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Distribution and Host Specificity of Erysiphe Cruciferarum (Powdery Mildew) Attacking Alliaria Petiolata (Garlic Mustard), in Southwestern Ohio

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DISTRIBUTION AND HOST SPECIFICITY OF *ERYsiphe CRUCIFERARUM* (POWDERY MILDEW) ATTACKING *Alliaria petiolata* (GARLIC MUSTARD), IN SOUTHWESTERN OHIO

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

VICTORIA L. CIOLA
B.S. The Ohio State University, 2005

2009

Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY Victoria L. Ciola ENTITLED
Distribution and Host Specificity of *Erysiphe cruciferarum* (powdery
mildew) Attacking *Alliaria petiolata* (garlic mustard), in Southwestern
Ohio, BE ACCEPTED IN PARTIAL FULFILLMENT OF THE
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ABSTRACT


Garlic mustard is an invasive Eurasian biennial that has rapidly spread throughout the United States. In Southwestern Ohio, many garlic mustard populations are infected with the powdery mildew *Erysiphe cruciferarum* that can reduce the growth and fitness of garlic mustard. *E. cruciferarum* was assessed using a Decision Tree in regards to its potential use as a biological control agent. I determined the distribution of *E. cruciferarum* on garlic mustard and I determined the potential risk of *E. cruciferarum* to native Brassicaceous species and selected crops. I surveyed 19 parks in Southwestern Ohio and recorded the number of diseased garlic mustard and aspect at every population and then made a distribution map of disease incidence of *E. cruciferarum* on garlic mustard using GIS ESRI (ArcMap) software. My distribution survey showed a random distribution of *E. cruciferarum* infection on garlic mustard with disease incidence decreasing away from Dayton, Ohio. Aspects with higher disease incidences were level and facing southeast and were significantly different than northwest facing aspects. Five native spring ephemeral species were surveyed in the field and then obtained from local wooded areas in May 2008. Individual plants were transplanted in the greenhouse and
then exposed to infected garlic mustard plants. The native plants surveyed showed no obvious signs of *E. cruciferarum* infection in the field. All of the native plants subjected to powdery mildew under greenhouse conditions became mildly infected with *E. cruciferarum*. Twelve crops in the family Brassicaceae along with 2 crops in the Fabaceae and Solanaceae families were planted and inoculated with *E. cruciferarum* and only one cultivar, Savanna Mustard (*Brassica juncea*), became infected with powdery mildew. A distribution survey showed the distribution of *E. cruciferarum* infection on garlic mustard with disease incidence decreasing away from Dayton, Ohio. Populations located on level ground or facing southeast had significantly higher disease incidence than northwest facing aspects. Results from the greenhouse study show that under optimal conditions, some native spring mustard species can become infected with powdery mildew. However, *E. cruciferarum* likely poses little threat to native Brassicaceaeous plants because the phenology of native plants allows them to escape infection. *E. cruciferarum* poses a slight threat to cultivated crops since only one crop became infected and Savanna Mustard is not a common cultivated variety in Ohio.
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INTRODUCTION

A. Garlic Mustard- The Problem

Garlic mustard, *Alliaria petiolata*, (M. Bieb) Cavara & Grande is an invasive Eurasian biennial that has rapidly spread throughout the Eastern United States and Canada (Nuzzo, 1999; Welk et al., 2002). Introduced by European settlers, garlic mustard was used as an herb, which was thought to have medicinal value (Cavers, 1979; Welk et al., 2002). In North America, garlic mustard is often found along roadsides, forest edges, and in forests (Nuzzo, 1993; Welk et al., 2002). Seedlings germinate in the spring and form basal rosettes by summer (Cavers, 1979; Drayton and Primack, 1999; Nuzzo, 2000). Second year rosettes, or adult plants, produce flowering stems, set seed, and die (Drayton and Primack, 1999; Nuzzo, 2000). Garlic mustard grows in mostly shaded areas and can grow in dense stands (Cavers et al., 1979; Cruden et al., 1996; Blossey et al., 2001). Dense stands of garlic mustard occur due to large volumes of seeds produced and low dispersal ability (Cruden et al., 1996). The dense stands of garlic mustard that invade forests can displace native plants (McCarthy, 1997), can have negative impacts on plant communities (McCarthy, 1997; Stinson, et al., 2007), can impact micorrhizal fungi (Roberts et al., 2001; Callaway et al., 2008; Wolfe et a., 2008) and can impact animal communities (Porter, 1994; Huang et al., 1995; Blossey et al., 2001) in North America.
Invasive species, such as garlic mustard, are managed to reduce the dominance of the invasive species and to increase the dominance of native species (DiTomaso et al., 2006). Many studies have been conducted to determine potential biological control agents and control methods for garlic mustard. Studies of glyphosate herbicide/Roundup (Carlson and Gorchov, 2004; Frey et al., 2007; Slaughter et al., 2007), controlled burning (Nuzzo et al., 1996; DiTomaso et al., 2006), and clipping and pulling (Pardini et al., 2008) are physical control methods for garlic mustard and potential biological control include herbivory by weevils (Blossey et al., 2001; Gerber et al., 2008). In two studies, Glyphosate was sprayed during winter months in order to avoid spraying native understory species (Carlson and Gorchov, 2004; Frey et al., 2007; Slaughter et al., 2007). Carlson and Gorchov (2004) found that applying Roundup in the fall reduced garlic mustard density by 85% in old growth stands and only 44% in second growth stands and there was a higher cover of native spring perennials in the old growth plots. Frey et al. (2007) used cold weather application of Glyphosate to manage garlic mustard. They found that spraying when temperatures are just below freezing reduced garlic mustard survival and also benefited native species. However, Glyphosate/ Roundup must be applied below a specific temperature, have good weather conditions, and without snow making it difficult and costly to use as a physical control for garlic mustard. In addition, the continuous use of herbicides can cause alternate problems such as the chemical moving and disturbing another site, plants evolving and becoming tolerant of herbicides, and altering nutrient levels (DiTomaso et al., 2006).

Nuzzo et al. (1996) used controlled fire regimes to determine if successive fires decreased garlic mustard abundance and increased native species richness. Fires were set
during the dormant season. One plot was burned in fall 1990, early spring 1992, and mid spring 1993, one plot was burned mid spring 1991, 1992, and 1993, and one plot was left unburned. Results show that the first fires decreased garlic mustard, but fires had to be sequential in order to prevent expansion (Nuzzo et al., 1996). When fire was set at an optimal time in autumn, it decreased garlic mustard abundance. Prescribed burns can be helpful in decreasing garlic mustard, but only if the soil is dry and if the burn happens sequentially (Nuzzo et al., 1996). Prescribed burns for garlic mustard must be repeated annually. This poses problems since some forest understory may not be able to recover from annual burns and garlic mustard seed can still be viable after many years in soil.

Pardini et al. (2008) examined whether clipping and pulling garlic mustard could impact garlic mustard survival. There were 4 main treatments in this study: unmanaged, pull whole plant and leave on ground, early in growing season clip stems at ground level, and early in growing season clip stems at midheight. Adult plants that were clipped at ground level were killed, but this aided in the survival of the juvenile population. The remaining 3 treatments did not work and all produced seeds (Pardini et al., 2008). Clipping and pulling garlic mustard is time consuming and must be performed at the right time in the season in order to effectively decrease garlic mustard populations.

