	6	1	7	0	0
8	MML	pumpkin	2	6	0	0	8
11	MMSK	pumpkin	0	11	11	0	0
13	MMSN	pepper	13	0	12	0	1
12	MMW	cucurbit	5	7	12	0	0
44	MMZ (field LI)	pumpkin	20	24	27	1	16
4	MMG	pumpkin	4	0	0	0	4
7	MMR	pumpkin	7	0	7	0	0
3	MMA	pumpkin	3	0	1	0	2
136	**All of Long Island**		**81**	**55**	**102**	**2**	**32**
262	**All of New York**		**131**	**131**	**184**	**2**	**76**

[a] Isolates were collected in 2007, unless otherwise noted

[b] Mefenoxam sensitivity: S = sensitive, I = intermediately sensitive, R = resistant

Table A4.2 Detailed list of all *Phytophthora capsici* isolates collected in New York State during 2006 and 2007. Isolations were made from surface disinfested symptomatic plant tissue on selective media. Single zoospore cultures were obtained from each isolate, and were tested for mefenoxam sensitivity and mating type. Date on which the plants were sampled from the field, host, and the region and county in which the fields were located, along with any other notes about the source of the isolates are included. Isolates that begin with the same few letters or numbers were collected from the same field (eg, 0752-, BE, BP, A, B, MMHA, MMZ), although isolates collected from western New York in 2007 have a slightly different naming scheme. Isolates are sorted by the region from which they were collected.

Isolate	Mef. Sens.[a]	MT[b]	From	Date Collected	Region	County	Additional Notes
0752-01A	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-02	S	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-03	R	A1	zucchini	7/24/07	Capital District	Herkimer	
0752-04A	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-05A	R	A1	zucchini	7/24/07	Capital District	Herkimer	
0752-06	R	A1	zucchini	7/24/07	Capital District	Herkimer	
0752-08A	R	A1	zucchini	7/24/07	Capital District	Herkimer	
0752-09	R	A1	zucchini	7/24/07	Capital District	Herkimer	
0752-10	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-12A	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-13	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-14	R	A2	zucchini	7/24/07	Capital District	Herkimer	
0752-15	S	A1	zucchini	7/24/07	Capital District	Herkimer	
BE-01A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	
BE-02A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	
BE-03A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 1, isolate 1
BE-04A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 1, isolate 2
BE-05A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 1
BE-06A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 2
BE-07B	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 1, isolate 1
BE-08A	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 1, isolate 2
BE-09B	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 2, isolate 1
BE-10B	S	A1	eggplant	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 2, isolate 2
BP-02A	S	A2	pepper	~9/25/07	Capital District	Rensselaer	
BS-01A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit1, lesion 1, isolate 1
BS-02A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 1, lesion 1, isolate 2
BS-03A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 1, lesion 2
BS-04A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 1, isolate 1
BS-05A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 1, isolate 2
BS-06A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 1

