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Exploring the Host Range, Impacts, and Distribution of Black Rot Disease on Alliaria Petiolata

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EXPLORING THE HOST RANGE, IMPACTS, AND DISTRIBUTION OF BLACK ROT DISEASE ON *ALLIARIA PETIOLATA*

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

by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Gabriela Ivette Harney-Davila ENTITLED Exploring the Host Range, Impacts, And Distribution, Of Black Rot Disease on Alliaria Petiolata BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Harney-Davila, Gabriela Ivette. M.S. Department of Biological Sciences, Wright State University, 2022. Exploring the host range, impacts, and distribution, of black rot disease on *Alliaria petiolata*.

Garlic mustard is an invasive Eurasian biennial spreading in deciduous forests of North America. Garlic mustard plants in Ohio can be infected with a strain of *Xanthomonas campestris*, the causal agent of black rot disease in brassicas. I examined variation in susceptibility to *X. campestris* among garlic mustard populations, several native wild species, and agricultural crop varieties. Twenty-four garlic mustard populations were universally susceptible to *X. campestris*, though disease severity varied. *Cardamine concatenata* and *Cardamine diphylla* were susceptible but can phenologically escape infection in the field. Of the 14 agricultural crops tested, three cultivars (*Raphanus sativus, Brassica rapa var. Rapa Hakurei*, and *cv - Brassica oleracea var. capitata*) were susceptible to the *X. campestris* strain that infects garlic mustards. Nutrient availability enhanced disease susceptibility and severity, but light had a limited effect. A survey of 31 garlic mustard populations in Ohio, Kentucky, and Indiana revealed that *X. campestris* is established throughout a 120 km radial distance from Dayton, Ohio. The strain of *X. campestris* infecting garlic mustard in the Wright State University woods was identified by sequencing as *X. campestris pv. incanae*.

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DEDICATION

I would like to dedicate this thesis to my mother, who passed on the wisdom that "Cada Guaraguao tiene su Pitirre".

I. INTRODUCTION

INVASIVE SPECIES AND INVASIVE PLANTS

When non-native introduced species become over-abundant and impair their new habitat (Torchin et al. 2001, Andersen et al. 2004, Colautti et al. 2004) they are considered invasive. After habitat loss, invasive species are the second leading threat to native wildlife (Dueñas et al. 2018). Global trade and travel are the primary pathways through which invasive plant species are introduced (Hulme 2009). Invasive species threaten native wildlife by disrupting ecosystem function, biodiversity, nutrient cycles, and species interactions (Andersen et al. 2004, Weber and Gut 2004, McCary et al. 2019, Crystal-Ornelas et al. 2021). Invasive species can have significant environmental and economic impacts, such as the invasive Zebra mussel (*Dreissena polymorpha)* which causes economic damage by clogging up water pipes in municipalities and power plants (Andersen et al. 2004). However, not all introductions of non-native species result in negative impacts in their new environment. Crops such as potatoes and maize originated from North and South America and rice which originated from both China and India (Crawford and Shen 1998), are considered integral food items around the world (Hawkes and Francisco-Ortega 1993, Benz 2001). While there are numerous examples of non-native introductions into new ecosystems with positive outcomes on agriculture, there are many cases where introductions of novel species can have detrimental impacts. Invasive species can diminish environmental goods and services for human health (drinking water

quality), disrupt ecosystem functions and biological diversity (through predation, competition, and parasitism), and have indirect impacts by altering nutrient cycles and relationships between species (Andersen et al. 2004, Weber and Gut 2004, McCary et al. 2019). In addition, their introductions have also brought infectious agents and disease vectors that present a risk to the public health of people, agriculture, fauna, and floral populations (Andersen et al. 2004). Invasive species have been known to exhibit ecological impacts either directly or indirectly upon introduction or later in time once they have acclimated and adapted to the new environment (Charles and Duke 2008). Direct impacts come from invasive species competing with native species, suppressing, or even replacing the native species population all together, sometimes via assistance from pathogens/parasites that native species are not adapted/immune to (Charles and Duke 2008). They can also disrupt mutualistic relationships that promote growth (McCary et al. 2019).