An effective means for controlling invasive species, such as garlic mustard, are biological control agents (Berner and Bruckart, 2005). The root crown weevil (Ceutorhynchus scrobicollis), a potential biological control, oviposit onto garlic mustard rosettes and the larvae feed on the overwintering rosettes (Gerber et al., 2007). Common garden plots were conducted in Switzerland and field sites were located Berlin, Germany. In this study, feeding by weevils decreased garlic mustard survival and plant height, but
in a common garden did not reduce seed production (Gerber et al., 2007). Gerber et al. (2008) studied the impact of two stem boring weevils (*Ceutorhynchus alliiariae* and *C. roberti*) on garlic mustard survival, growth, and reproduction in common garden plots. Results showed that weevil attack significantly reduced garlic mustard height and negatively impacted seed production, but only when weevil density was high. Reduction in plant height only occurred when weevil density was high and only significant at high rates of attack (Gerber et al., 2008).

Evans and Landis (2007) objectives were to describe the sites that were invaded with garlic mustard plants and determine to what degree herbivores were affecting garlic mustard. They found that garlic mustard spreads within sites and that damage by herbivores was frequent but not extensive enough to damage the plant (Evans and Landis, 2007). While surveying quadrats, they noticed diseased garlic mustard plants that had symptoms that were similar to cucumber mosaic virus (CMV). The pathogen was later identified as *Sclerotinia sclerotiorum* (white mold). Diseased garlic mustard plants were weak at the stem, but the pathogen was not frequently encountered across populations of garlic mustard and seemed not to warrant exploration as a potential biological control (Evans and Landis, 2007).

**B. Garlic Mustard with Powdery Mildew- The Observation**

Infected plants that have foliar pathogens often show signs of decreased survivorship, reproduction, growth, and competitive ability (Jarosz and Davelos, 1995). In Southwestern Ohio, many garlic mustard populations have the powdery mildew *Erysiphe cruciferarum* (Opiz ex L. Junell) that can reduce the growth and fitness of garlic mustard (Enright and Cipollini, 2007).
*Erysiphe cruciferarum* is the major causal agent of powdery mildew in crucifers (Koch and Slusarenko, 1990). *Erysiphe cruciferarum* is a biotrophic pathogen that needs living plant tissue in order to grow and reproduce (Vogel et al., 2000; Chaure et al., 2000; Eckardt, 2002). It is an obligate parasite that grows all over the world in countries such as France, Germany, India, Japan, Sweden, Turkey, UK, and USA (Saharan et al., 2005). Powdery mildew infects above ground plant parts, grows in white circular patches, and increases in severity when temperature and humidity increase (Saharan et al., 2005). The life cycle of *Erysiphe* species have two distinct stages, asexual spores (conidial) and sexual spores (ascospores) (Deacon, 1997). During the mycelium and conidial stage, the mycelium is external to plant tissues and is hyaline (Reed, 1913). Hypha, on the mycelium, grows toward the plants tissue, penetrates a cell of the plant, and forms haustorium. The haustoria are nutrient-absorbing structures that take in nutrients from the host plant, but do not kill the host plant (Reed, 1913; Deacon, 1997). As the mycelium develops, branches are produced and grow away from the host plants leaf and the hypha form conidiophores. At the ends of conidiophores, conidium is cut off and when hypha extend, the conidium get cut off again. This process continues and results in a chain-like row of conidia (Reed, 1913). Conidia are released when they mature and get dispersed by air currents. The primary source of inoculum to crops is off season *Brassica* species that have the fungal mycelium and conidia, while a secondary source of the pathogen occurs through air-born conidia (Saharan et al., 2005). During the sexual stage, the sexual fruiting body (ascocarp) is the cleistothecium, which encloses the asci (Silverside, 2001). The wall of the cleistothecium has to break open to release spores. The
clesitothecia are developed at the end of the season as minute brown/black dots between mycelium, which is the main mode of surviving overwinter (Silverside, 2001).

*Erysiphe cruciferarum* has been reported infecting garlic mustard (*Alliaria petiolata*) in the British Isles (Newsletter of the Warwickshire Fungus Survey, 2004), in Germany (Erysiphales Collection at the Botanische Staatssammlung München, Global Diversity Information Facility, http://data.gbif.org/datasets/resource/1440 [accessed 25 April 2009]), in Armenia (Erysiphales Collection at the University Halle-Wittenberg, http://www.gbif-mycology.de/DatabaseClients/HALcoll/index.html [accessed 25 April 2009]), and in Indiana and Ohio in the US (Blossey et al., 2001; Enright and Cipollini, 2007). Enright and Cipollini (2007) recognized that garlic mustard populations had the fungus *E. cruciferarum* around the Dayton, Ohio region. Experiments were conducted on garlic mustard in both the field and the greenhouse. For the field component, garlic mustard populations at Taylorsville Metropark (Dayton, Ohio) were studied to determine if the populations were impacted by powdery mildew in the field. Results showed that the moderate and severely diseased plants were smaller than the mildly diseased plants. The mildly diseased plants produced twice as many siliques per plant and twice as many seeds per plant as the more severely diseased plants. For the greenhouse component, first year seedlings of garlic mustard were inoculated with powdery mildew. The growth of young plants was greatly reduced when they became diseased, which led to mortality. Overall, Enright and Cipollini (2007) demonstrated the potential of *E. cruciferarum* as a biological control agent in controlling garlic mustard. However, a risk assessment must be conducted to determine if this strain of *E. cruciferarum* could be developed as a control agent.
C. Biological Control and Risk Assessment- The potential solution and risk assessment

Environmental assessments identify environmental impacts caused by the project and rate the impacts as significant or insignificant (Kwiatkowski, 1998). All biological control projects must be accompanied by an environmental assessment (Kwiatkowski, 1998). Ecological risk assessments are used to evaluate and organize data, information, assumptions, and uncertainties so that relationships between the environment and stressors can be used in decision making (EPA, 1998). After a risk assessment is performed, risk managers determine the course of action that must be taken to protect or fix the problem. The response of the risk manager is based on social, legal, political, or economic factors (EPA, 1998). Legal factors are included when the air or water have risks due to legislation such as the Clean Air and Clean Water Acts. Moreover, risk assessments are primarily used to aid in the decision-making process. An example of a proposed biological control agent that assessed environmental risks was Charudattan (2007). This study assessed the risk of the use of Tobacco mild green mosaic virus on the invasive tropical soda apple to nontarget plants, to humans, and to the environment, with the goal of marketing the pathogen as a bioherbicide (Charudattan, 2007).