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
BS-07A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 2
BS-08A	R	A1	s. squash	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 1
BWS-01A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 1
BWS-02A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 1
BWS-03A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 2, isolate 2
BWS-04B	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 3, isolate 1
BWS-05A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 2, lesion 3, isolate 2
BWS-06A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 1, isolate 1
BWS-07B	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 1, isolate 2
BWS-08A	R	A2	s. squash	~9/25/07	Capital District	Rensselaer	fruit 3, lesion 2
GK-01A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
GP-05A	R	A2	pepper	~9/25/07	Capital District	Schenectady	
GP-07A	R	A1	pepper	~9/25/07	Capital District	Schenectady	
GT-01A[c]	R	A1	tomato	~9/25/07	Capital District	Schenectady	
GT-02A[c]	R	A1	tomato	~9/25/07	Capital District	Schenectady	
GT-03A[c]	R	A1	tomato	~9/25/07	Capital District	Schenectady	
GT-04A[c]	R	A1	tomato	~9/25/07	Capital District	Schenectady	
SC-02A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-03A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-04A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-05A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-06A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-07A	R	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-08A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-09A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-10A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-11B	R	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-12A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
SC-13.1A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-13.2A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-14.1A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-14.2A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-15A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-17A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-18A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-19A	S	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-20A	R	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-21A	R	A1	pumpkin	~9/25/07	Capital District	Schenectady	
SC-22A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
SC-23A	S	A2	pumpkin	~9/25/07	Capital District	Schenectady	
0664-1	S	A1	pepper	8/14/06	central New York	Monroe	
0759-08	S	A1	pumpkin	7/31/07	central New York	Ontario	
0759-11	S	A1	pumpkin	7/31/07	central New York	Ontario	
A1-1.1	S	A2	w. squash	9/18/06	central New York	Ontario	
A1-2.1	S	A2	w. squash	9/18/06	central New York	Ontario	
A1-3.1	S	A2	w. squash	9/18/06	central New York	Ontario	same plant as A1-3.2 & A1-3.3
A1-3.2	S	A2	w. squash	9/18/06	central New York	Ontario	same plant as A1-3.1 & A1-3.3
A1-3.3	S	A2	w. squash	9/18/06	central New York	Ontario	same plant as A1-3.1 & A1-3.2
A2-2.1	S	A2	w. squash	9/18/06	central New York	Ontario	
A2-3.1	S	A2	w. squash	9/18/06	central New York	Ontario	
A2-6.1[c]	S	A2	w. squash	9/18/06	central New York	Ontario	
A2-6.2[c]	S	A2	w. squash	9/18/06	central New York	Ontario	
A3-3.1	S	A2	w. squash	9/18/06	central New York	Ontario	
B1-1.1	S	A2	w. squash	9/18/06	central New York	Ontario	
B1-3.1	S	A2	w. squash	9/18/06	central New York	Ontario	
B2-1.1	S	A2	w. squash	9/18/06	central New York	Ontario	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
B2-2.1	S	A2	w. squash	9/18/06	central New York	Ontario	
B2-3.1	S	A2	w. squash	9/18/06	central New York	Ontario	
B2-4.1	S	A2	w. squash	9/18/06	central New York	Ontario	
MM1-1C	S	A2	pepper	8/28/07	Long Island	Suffolk	section 1 of field
MM1-2B	S	A1	pepper	8/28/07	Long Island	Suffolk	section 1 of field
MM1-3A	S	A1	pepper	8/28/07	Long Island	Suffolk	section 1 of field
MM1-4A	S	A1	pepper	8/28/07	Long Island	Suffolk	section 1 of field
MM1-5A	S	A2	pepper	8/28/07	Long Island	Suffolk	section 1 of field
MM2-1A	S	A1	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MM2-2A	S	A1	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MM2-3A	S	A1	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MM2-4B	S	A1	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MM2-5A	S	A2	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MM2-6A	S	A2	pepper	8/28/07	Long Island	Suffolk	section 2 of field
MMA-01A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMA-02A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMA-03A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMG-01A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMG-02A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMG-03C	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMG-04A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MMH-1A	S	A2	pumpkin	8/30/07	Long Island	Suffolk	
MMH-2A	S	A1	pumpkin	8/30/07	Long Island	Suffolk	
MMH-3C	S	A1	pumpkin	8/30/07	Long Island	Suffolk	
MMH-4A	S	A1	pumpkin	8/30/07	Long Island	Suffolk	
MMH-5B	S	A1	pumpkin	8/30/07	Long Island	Suffolk	
MMH-6A	S	A1	pumpkin	8/30/07	Long Island	Suffolk	
MMH-7A	S	A1	pumpkin	8/30/07	Long Island	Suffolk	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
MMHA-01A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-02A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-03B	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-04A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-05C	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-06a-A[c]	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-06b-B[c]	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Northwest section of field HA
MMHA-07A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Southwest section of field HA
MMHA-08A	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Southwest section of field HA
MMHA-09B	S	A1	pumpkin	9/4/07	Long Island	Suffolk	Southwest section of field HA
MML-01A	R	A1	pumpkin	9/4/07	Long Island	Suffolk	
MML-02A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-03A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-04A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-05A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-06A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-07A	R	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MML-08A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMR-02A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field[d]
MMR-03A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMR-04A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMR-06A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMR-08A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMR-09A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMR-10 -2A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	LIHREC field
MMS2-1A	R	A2	unknown	8/30/07	Long Island	Suffolk	
MMS2-2A	S	A1	unknown	8/30/07	Long Island	Suffolk	
MMS2-3A	S	A1	unknown	8/30/07	Long Island	Suffolk	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
MMS2-4A	I	A2	unknown	8/30/07	Long Island	Suffolk	
MMS2-5B	S	A1	unknown	8/30/07	Long Island	Suffolk	
MMS2-6C	S	A1	unknown	8/30/07	Long Island	Suffolk	
MMSK-01A	S	A2	cucurbit	8/30/07	Long Island	Suffolk	
MMSK-03A	S	A2	cucurbit	8/30/07	Long Island	Suffolk	
MMSK-04A	S	A2	cucurbit	8/30/07	Long Island	Suffolk	
MMSK-05A	S	A2	cucurbit	8/30/07	Long Island	Suffolk	
MMSK-06A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-07A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-08A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-09A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-10A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-11A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSK-12A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMSN-01B	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-02A	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-03C	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-04C	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-05A	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-06C	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-09A	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-10B	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-12B	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-13A	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-14B	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-15a-A[c]	R	A1	pepper	8/30/07	Long Island	Suffolk	
MMSN-15b-A[c]	S	A1	pepper	8/30/07	Long Island	Suffolk	
MMW-02A	S	A1	cucurbit	8/20/07	Long Island	Suffolk	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
MMW-03A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-04A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-05A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-06A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-07A	S	A1	cucurbit	8/20/07	Long Island	Suffolk	
MMW-08 A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-09A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-10A	S	A2	cucurbit	8/20/07	Long Island	Suffolk	
MMW-11A	S	A1	cucurbit	8/20/07	Long Island	Suffolk	
MMW-12B	S	A1	cucurbit	8/20/07	Long Island	Suffolk	
MMW-14A	S	A1	cucurbit	8/20/07	Long Island	Suffolk	
MMZ-01B	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-02A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-03A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-04A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-05A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-06A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-07A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-08A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-09A	I	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-10A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-11A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-12A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-13A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-14A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-15A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-16A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-17C	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
MMZ-18A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-19A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-20A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-21A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-22A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-23A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-24A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-25A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-26A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-27A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-28A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-29A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-30A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-31C	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-32A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-34A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-35A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-36A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-37A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-39A	S	A2	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-40A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-41A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-42A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-43A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-44A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-45A	R	A1	pumpkin	~11/1/07	Long Island	Suffolk	
MMZ-46A	S	A1	pumpkin	~11/1/07	Long Island	Suffolk	
06120-1	S	A1	pepper	9/12/06	western New York	unknown	