Various hypotheses have been proposed to explain the mechanisms of success of invasive species. Catford et al. (2009) looked at several hypotheses that could explain why some species become invasive (empty niche hypothesis, opportunity windows hypothesis, invasion window hypothesis, resource availability, etc.) whereas Stohlgren et al. (2002) looked at hypotheses that could explain why some habitats become or are vulnerable to invasion (vegetation richness and environmental factors such as light, water, and temperature). van Kleunen et al. (2010) tried to identify traits that promoted

invasiveness in plants species other than the mechanisms mentioned earlier. They found, from the 125 invasive and 196 noninvasive species examined, that invasive species had higher physiological traits such as growth rate, fitness, and shoot allocation, compared to the non-invasive species. They concluded that invasive plants in general had higher values of physiological traits compared to non-invasive species and that the traits promoting invasiveness can occur under various conducive circumstances. van Kleunen (2010) summarized that though there are studies that assess a few traits that promote invasiveness, further studies focusing on traits that directly or indirectly promote invasiveness should be the focal point.

An often-cited hypothesis for the success of invasive species is the enemy-release hypothesis. Invasive species may thrive in their new environment since there is little population regulation by predators or pathogens whereas in their native environment their population density is regulated by the natural predators/pathogens from their home range. The enemy-release hypothesis (ERH) states that upon introduction of invasive species to a new nonnative habitat, they should experience a decline in regulation by specialist herbivores or pathogens and therefore result in an increase in abundance and distribution (Keane and Crawley 2002). In the native range of plant species, there are on average about 16 parasites whereas that number drops to three parasites in their introduced range (Middleton 2019). This can be seen in a study by Torchin et al. (2001) where the body size and biomass of the European green crab (*Carcinus maenas*) was compared between

their native and their introduced ranges. Green crabs in the introduced ranges (Australia, South Africa, United States east and west coast) were found to have fewer parasites with each new introduction resulting in larger body size and biomass. An experiment done by Wolfe (2002) also tested the ERH using the perennial plant *Silene latifolia* (white campion), which is native to Europe and was introduced to North America. White campion experienced greater regulation by generalists in its native range versus its introduced range and benefited from the absence of two specialists in the introduced range that greatly reduced the fitness of the plant in the native range. Wolfe concluded that white campion's success could partially be explained by escape from enemies. Keane and Crawley (2002) did a similar study looking at the ERH but focused more on understanding which species in particular would most likely benefit from the enemy release. While a number of studies support enemy release as a primary mechanism for invasion (Wolfe 2002, Torchin et al. 2001, Meijer et al. 2016), there are those that reject or are critical of the ERH in some manner (Colautti et al. 2004, Keane and Crawley 2002). Colautti et al. (2004) and Keane and Crawley (2002) both concluded that the enemy-release is most fitting for species that lack dominant specialists and are attacked only by generalists with low impact.

Although invasive plants often lack predators that would normally regulate their population density and distribution, there is mounting evidence that herbivores, predators, and pathogens are adapting to some invasive plants and could reduce fitness and impacts of the invasive plant. Pathogen accumulation is the process by which over time, native

pathogens adapt and successfully infect the novel invasive host species with increasing frequency resulting in the decline of the invasive host's population density and distribution (Flory and Clay 2013). There are numerous examples of pathogen accumulation whereby invasive species have become infected by pathogen(s) in their introduced range. Some examples are attack of *Alliaria petiolatae* (garlic mustard) by *Erysiphe cruciferarum* (powdery mildew) (Ciola and Cipollini 2011, Enright and Cipollini 2009), *Pueraria lobate* (Kudzu) by *Phakopsora pachyrhizi* (Asian soybean rust) (Fabiszewski et al. 2010), *Microstegium vimineum* (Japanese stiltgrass) by *Bipolaris* fungi (Kleczewski and Flory 2010), and currently, *Alliaria petiolatae* (garlic mustard) by *Xanthomonas campestris* (black rot). These examples of pathogen accumulation on invasive species suggest that this phenomenon is not local to any one region in particular but is widespread and may be a means for invasive species regulation in the future (Flory and Clay, 2013). It is important to note that to have a successful suppression of an invasive plant using biological controls, will require host-specific control agents that are able to reduce plant fitness faster than the population can be replaced (Davis et al. 2006). In the case of garlic mustard, which has shown relevant evidence in fostering and justifying use of bio controls via pathogen accumulation, there have been numerous proposals on biocontrol agents to regulate population growth and expansion.