Berner and Bruckart (2005) constructed a “Decision Tree” (Fig. 1) to evaluate exotic pathogens for biological control of introduced invasive weeds in which Phase I shows how the pathogen is damaging to the pest, Phase II evaluates the safety of the pathogen, and Phase III determines the regulation and implementation of the pathogen. *Erysiphe cruciferarum* could be used as a biological control agent for garlic mustard if it meets the criteria for usefulness, safety, and deployment (Berner and Bruckart, 2005).
Other fungi have been studied for their use as biocontrols. Dyer’s woad (*Isatis tinctoria* L.) is in the family Brassicaceae and is an annual, biennial, or perennial native to central Asia (Hansen and Bloem, 2006). A native rust fungus, *Puccinia thlaspeos*, has infected dyer’s woad throughout the western US. This rust fungus may decrease growth and seed production of dyer’s woad which makes it a potential biocontrol candidate (Hansen and Bloem, 2006). *Erysiphe cynoglossi* is an endemic fungus found on the invasive plant houndstongue (Asteraceae family: *Hieracium cynoglossoides* Arv.-Touv.) in the western US (De Clerck-Floate, 1999). Field observations showed a significant negative relationship between the percentage of seed set and percentage of plant diseased. A common garden experiment showed that *E. cynoglossi* infection of houndstongue significantly reduced seed production, which suggests that this powdery mildew could be used as a potential biocontrol (De Clerck-Floate, 1999). In Europe, investigations were conducted on a potential biological control, *Erysiphe cichoracearum*, for skeleton weed (*Hasan*, 1974). *E. cichoracearum* attacks skeleton weed (*Chondrilla juncea* L. (Asteraceae family), killing severely infected plants and reducing flowering and seeding in stressed plants. *Hasan* (1974) conducted host specificity tests of 65 cultivated species, which included the Compositae, Cucurbitaceae, Solanaceae, and Leguminosae families that are known to be hosts for this pathogen. Results showed that the strain of *E. cichoracearum* was highly specific to the skeleton weed (*Hasan*, 1974). Additional study of the *E. cruciferarum* strain attacking garlic mustard is needed.

**D. Distribution of Garlic Mustard with Powdery Mildew**

*Erysiphe cruciferarum* has been found on garlic mustard in Indiana (Blossey et al., 2001) and Southwestern Ohio (Enright & Cipollini, 2007) as well as in the British
Isles (Newsletter of the Warwickshire Fungus Survey, 2004), in Germany (Erysiphales Collection at the Botanische Staatsammlung Mu¨nchen, Global Diversity Information Facility, http://data.gbif.org/datasets/resource/1440 [accessed 25 April 2009]), and in Armenia (Erysiphales Collection at the University Halle-Wittenberg, http://www.gbif-mycology.de/DatabaseClients/HALcoll/index.html [accessed 25 April 2009]). The local distribution of garlic mustard with powdery mildew has never been examined systematically. Few surveys of powdery mildew distribution exist at all. Laine and Hanski, (2006) conducted a large scale population survey of a powdery mildew, *Podosphaera plantaginis*, that infects *Plantago lanceolata* on the Åland Islands in Finland to determine the spatial distribution and dynamics of powdery mildew infection. Results showed that the incidence of powdery mildew across host populations was significantly aggregated. The population size of *P. lanceolata* and the proximity to roads were very significant in explaining incidence of powdery mildew (Laine and Hanski, 2006). Host population size had a positive effect on powdery mildew colonization and plants in meadows connected by roads on the Island had a higher probability of becoming infected (Laine and Hanski, 2006). Further understanding the distribution of powdery mildew on garlic mustard must be determined in order to understand the spread of *E. cruciferarum*, understand its current locations of infection, and understand its potential use as a biocontrol for of garlic mustard. Understanding its current distribution aids in determining where *E. cruciferarum* may need to be released (if approved). I determined the distribution of garlic mustard with powdery mildew by sampling local natural areas in order to establish the current regional distribution of garlic mustard with *E. cruciferarum*. 
E. Native Brassica species

The family Brassicaceae has around 340 genera and over 3000 species. Ohio has approximately 30 native Brassicaceous species (Easterly, 1964) of which 10 occupy habitats similar to garlic mustard. In Southwestern Ohio, these species include *Arabis laevigata*, *Cardamine bulbosa*, *C. concatenata*, *C. douglassii*, and *Cardamine diphylla*. The susceptibility of these species to *E. cruciferarum* is important to know because infection by pathogens is not currently observed and this mildew may be detrimental to the growth and survival of these native species. Smooth rockcress, *Arabis laevigata*, is a facultative biennial that grows in woodlands (Bloom et al., 1990; Bloom et al., 2001). *Arabis laevigata* does not have persistent basal leaves and has white flowers which bloom from April to June in dry woods, rocks, and hillsides (Rhoades and Block, 2000). *Cardamine bulbosa*, spring cress, is a perennial ephemeral species with simple leaves, grows in wet areas near woods, and has white flowers from April to July (Rhoades and Block, 2000). *Cardamine concatenata*, cut-leaved toothwort, is a perennial ephemeral common in Ohio woods, has deeply palmately-lobed leaves, and has white to purple flowers that bloom from March to early May (Rhoades and Block, 2000). *Cardamine douglassii*, purplecress, is a perennial ephemeral that has pink to purple flowers from April to early May (Rhoades and Block, 2000). *Cardamine diphylla*, two-leaved toothwort, is a perennial ephemeral that has nearly opposite cauline leaves, leaflets that are coarsely toothed with minute marginal hairs, grows in woods and floodplains, and has white flowers from April to June (Rhoades and Block, 2000).

There is no known survey of these native Brassicaceae plants in Ohio to have considered pathogens. Infected garlic mustard plants start to show signs of powdery
mildew infection in early July due to the powdery mildews need for warm and humid conditions. Thus, native Ohio spring ephemeral species may be able to escape infection. *Brassica* crops sown in late October are attacked more heavily by *E. cruciferarum* (Saharan et al., 2005). I determined if the strain of *E. cruciferarum* on garlic mustard in Southwestern Ohio can infect *Arabis laevigata, Cardamine bulbosa, C. concatenata,* or *C. douglassii* both in the greenhouse under favorable conditions and whether natural populations of these plants exhibit evidence of powdery mildew disease.

**F. Brassica Crops**

Brassicaceae is a family that includes many important agricultural *Brassica* cultivars (Koch et al., 2001). Rapeseed-mustards can become severely infected by foliar pathogens, which deteriorate the quality and quantity of seed and oil content in crops and cause yield losses (Williamson & MacFarlane, 1986; Penaud, 1999; Dange et al., 2002; Kumar & Saharan, 2002).

Broccoli, cauliflower, cabbage, Brussels sprouts, canola, and turnips are grown in Ohio on family farms or by home gardeners. Cabbage production is a significant industry in northwest and south central Ohio (The Ohio State University Extension, 2009). Nearby states such as Pennsylvania have significant *Brassica oleracea* crops such as cabbage. The main pathogens affecting cabbage are white mold, black rot, and downy mildew (The Ohio State University Extension, 2009). Powdery mildew is not listed as a concern for most *Brassica oleracea* crops. Powdery mildews are fairly well known pathogens of crops. *Erysiphe cruciferarum* can infect *Brassica rapa* (turnip and broccoli raab) and *Brassica napus* (canola) (Karakaya et al., 1993; Koike et al., 1997; OK website). Many *Brassica* cultivars can host the powdery mildew *E. polygoni* (Tongue and
Griffiths, 2004), including Brussels sprouts and cabbage. Crops sown in late October are more severely attacked by powdery mildew than those sown earlier (Saharan et al., 2005). We know that *Erysiphe* spp. can infect *Brassica* cultivars, but we do not know if the strain of *E. cruciferarum* on garlic mustard in Southwestern Ohio can infect *Brassica* species. It is important to know what this strain of mildew can infect since farming is a large industry in Ohio and knowing what it can infect will help determine if this powdery mildew could be developed as a control agent. I determined if the strain of *E. cruciferarum* on garlic mustard in Southwestern Ohio can infect *Brassica* crop cultivars.

**HYPOTHESES**

1. The distribution of garlic mustard with powdery mildew will be more abundant in southwestern Ohio, the epicenter, and radiate outwards. The incidence of powdery mildew on garlic mustard will decrease when moving away from southwestern Ohio. No aspect will favor garlic mustard plants infected with powdery mildew.