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
E1.02[e]	S	A2	s. squash	6/19/07	western New York	Erie	rogued area
E1.04	S	A2	s. squash	6/19/07	western New York	Erie	rogued area
E1.08	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.09	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.10	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.11	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.12A	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.13	S	A1	s. squash	6/19/07	western New York	Erie	rogued area
E1.15	S	A2	s. squash	6/19/07	western New York	Erie	rogued area
E1.16	S	A2	s. squash	6/19/07	western New York	Erie	rogued area
1.1B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 1
2.2	S	A1	s. squash	6/26/07	western New York	Erie	mapped - area 2
2.3	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 2
5.2a-C[c]	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 5
5.2b-C[c]	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 5
5.2E	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 5
5.4A	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 5
6.1C	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 6
7.1C	S	A1	s. squash	6/26/07	western New York	Erie	mapped - area 7
7.2C	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 7
7.3A	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 7
7.6B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 7
8.1	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8
8.2-1A[c]	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8
8.2-2A[c]	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8
8.3	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8
8.4	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8
8.5	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 8

Table A4.2 (Continued)

Isolate	Mef. Sens.	MT	From	Date Collected	Region	County	Additional Notes
9.1B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 9
10.1b-C	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 10
10.3A	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 10
10.4B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 10
11.1b-A	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 11
11.2B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 11
11.5B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 11
11.6B	S	A2	s. squash	6/26/07	western New York	Erie	mapped - area 11

[a] S = sensitive, I = intermediately sensitive, and R = resistant to the fungicide mefenoxam.
[b] MT = mating type.
[c] The following pairs of isolates were recovered from distinct lesions on the same plant: GT-01A and GT-02A, GT-03A and GT-04A, A2-6.1 and A2-6.2, MMHA-06a-A and MMHA-06b-B, MMSN-15a-A and MMSN-15b-A, 5.2a-C and 5.2b-C, 8.2-1A and 8.2-2A.
[d] LIHREC = Long Island Horticultural Research and Extension Center, in Riverhead, NY.
[e] All isolates collected from western New York in 2007 were from the same field.