Treatment methods to control and reduce distribution of garlic mustard have included non-biological and biological control. Non-biological methods consist of herbicide, prescribed fire (scorch), hand-pulling, and combinations of those treatments

(Nuzzo 1991, 1994, Slaughter et al. 2007, Shartell et al. 2012). Treatment efficacy has varied, with some reduction of adult garlic mustards by herbicide, prescribed fire, hand-pulling, and combinations of those treatments (Nuzzo 1991, Nuzzo 1994, Shartell et al. 2012). Herbicide, prescribed fire, hand-pull/herbicide, and hand-pull/scorch treatments decreased the abundance of seedlings, however there was an increase in abundance of garlic mustard the following year (Shartell et al. 2012). Garlic mustard seeds can be viable in the soil for up to five years and require treatments to be done yearly until garlic mustard have been removed and vacant for a minimum of three years (Nuzzo 1991). Non-biological methods though effective, are labor and cost intensive (Nuzzo 1991) and may not be a practical method to control garlic mustard.

Biological methods include the use of insects to control garlic mustard. Garlic mustard, in its native range, was attacked by approximately 70 insects and seven fungi (Blossey et al. 2001). Two European specialist weevils were tested in field studies in Illinois and Michigan from 2005 to 2008 that reduced garlic mustard seed production (Evans et al. 2012). One species controlled 63% of garlic mustard alone, while together controlled 88%. Despite the potential success and lower cost of using these biocontrol agents, research and regulation practices have hindered the import and release of these biological controls (Orion 2015).

GARLIC MUSTARD

Garlic mustard (*Alliaria petiolata*; Brassicacaea) is a biennial invasive shade-tolerant plant species that occurs throughout the northeastern and Midwestern U.S., Alaska (Blossey et al. 2003) and in southern Canada (Figure 1) (Cavers et al. 1979, Byers and Quinn 1998). Garlic mustard was introduced from Europe into North America in the mid-1800s for ornamental and culinary uses (Rodgers et al. 2008, Cipollini and Cipollini 2016), as well as medicinal properties (Cavers et al. 1979). Garlic mustard is commonly found in disturbed sites like trails, roadsides, and edges of forest and gardens (Cavers et al. 1979) and can be distinguished by its large heart-shaped leaves (Rodgers et al. 2008). In its native range in Eurasia, garlic mustard species is a winter annual or biennial, however in the North America it is biennial (Dhillion and Anderson, 1999, Cipollini et al. 2020, Byers and Quinn 1998). Seeds germinate in the early spring forming rosettes, a cluster of leaves, which will grow throughout the year enduring the winter months as well as keeping its dark green color (Stinson et al. 2007, Cavers et al. 1979). Rosettes are vulnerable to summer droughts with a 60-90% mortality rate by the fall (Driesche et al. 2002). Rosettes mature by spring of their second year and produce a tall flowering stem (0.304-1.22m) and siliques (seed pods) before dying (Cavers et al. 1979, Drayton and Primack 1999). Though historically garlic mustard has preferred moderate exposure to light, moist areas, and cool temperatures, which has allowed it to grow in forest understories with full or partial shade (Lankau et al. 2009, Cavers et al. 1979), it can be

found in drier and full-sun areas (Byers and Quinn 1998, Cavers et al. 1979), being the possible result of selection for different life histories. Garlic mustard becomes a permanent member of the community once it is established (Davis et al. 2006, Rodgers et al. 2008) and is more likely to invade areas with high species richness (Driesche et al. 2002). Its high seed production and low dispersal distances allow garlic mustard to grow in highly dense strands (Cruden et al. 1996), spread, and reduce biodiversity in the surrounding area. Rodgers et al. (2008) suggested that garlic mustard's success may come from its mating system with three aspects paving the way as a successful invader. First, Rodgers et al. (2008) stated that their flowers are adapted to be pollinated by generalist or self-pollinated by the second day if not visited by a pollinator. Second, every pollinated ovule will develop into a viable seed (hence high seed production), and thirdly garlic mustard is able to cross-pollinate with other populations ensuring genetic variability. Overall, garlic mustard can disrupt ecosystems, alter food webs, and affect biodiversity in many respects (Stinson et al. 2007).