2. Native plants will not get powdery mildew in the wild because of the differences in phenology. Native plants grown in the greenhouse will become infected with powdery mildew.

3. Powdery mildew will infect the *Brassica* crops (note: Some crops could be immune to the powdery mildew due to breeding techniques implemented to avoid a decrease in crop yield).
MATIERIALS AND METHODS

A. Distribution of garlic mustard with powdery mildew

I surveyed 19 parks and reserves to determine the incidence of powdery mildew disease within garlic mustard populations in Ohio (and one park in Indiana) between July 2008 and August 2008 since *E. cruciferarum* show visible signs of infecting host plants mid-summer. It appears that the epicenter of garlic mustard with powdery mildew is in Dayton, Ohio (D. Cipollini, personal observation). I surveyed plants in 17 areas within a 96 kilometer radius of the epicenter (Dayton, Ohio), and 2 outlier parks that were far from the epicenter. The parks surveyed are shown in Table 1. I surveyed garlic mustard populations with powdery mildew using a rapid sampling technique, modified from Benkendorff and Davis (2002). I modified the 4 hour survey time from Benkendorff and Davis (2002) and determined the survey time based on the size of the park. Smaller parks and parks with minimal trails were surveyed the shortest amount of time and larger parks with extensive trails were surveyed the longest amount of time. All parks were surveyed in less than 6 hours. Before surveying the parks, a map was obtained and trials were selected based on access and length of trails due to a limit in survey time. At each park, I went to as many sides of the park as possible, depending on trail locations, so that all sides of the park were included. I used a haphazard sampling technique since the survey was conducted only at parks with public access that had trails. For each park surveyed, latitude and longitude were taken using a Garmin GPS unit, how many garlic mustard plants within each population had powdery mildew, and habitat aspect at each garlic mustard population. Aspect was determined using a compass at each population (level, N, S, E, W, NE, NW, SE, and SW). Disease
incidence was measured by counting the number of individuals in smaller populations of garlic mustard and counting how many in each population were diseased, whereas disease incidence of larger populations was estimated by counting a small area and estimating the remaining area. I entered data in a spreadsheet from all parks including the longitude and latitude of each park, disease incidence and aspect at every garlic mustard population within each park. Disease incidence was first determined for each population. Then, the disease incidence at every population within each park was averaged to determine the average disease incidence of each park. I used ESRI GIS (ArcMap) software to produce a map of the distribution of garlic mustard with the park incidences of powdery mildew. The program SAS (version 9.2, SAS Institute, Cary, North Carolina, USA) was used for statistical analyses. Means were compared using Tukey’s test. A one-way ANOVA was used to examine whether disease incidence varied by park and whether it varied by aspect. For the analysis of aspect, I took 7 parks that had no disease incidence out of the one-way ANOVA in order to properly show if aspect affected disease incidence. Additionally, I resurveyed Wright State University Forest Preserve and Eastwood Metropark to determine if disease incidence had changed one month after they had been initially surveyed. A linear regression was used to determine whether the distance from Dayton, Ohio (WSU) and disease incidence decreased the further away from the suspected epicenter (Dayton, Ohio).

B. Susceptibility of native Brassicaceous plants to powdery mildew disease in the field and greenhouse

From April 2008 to May 2008, I conducted field observations of populations of *Arabis laevigata, Cardamine bulbosa, Cardamine concatenata, and Cardamine*
*Cardamine douglassii* to determine if *E. cruciferarum* infected these native species. *Cardamine bulbosa* was surveyed at John Bryan State Park (Yellow Springs, Ohio), Glen Helen Nature Preserve (Yellow Springs, Ohio) and Cedar Bog (Urbana, Ohio). *Cardamine concatenata* and *C. douglassii* were surveyed at John Bryan State Park and Glen Helen Preserve, and also Wright State University Forest Preserve (Dayton, Ohio). I observed *C. concatenata* by walking a trail along a 100-150 meter transect and recorded the individuals that I saw within 2 meters on each side and recorded the number of plants observed, if they showed any signs of powdery mildew infection, and if nearby garlic mustard plants were diseased (Mack and Quang, 1998; Larson et al., 2001; Merriam, 2003). *Cardamine douglassii* and *Arabis laevigata* were surveyed by locating rock surfaces and counting the number of plants, noting the number showing powdery mildew, and determining if garlic mustard populations were diseased. *Cardamine douglassii* was also surveyed by covering a 60x10 meter swath and counting the number of plants and counting the number infected with powdery mildew. *Cardamine bulbosa* was surveyed similar to *C. douglassii* by covering a 60x10 meter swath, but observations were restricted to wet, seep areas where this species is restricted.

Native species (that had not flowered) were transplanted from the wild to the greenhouse, since seed was not readily available. *C. bulbosa* was obtained from Glen Helen Nature Preserve, *C. douglassii* and *C. concatenata* were taken from Wright State University Forest Preserve, and *C. diphylla* was obtained near Cleveland, Ohio and near Brush Valley, Pennsylvania. I transplanted 10-15 of each species in ProMix-BX with micorrhizae potting soil in 18-cell flats (280 mL per cell). After native plants are transplanted, acclimated to the new environment for 3 days, and had enough humidity,
they were inoculated. I inoculated the native plants by holding an infected garlic mustard plant above the native species and knocking the conidia off the infected plant onto the native species. Native species were placed in containers with lids to simulate shade and 2 diseased garlic mustard plants were placed at opposite ends of the containers to keep fresh inoculum sources. One to two weeks after inoculation, I rated the severity of disease in each plant on a 0-5 class scale: 0= no disease, 1=1-10% leaf areas have powdery mildew, 2=11-20% leaf area have powdery mildew symptoms, 3= 21-50% leaf area have powdery mildew symptoms, 4=50-75% leaf area have powdery mildew symptoms, 5= more than 75% leaf area have powdery mildew symptoms (Kumar et al., 2002, Saharan et al., 2005).

C. Susceptibility of Brassica crops to powdery mildew disease in the greenhouse

In trial 1, I planted 18 of each of several Brassica species and cultivars, whereas in trial 2 and 3, I planted 9 of each: Crops used were Brassica oleracea: Broccoli- cv. Packman Hybrid, cv. Coronado Crown Hybrid, and cv. Early Dividend Hybrid; Cauliflower- cv. Amazing and cv. Cassius Hybrid; Cabbage- cv. Cairo Hybrid and cv. Everlast Hybrid; Collard Greens- cv. Georgia; Brussels sprouts- cv. Diablo Hybrid; Brassica rapa: Turnip- cv. Hakurei; Brassica napus: Canola-cv. Westar and cv. Gido; Brassica juncea: Mustard Greens- cv. Savanna Hybrid. Seeds for all crops except B. napus (obtained from Cipollini laboratory) used were obtained from Park Seed (Greenwood, SC). Uninfected garlic mustard plants were collected from WSU Forest Preserve and transplanted in the greenhouse and then acclimated to the new environment for 3-5 days in order to serve as a control. Spores and conidia collected from previously infected garlic mustard plants were used to inoculate garlic mustard seedlings. Also,
plants from the field with *E. cruciferarum* were collected and transplanted in order to be used for inoculations. *Brassica* crop cultivar seeds were planted in ProMix-BX with microrhizae potting soil in 18-cell flats (280 mL per cell). Uninfected garlic mustard was transplanted from Wright State University Forest Preserve into the greenhouse to serve as a control. At 4-6 weeks of age, inoculations were performed using settling towers according to Adam et al. (1999). I made a 75 cm settling tower by using a box that surrounded one flat of plants. Plants were watered before inoculation. In a draft free environment, I obtained 2 garlic mustard plants infected with *E. cruciferarum* and shook the leaves 0.5-1.0 meter above the crops and garlic mustard control plants, which ensured even distribution of inoculum. In trial 3, the inoculum of the infected garlic mustard did not come off the leaves, so I had to rub the infected garlic mustard leaf onto every plant in each cell. The flats of plants and infected garlic mustard plants (used as inoculum) were left in the back prep room area, located in a closed room at the back of the greenhouse, in a draft free environment for 24 hours. After one week, I rated the disease incidence every week for 3 weeks from 0-5 following the same criteria as the native species disease class.