The continued spread and increase in abundance of garlic mustard is likely partly due to its ability to escape herbivores and pathogens (ERH), among other plant traits (Ciola and Cipollini 2011). Garlic mustard has a variety of secondary plant compounds such as flavonoids, glycosides, and glucosinolates (responsible for the sharp taste) allowing it to deter herbivory and contributing to its success as an invasive species (Rodgers et al. 2008). In North America there is very little herbivore pressure (Blossey et al. 2001). Its fast growth rate and ability to adapt to a variety of habitats, coupled with

enemy escape, will allow the continued spread and further impacts of garlic mustard on native microbial, floral, and faunal abundance and diversity.

Faunal impacts of garlic mustard can be seen with *Pieris virginiensis* (West Virginia white butterfly) and *Pieris oleracea* (Mustard white butterfly), two native insects that are unable to distinguish garlic mustard from their native host plants (such as *Dentaria laciniata*) which results in the death of their larval progeny (Davis and Cipollini 2014, Keeler and Chew 2008, Davis et al. 2015, Augustine and Kingsolver 2017). Courant et al. (1994) reported that the mustard white butterfly populations in New England appear to be adapting to garlic mustard despite continued harm to fitness. Garlic mustard is also known to disrupt the mutualistic relationship between mycorrhizal fungi and herb and tree seedlings (McCary et al. 2019, Roberts and Anderson 2001), with devastating impacts on competing plant success in areas where garlic mustard is found. However, there is mounting evidence (Ciola and Cipollini 2011, Enright and Cipollini 2009, Tancos and Fredericks 2020) that pathogens such as powdery mildew and black rot are accumulating on garlic mustard and having negative impacts on its fitness and therefore show potential for garlic mustard population regulation. For example, powdery mildew disease caused by *Erysiphe cruciferarum* has been found in garlic mustard populations by Enright and Cipollini (2007, 2009) and Ciola and Cipollini (2011). Enright and Cipollini (2007) reported that powdery mildew could reduce garlic mustard populations because infected first year plants experience high declines in growth and increased mortality and second year plants were significantly smaller with greatly

reduced seed production. In a further study Enright and Cipollini (2009) examined if powdery mildew infection could alter garlic mustard's competitive impact on a native species, *Impatiens pallida* (jewelweed). They found that when garlic mustard was inoculated with powdery mildew disease it nullified its competitive impact on *I. pallida*.

XANTHOMONAS CAMPESTRIS: CAUSAL AGENT OF BLACK ROT

Xanthomonas campestris is a gram-negative mesophilic bacterial plant pathogen that causes black rot disease in cruciferous plants (Dow et al. 2003). It can be a highly destructive disease (Vicente and Holub 2012). The genus *Xanthomonas* alone has a vast host range including over 66 genera of nine monocotyledonous families and 160 genera of 49 dicotyledonous families (Vicente et al. 2001). *Xanthomonas campestris* originally was divided into 123 pathovars based on host-specificity, but since has been proposed to be reclassified based on DNA-DNA hybridization due to the idea that *X. campestris* species should be restricted (Vicente et al. 2001, Fargier and Manceau 2007). Xanthomonas campestris has been restricted to only six pathovars that causes disease in Brassicaceae family: *X. campestris pv. campestris*, *X. campestris pvs. aberrans (Knösel) Dye, armoraciae (McCulloch) Dye, barbareae (Burkholder) Dye, incanae (Kendrick & Baker) Dye,* and *raphani (White) Dye* (Vicente et al. 2001, Fargier and Manceau 2007).