**D. Decision Tree**

The Decision Tree by Berner and Bruckart (2005) was used to guide our determinations of whether *E. cruciferarum* can be used in managing garlic mustard. Phase I of the Decision Tree has mostly been determined. For example, we know that powdery mildew decreases the growth and fitness of garlic mustard (Enright and Cipollini, 2008). If criteria are met in Phase I, Phase II may proceed. Phase II tests the potential control agent for safety. I tested native Brassica plants in the wild and the
greenhouse to determine if they are adversely affected by *E. cruciferarum*. I also tested common *Brassica* cultivars in the greenhouse for powdery mildew infection. If native species are capable of infection and are severely impacted, the powdery mildew may not meet qualifications for use as a biological control agent. If native species get low infection rates or miss the mildew phenologically in the field, the powdery mildew may still be considered a potential candidate. If *Brassica* crops become infected, *E. cruciferarum* could still be considered for use since many farmers spray for mildews and it may not be deemed as a concern. Also, many crops show genetic resistance to mildews and other plant pathogens, which suggests that powdery mildew may not be a potential problem. Finally, many garlic mustard-infested areas are geographically separated from crop growing areas. Thus, if Phase I and Phase II of Berner and Bruckart (2005) Decision Tree are successful, Phase III must be determined United States regulatory agencies such as TAG and APHIS. If APHIS issues a “Finding of No Significant Impact” (FONSI), a permit is issued for the pathogens release. If criteria are not met, often the proposed study of the biocontrol candidate is dropped. A proposal must be written so that regulators can make decisions about the safe release of a biocontrol (Berner and Bruckart, 2005).
RESULTS

A. Distribution of garlic mustard with powdery mildew disease

The distribution of the incidence of garlic mustard with powdery mildew varied across the region (Fig 2). Oak Openings and Mohican State Park were outliers in order to determine if there was a regional difference of *E. cruciferarum* distribution. The outliers and Whitewater Memorial State Park (Indiana) were excluded from Fig. 3 since no powdery mildew was found and so that Southwestern Ohio could be shown more clearly on the map. There were strong significant differences between disease incidence means across parks (F=13.515, P ≤ 0.05). Fig. 4 shows disease incidence of parks from lowest to greatest and shows which parks are significantly different from each other. Deer Creek State Park, Highbanks Metropark, Hueston Woods State Park, Mohican State Park, Sharon Woods, and Whitewater Memorial State Park (Indiana) had no signs of powdery mildew infection. Kiser Lake State Park had the highest average disease incidence (51.55%). Average disease incidence at parks varied from no powdery mildew attacking garlic mustard to 51.55%. Fig. 5 shows the relationship between distance (natural log) of parks from Wright State University (Dayton, OH) and with disease incidence (Decay function= -1.93(x) +11.65, $R^2=0.1793$, F= 3.71, P=0.0708). Since Kiser Lake appears to be an outlier, I removed Kiser Lake from the data and ran a one way ANOVA and it showed that the distance from Wright State was significant (p≤0.05, F=13.51, $R^2=0.4578$). Fig. 6 shows if Kiser Lake was an epicenter the relationship between distance and disease incidence (Decay function= -4.94(x) +23.35, p≤0.0001, F=24.37, $R^2= 0.5890$).
Aspect was also determined at each population and analyzed. There were significant differences between disease incidences on southeast and level aspects and northwest facing aspects (F=3.451, P \leq 0.05). Southeast facing aspect had the highest average percent disease incidence, whereas northwest facing aspect had the lowest percent incidence (Fig. 7).

B. Susceptibility of native Brassicaceous plants to powdery mildew disease in the field and greenhouse

The native species *C. bulbosa*, *C. concatenata*, *C. douglassii*, and *A. laevigata* had no visible signs of powdery mildew infection in the field (Table 2). Garlic mustard plants nearby were also observed and showed no signs of powdery mildew infection at the time of the survey. In the greenhouse study, *C. bulbosa*, *C. concatenata*, *C. douglassii*, and *C. diphylla* were susceptible to becoming infected under optimal conditions for temperature and humidity (Table 3). *C. douglassii* had the highest average disease class (2.3), followed by *C. concatenata* (1.92), *C. bulbosa* (1.0), and *C. diphylla* (0.083).

C. Susceptibility of Brassica crops to powdery mildew disease in the greenhouse

Only Savanna Mustard (*Brassica juncea*) became infected with *E. cruciferarum* in the greenhouse study (Table 4). The average disease class for Savanna Mustard in Trial 1 was 1.88 and 1.22 in Trial 3. Garlic mustard also inoculated with *E. cruciferarum* was used as a control and its disease class average was 1.24. The other crop varieties tested showed no signs of infection. Trial 2 was abandoned due to several issues. The plants became infected in the greenhouse without being infected using the settling tower technique. When it was time to infect the crops, the garlic mustard plants became
severely infested with blue-black aphids. This disrupted the timing and the crops were much older than trial 1 plants by the time they were able to be inoculated (once garlic mustard plants with aphids were treated). I did infect the crop cultivars, but they soon died due to heat stress. Cabbage cv. Everlast had small knots on the leaves after inoculations with powdery mildew (Fig. 8). It is not known if the knots on the leaves were a sign of fighting infection, if they are normal during plant development, or if a pest affected the leaves.
DISCUSSION

In southwestern Ohio, garlic mustard is attacked by a powdery mildew fungus, *E. cruciferarum*, the can reduce the growth and fitness of garlic mustard (Enright and Cipollini, 2007). Distribution surveys of garlic mustard with powdery mildew were conducted primarily in southwestern Ohio to determine the disease incidence at parks. Nineteen parks were surveyed and disease incidence and aspect at each garlic mustard population were determined. This strain of *E. cruciferarum* has not been tested to determine its risks to native Brassicaceous species or Brassica crop cultivars in Ohio. I also surveyed local parks to determine if native spring ephemerals in the field were infected with powdery mildew. In the greenhouse test, native species were transplanted from the wild to establish if *E. cruciferarum* could infect these species under optimal conditions. Brassica crop cultivars were grown in the greenhouse and inoculated with powdery mildew to determine if they could become infected with this strain of *E. cruciferarum*.

A. Distribution of garlic mustard with powdery mildew

The distribution survey showed the locations that have garlic mustard infected with powdery mildew and locations where no infection exists. Parks that were the furthest distance from Dayton, Ohio had no signs of *E. cruciferarum* infection. The disease incidence at parks that had infected garlic mustard plants was apparently randomly distributed. The incidence of disease by *E. cruciferarum* may increase with host population size (Laine and Hanski, 2006). Many parks that showed zero to low disease incidence had much smaller host populations than parks with higher disease incidences. There was no obvious trend in distribution of *E. cruciferarum* infection near
what was considered to be the epicenter (Dayton, Ohio). Kiser Lake State Park had the highest disease incidence, even though it was thought that Dayton, Ohio was the epicenter. Fig. 6 shows that there is a relationship between disease incidence and distance if Kiser is considered the epicenter; however, there is a large gap between Kiser Lake and the closest park. Surveys should be conducted in a radius around Kiser Lake to determine if that is the epicenter or to determine if infection is random. If more parks near Kiser Lake had higher rates of disease incidences then Dayton, Ohio, then it could be determined that the epicenter is around Kiser Lake not Dayton, Ohio. The Dayton area does have many parks with infected garlic mustard plants, but not many parks had a high disease incidence. Several parks in the Dayton area that had low disease incidences had low host population sizes.

Parks were surveyed between 8 July 2008 and 13 August 2008. The gap in time may have caused parks surveyed in August to have slightly higher disease incidences than those surveyed in July, due to more time for the powdery mildew disease to spread infecting more garlic mustard plants (note: most of the parks surveyed in August had no powdery mildew infection). A resurvey of Wright State University Forest Preserve and Eastwood Metropark in mid August 2008 revealed a large increase in disease incidence at WSU (now 64%) relative to July (23%) but only a small change at Eastwood Metropark. It is likely that disease incidence rose through time in most parks since many pathogen spore generations are short (5-10 days) enabling them to expand to new individuals and host populations (Aylor, 2003). Disease incidence at WSU increasing over time and not having a high disease incidence initially could also be attributed to host population sizes (Gilbert, 2002; Laine and Hanski, 2006). Eastwood Metropark had large host size
populations, whereas populations at WSU were not as large and spread out. In addition, local parks that have had reports of powdery mildew infection may also vary in disease incidence from year to year since it is an aerially dispersed pathogen (Jarosz and Burdon, 1992). This variability may have shown parks such as Taylorsville Metropark to have a low disease incidence even though it was thought to have a higher disease incidence in previous years. Variability of disease incidence may occur if heavily diseased populations decline.

The weather was the main factor in the length of time of the distribution survey. When it rained, I had to wait at least 3 days for the powdery mildew to show signs of infection before I could start surveying again. Weather conditions may affect powdery mildew at different sites as well as within sites due to differences in local microclimate and soil conditions (Ovaskainen and Laine, 2006). The distance between parks was also a factor in the amount of time it took to conduct the survey and made it difficult to survey multiple parks in a day. Future surveys should go to at least 2 parks in a day to avoid a gap in survey time. Also, more time could be spent at larger parks where not all areas were surveyed (those with longer trails). This could also affect results as infected populations of garlic mustard may have only been on one side of the park and missed in a survey. Working with more people and splitting up within parks may also speed up survey time while covering a larger area.

Aspect was determined while conducting the survey at parks. The relationship of aspect and disease incidence was examined and showed that level and southeast facing aspects had higher disease incidence than northwest aspects. This could be caused by climatic differences since powdery mildew increases in severity when temperature and
humidity increase (Saharan et al., 2005). In the summer in the Northern Hemisphere, the sun tends to dry out south facing aspects since they receive greater intensity and duration of solar radiation (Warren, 2008). Solar radiation can change climatic conditions such as soil temperature and moisture (Galicia, et al., 1999). There was a significant difference between northwest facing aspect and southeast facing and level aspects, which slightly differs from what Warren (2008) explains. Warren (2008) did not account for the morning sun being less intense in the southeast. Northwest and southwest aspects would receive more intense sunlight during the day drying out those soils. Westward winds could be the main factor in this situation since it can dry out northwest and southwest facing aspects and spread more spores to the southeast and northeast. Populations downwind from infected populations (northeast, southeast, east, and level aspects) are more likely to be infected with powdery mildew (Laine and Hanski, 2006). Parks that were surveyed may have had certain soil characteristics not considered in the distribution survey. Fast water infiltration is associated with loose, dry, and sandy soils, whereas compact, wet, clay soils slowly absorb water (Hudak, 2005). Flat landscapes would facilitate water infiltration, but steep landscapes would support runoff (Hudak, 2005). Therefore, in my study, level aspects were similar to southeast facing aspects since due to higher water infiltration creating a moist and humid area conducive to *E. cruciferarum*. Soil moisture tests and degree of slope should be included in any future surveys to determine if the aspect and slope are affecting the disease incidence of garlic mustard.
B. Susceptibility of native Brassicaceous plants to powdery mildew disease in the field and greenhouse

No native Brassicaceous species surveyed in the wild showed symptoms of disease caused by *E. cruciferarum*. *E. cruciferarum* is the primary source of powdery mildew infection in crucifers (Koch and Slusarenko, 1990). However, the native species surveyed were spring ephemerals and likely escaped powdery mildew infection due to differences in phenology. *Cardamine bulbosa* has one of the latest flowering times of the species studied which extends from April to July, but spring cress in southwestern Ohio finished flowering around June (Rhoades and Block, 2000). The latest flowering time of *C. bulbosa* indicates a gap of about a month from when the last spring ephemeral finishes blooming and senescence and *E. cruciferarum* starts to show visible signs of infecting garlic mustard plants.

The native Brassicaceous species did become infected under greenhouse conditions. Since powdery mildew needs warm, humid conditions to infect plants, the greenhouse offered *E. cruciferarum* the optimal setting for infecting these native species. The greenhouse was used as a comparison to the wild survey to show that under optimal conditions, *E. cruciferarum* infection is possible of native species if inoculum is present. Weather influences the life cycles of both the host and pathogen (Chakraborty et al., 2000). If climate change persists, then pathogens like powdery mildew may be able to infect species of plants it was not previously capable of infecting. Climate change could increase the risks to native Brassicaceous species developing powdery mildew and could greatly affect populations of native species. Climate change could impact native species with powdery mildew disease by decreasing their growth and fitness, hence decreasing
their populations allowing other species, such as garlic mustard, to displace them. However, their phenology could change too. Plants could evolve and flower earlier than previously recorded. If their phenology changed with climate change, then these native species would continue to escape powdery mildew infection. But, if these species flower too early, they may not have insects to pollinate their flowers, causing this species to decline (USA National Phylogeny Network. Phylogeny: The Seasonal Pulse of the Biosphere, http://www.usanpn.org/files/shared/NPN_Fact_Sheet-med.pdf [Accessed 11 June 2009]).

Native spring ephemerals were chosen since they co-occur in forest understory with garlic mustard. Additional species including summer species and other invasive species in the Brassicaceae family need to be tested on a broader scale. Native species such as *Arabis canadensis* and invasive species such as *Brassica rapa* (field mustard) and *Hesperis matronalis* should be examined. The strain of *E. cruciferarum* in Ohio may be able to infect other invasive species such as those previously mentioned.

Currently, native spring ephemerals in the wild are not infected by powdery mildew, but they do compete with garlic mustard. Full removal of garlic mustard increased abundance of native understory plants such as *Viola papilionacea* and *Aster divaricatus* in a study conducted by Stinson et al. (2007). Garlic mustard makes management efforts difficult since it is able to outcompete and displace native species (Murphy, 2005). Control of garlic mustard with herbicide application and manually pulling and clipping garlic mustard are time consuming and costly techniques (Carlson and Gorchov, 2004; Frey et al., 2007; Slaughter et al., 2007; Pardini et al. 2008). Studies must focus on controlling garlic mustard in order to decrease competition for native
spring ephemerals to successively maintain populations. If *E. cruciferarum* can impact garlic mustard in the summer and falls seasons, then native spring ephemerals may benefit due to a decrease in garlic mustard abundance. Plant pathogens can assist in maintaining plant diversity (Gilbert, 2002).

C. Susceptibility of Brassica crops to powdery mildew disease in the greenhouse

Savanna Mustard (*B. juncea*) was the only crop to become infected by *E. cruciferarum*. *E. cruciferarum* has been shown to have the ability to infect turnip and broccoli raab (*B. rapa*) and canola (*B. napus*) (Karakaya et al., 1993; Koike et al., 1997; OK website). The varieties of *B. napus* and *B. rapa* used in the study did not show signs of infection.

In a study conducted by Adam et al. (1999) they inoculated similar Brassica crop cultivars, but obtained different results with *E. cruciferarum* (UEA1- isolate). Their study ranked plants 0-3 (3 showing profuse mycelial development and conidiation). The only Brassica crop cultivar to have similar results to my study was *B. juncea* var. rugosa (Savanna Mustard). In my study, the average disease class of Savanna Mustard was 1.88 and 1.24 on a 0-5 scale, whereas Adam et al. (1999) scored theirs as a 3 (on 0-3 Disease Reaction score). Adam et al. (1999) disease reaction (DR) scores are as follows: “0= limited hyphal development with no conidiation (i.e., fully resistant phenotype); 1, low to moderate hyphal growth with sporadic conidiation; 2, abundant mycelial development with moderate or delayed conidiation; and 3, profuse mycelial development and conidiation (i.e., fully susceptible phenotype).” The difference in disease class scores is likely due to different strains of *E. cruciferarum*. Our isolate of *E. cruciferarum* has not been determined, but our powdery mildew did not infect the same crop varieties. Also, I
determined disease class macroscopically (by eye) and Adam et al. (1999) determined disease incidence microscopically, which could cause discrepancies in rank scores. Not all Savanna Mustard in my study became profusely attacked by *E. cruciferarum*, but most plants that did become infected were moderately to severely infected. Also, some plants senesced which altered disease class from week to week occasionally lowering final classes. Nothing else in my study became infected by *E. cruciferarum*, which differed from Adam et al. (1999). In their study, collards (*B. oleracea* var. acephela) was classified as 1, cauliflower (*B. oleracea* var. botrytis) was classified as 1, cabbage (*B. oleracea* var. capitata) were classified as 1, 2, and 3, Brussels sprouts (*B. oleracea* var. gemmifera) was classified as 2, and *B. rapa* turnips (*B. rapa* var. rapifera) were all classified as 3. Most of the varieties Adam et al. (1999) tested did not match my cultivated varieties which could also explain the differences in disease classes. Adam et al. (1999) did not test *B. napus* (canola), *B. oleracea* var. italica (broccoli), *Glycine max* (soybeans-Fabaceae family), or *Lycopersicon esulentum* (tomato-Solanaceae). The strain of *E. cruciferarum* in Southwestern Ohio does appear to be particularly infective on garlic mustard.

The greenhouse study of Brassica crops infected with powdery mildew is important to local farmers. Farmers can look at the distribution survey to determine if *E. cruciferarum* is in their area and determine if the Brassica species of crop they plant will become infected. They also need to know if this powdery mildew can move or if it is already present in other Brassica growing regions. Determining how far spores of *E. cruciferarum* can travel must be determined in order to establish if this powdery mildew could reach nearby crops. Cabbage farmers need not worry since cabbage in
northwestern and south central Ohio are prone to other diseases (The Ohio State University Extension, 2009). Farmers take precautions and spray fungicides on plants known or suspected of becoming infected. Farmers purchase altered cultivars that resist pathogens (Farnham et al., 2002) such as downy mildew and powdery mildew in order to reduce both pathogens and cost of fungicides as well as increase crop yield. All of these steps can minimize crop loss by powdery mildew infection for local farmers.

D. Decision Tree

Based on results from the greenhouse tests and field studies, the Decision Tree by Berner and Bruckart (2005) was used to determine the potential for *E. cruciferarum* to be used in the management of garlic mustard. Before the study, the majority of Phase I was determined. The Decision Tree had steps in each Phase in order to determine if the proposed pathogen may be suitable as a biological control agent (Fig. 1). Note: the Decision Tree is generally used for exotic pathogens and not all steps relate to our endemic pathogen.  

1. The source and host is known for this pathogen.  
2. Koch’s postulates have been fulfilled.  
3. The pathogen can be maintained in a distinguishable state in the greenhouse.  
4. The pathogen has been identified to species.  
5a. The pathogen has been reported in area of potential release (distribution survey), but it appears to be randomly distributed and potentially infrequent.  
7b. The pathogen has been reported in area of potential release (distribution survey), but it appears to be randomly distributed and potentially infrequent.  
8. The pathogen was tested in the greenhouse on native Brassicaceaeous spring ephemerals and Brassica crop cultivars.  
9. Transmission is independent of a vector.  
10. Infection is accomplished without wounding the plant.  
11. The pathogen causes significant disease severity (on garlic mustard) in greenhouse tests.
Parts of Phase II was determined: testing the severity of disease on non-target plants in the greenhouse, but it was not determined if the disease was damaging to growth or reproduction of non-target species. Part of Phase II was determined: \(^{12b}\) The pathogen appeared to parasitize plants other than the target (garlic mustard). I studied native Brassicaceous spring ephemerals and Brassica crops in the greenhouse. Every native spring ephemeral got infected by powdery mildew under greenhouse conditions. As stated previously, the native Brassicaceous species studied have a different phenology than \(E.\ cruciferarum\). Thus, the greenhouse study merely showed that if the native species and powdery mildew occurred simultaneously, under optimal conditions, infection may occur. Savanna Mustard (\(B.\ juncea\)) was the only crop variety to become infected with \(E.\ cruciferarum\). The other varieties may not have become infected in the greenhouse because it may not have been the right conditions for them to become infected or because of resistant crops being produced, or because this strain is incapable of infecting many Brassica species. Savanna Mustard is not a popular variety grown or sold by local farmers and many farmers spray fungicides to prevent the spread of diseases like \(E.\ cruciferarum\).

The final consideration from the Decision Tree was if the disease is damaging to non-target species. The greenhouse tests for crops show that it is damaging to Savanna Mustard (\(B.\ juncea\)), but as previously mentioned it is not a large threat as it is not a commonly grown crop variety in Ohio. The greenhouse tests to determine if \(E.\ cruciferarum\) infection is damaging to native spring ephemerals were not conducted. The native species did become infected; however, seed production or growth was not studied.
There are no known studies of seed production or growth for the native Brassicaceous species.

The native spring ephemerals were chosen because they co-occur with garlic mustard in forest understory. They were tested in the greenhouse to determine if under optimal conditions they could become infected with powdery mildew. The Brassica crops tested were chosen based on popular varieties. It was known that cv. Cabbage and Brussels sprouts could become infected by *E. polygni* (Tonguc and Griffiths, 2004) and that turnip, broccoli, and canola could become infected by *E. cruciferarum* (Karakaya et al., 1993; Koike et al., 1997; OK website). Thus, the crops chosen were based off of previous studies that showed *Erysiphe* spp. infecting popular crops such as canola. Future studies should test more Brassica crop varieties and study native species and non-native species.

The strain of *E. cruciferarum* that attacks garlic mustard in southwestern Ohio could be used as a biocontrol. This pathogen poses little risk to native species or Brassica crops. The native species in the field escape infection and Brassica crops such as cabbage become infected by other pathogens (The Ohio State University Extension, 2009). Most farmers worry more about downy mildew and black rot than powdery mildew. In order for this strain of *E. cruciferarum* to be further promoted as a biological control agent, more native species and crop varieties need to be tested. It may be useful to test other invasive species in order to determine if this powdery mildew could reduce the abundance of other problem species. Additional crop varieties need to be tested including the more common species of cabbage that are grown in Ohio (Almanac,
Fortress, Genesee, Megaton, and Hinova). Once further testing occurs, it could be
determined if our strain of *E. cruciferarum* would be a successful biocontrol.
Fig. 1. Decision Tree D.K. Berner, W.L. Bruckart / Biological Control 34 (2005) 222–32
Table 1. Parks surveyed for garlic mustard infected with *E. cruciferarum*.

<table>
<thead>
<tr>
<th>Park</th>
<th>Abbreviation</th>
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<td>Forest Preserve</td>
<td></td>
<td></td>
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<td>Eastwood Metropark</td>
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<td>39.78275</td>
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<td>-84.0293</td>
<td>39.69083333</td>
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<td>Glen Helen Nature Preserve</td>
<td>Gln</td>
<td>-83.55188333</td>
<td>39.80098333</td>
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<td>39.7907</td>
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<td>39.80268333</td>
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<td>39.88155</td>
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<td>Caesars Creek State Park</td>
<td>CC</td>
<td>-84.05868333</td>
<td>39.48963333</td>
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<tr>
<td>Sharon Woods (Hamilton Co. Parks)</td>
<td>Sha</td>
<td>-84.40378333</td>
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<td>Deer Creek State Park</td>
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<td>39.62296667</td>
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<td>39.95681667</td>
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<td>Moh</td>
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<td>Greenville Falls Scenic Area</td>
<td>Gre</td>
<td>-84.37606667</td>
<td>40.10928333</td>
</tr>
<tr>
<td>Hueston Woods State Park</td>
<td>Hue</td>
<td>-84.7416</td>
<td>39.57291667</td>
</tr>
<tr>
<td>Whitewater Memorial</td>
<td>Whi</td>
<td>-84.96533333</td>
<td>39.6108</td>
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<td>State Park Indiana</td>
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<td>Highbanks Metropark</td>
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<td>Kis</td>
<td>-83.953855</td>
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<td>Oak Openings Metropark</td>
<td>Oak</td>
<td>-83.84525</td>
<td>41.54486667</td>
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</table>
Fig. 2. Distribution of garlic mustard (*Alliaria petiolata*) at 19 parks naturally infected with powdery mildew (*E. cruciferarum*).
Fig. 3. Distribution of garlic mustard (*Alliaria petiolata*) in southwestern Ohio infected with powdery mildew (*E. cruciferarum*).
Fig. 4. Average disease incidence of garlic mustard (*Alliaria petiolata*) naturally infected with powdery mildew (*E. cruciferarum*) at each park location. Means ±1 SE are shown. Means with a different lower case letter are significantly different at P≤0.05. Number of garlic mustard populations at each park is located above lower case letters.
Fig. 5. Relationship between average disease incidence and distance (natural log) of parks from Wright State University (Dayton, OH).
Fig. 6. Relationship between average disease incidence and distance (natural log) of parks from Kiser Lake State Park (Saint Paris, OH).
Fig. 7. Average disease incidence at each aspect. Means with a different lower case letter are significantly different at $P \leq 0.05$. Number of garlic mustard populations at each aspect are within the bars.
Table 2. Disease incidence of native Brassicaceae plants with powdery mildew (*E. cruciferarum*) observed in the field

<table>
<thead>
<tr>
<th>Family/species</th>
<th>Location</th>
<th>Number Observed</th>
<th>Disease Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brassicaceae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arabis laevigata</em></td>
<td>Cedar Falls</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(Cedarville, OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glen Helen Nature Preserve</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(Yellow Springs, OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>John Bryan State Park</td>
<td>153</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(Yellow Springs, OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cardamine bulbosa</em></td>
<td>Cedar Bog</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(Urbana, OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glen Helen Nature Preserve</td>
<td>1039</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>John Bryan State Park</td>
<td>122</td>
<td>0</td>
</tr>
<tr>
<td><em>Cardamine concatenata</em></td>
<td>Glen Helen Nature Preserve</td>
<td>101</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>WSU Biological Preserve</td>
<td>429</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(Dayton, OH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cardamine douglassii</em></td>
<td>Glen Helen Nature Preserve</td>
<td>173</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>John Bryan State Park</td>
<td>139</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>WSU Biological Preserve</td>
<td>151</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Greenhouse study of native species inoculated with powdery mildew (*E. cruciferarum*) indicate * Number of plants that became infected by *E. cruciferarum*  **
The number of plants that were in each disease score are in parentheses and ranks are based on percentage of the leaf area of the plant that became infected: 0=0%, 1=1-10%, 2=11-20%, 3=21-50%, 4=51-75%, 5=>75%

<table>
<thead>
<tr>
<th>Family/species</th>
<th>Number infected*</th>
<th>Score**</th>
<th>Ave. Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brassicaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cardamine bulbosa</em></td>
<td>15/18</td>
<td>0(3), 1(12), 2(3)</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Cardamine concatenata</em></td>
<td>12/12</td>
<td>1(5), 2(5), 4(2)</td>
<td>1.92</td>
</tr>
<tr>
<td><em>Cardamine diphylla</em></td>
<td>1/13</td>
<td>0(12), 1(1)</td>
<td>0.083</td>
</tr>
<tr>
<td><em>Cardamine douglasii</em></td>
<td>10/10</td>
<td>1(3), 2(2), 3(4), 4(1)</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Table 4. Greenhouse test of crops inoculated with powdery mildew (*E. cruciferarum*).

<table>
<thead>
<tr>
<th>Cultivated</th>
<th>Commercial Variety</th>
<th>Disease Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td><strong>Brassicaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brassica juncea</em> (L.) Czerniak var. <em>rugosa</em></td>
<td>Savanna (mustard)</td>
<td>1.88</td>
</tr>
<tr>
<td><em>Brassica napus</em> (L.)</td>
<td>Canola</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>acephala</em></td>
<td>Georgia (collards)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>botrytis</em></td>
<td>Amazing (cauliflower)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>botrytis</em></td>
<td>Cassius (cauliflower)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>capitata</em></td>
<td>Cairo (cabbage)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>capitata</em></td>
<td>Everlast (cabbage)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>gemmifera</em></td>
<td>Diablo (Brussels sprouts)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>italica</em></td>
<td>Coronado Crown (broccoli)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>italica</em></td>
<td>Early Dividend (broccoli)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. oleracea</em> (L.) var. <em>italica</em></td>
<td>Packman (broccoli)</td>
<td>0</td>
</tr>
<tr>
<td><em>B. rapa</em> (L.) var. <em>rapifera</em></td>
<td>Hakurei (turnip)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Fabaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glycine max</em> (L.) Merr.</td>
<td>Williams (soybean)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Solanaceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lycopersicon esulentum</em> Mill.</td>
<td>Better Boy (tomato)</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 8. Native brassicaceous plants that became infected by *E. cruciferarum* in the greenhouse test.
Fig. 9. Savanna Mustard infected with *E. cruciferarum* in greenhouse study.
Fig. 10. Cabbage Everlast showing knots after *E. cruciferarum* infection in greenhouse study.
LITERATURE CITED