Though found globally, particularly where brassica crops are cultivated, black rot thrives best in humid tropical regions such as Southern and eastern Africa (Vicente and

Holub 2012) where *X. campestris* is the largest agricultural threat. It has caused severe crop damage in the United States, Japan, India, and Turkey (Vicente et al. 2006) as well. Warming temperatures due to climate change may lead to weaker constraints allowing for distribution expansion. Black rot was first reported in North America in New York, Kentucky, Iowa, and Wisconsin from 1893 to early 1900s affecting cabbage, rutabaga, and turnips (Vicente and Holub 2012).

This bacterium spreads to plants primarily through infected seeds or in water droplets and invades through the pores in the leaf margins, called hydathodes (Hugouvieux et al. 1998). *Xanthomonas campestris* has the pathogenicity to suppress plant host defense (Weber et al. 2005). Symptoms of *X. campestris* infection are easy to identify by the darkening V-shaped chlorotic lesions extending from the leaf margins (Hugouvieux et al. 1998) (Figure 6). The disease eventually spreads to the plant's vascular system in the leaves and stems, leading to heavily reduced growth and often, death of the plant (Swings and Civetta 2012). Though *X. campestris* thrives best in warmer climates, it can persist in low temperatures where symptoms of infection are not as evident (Cook et al. 1952). The host range of many strains of *X. campestris* is known in agricultural settings, but incomplete when it comes to wild plant species (such as garlic mustard) since there is less economic interest in them (Swings and Civetta 2012). Understanding the impacts on non-agricultural plants is important, however, in part to learn if they may serve as reservoir host for the black rot pathogen which sustains the threat to agricultural species.

Environmental factors and their influence on disease susceptibility and development in plants have been well studied (Jones 1924, Colhoun 1973, Schoeneweiss 1975, Roden and Ingle 2009, Gullino and Garibaldi 2018). High humidity, coupled with warm temperatures can increase the susceptibility and severity of bacterial blight, caused by *Xanthomonas hortorum pv. pelargonii* on geranium (Gullino and Garibaldi 2018). Symptoms of bacterial blight can develop in 7 days at 27 °C, in 21 days at 16 °C, with symptoms most prominent between 21-23 °C, but symptoms are suppressed at 10–15 °C or 32-38 °C (Gullino and Garibaldi 2018). Jones (1924) found that soil disease agents of various plant species often shared optimal temperature for growth as well as how environmental factors alter susceptibility to disease. An example given by Jones (1924) is orchard disease apple scab, caused by the ascomycete fungus *Venturia inaequalis*, a low temperature disease, which is common in the Rosaceae (Bowen et al. 2009). The fungus is present in dead host plant leaves during winter and will mature to produce ascospores for secondary infection in the same host come early spring. However, the progress of apple scab disease is hindered by increasing temperatures as summer approaches. As for *X. campestris*, the optimal temperature range is 25-30 °C, with symptoms less evident or not present at temperatures below 18 °C (Carisse et al. 1999).

Environmental factors like light and nutrient availability will likely prove important in determining disease susceptibility and severity in garlic mustard populations. Light can affect the host's defense response and the pathogen's virulence (Roden and Ingle 2009) while light intensity and duration of light can influence fungal

spore germination, penetration, infection type, and vitality (Colhoun 1973). Mineral nutrient deficiency or excess can impact host vitality and alter defense responses (Schoeneweiss 1975). Different concentrations of potassium, nitrogen, and phosphorus have been shown to enhance or diminish the susceptibility of rice cultivars to *X. campestris pv. oryzae*, the causal agent of bacterial leaf blight in rice. Mohanty, Reddy, and Sridhar (1983) found nitrogen concentrations at 60 and 120 mg/l and phosphorus concentration at 25 mg/l enhanced susceptibility to the disease, but symptoms were significantly reduced at nitrogen concentration of 100 mg/l and potassium at 160 mg/l. Major plant nutrients like nitrogen, phosphorus, and potassium can increase or decrease susceptibility of plants to pathogens which may be ascribed to effects on plant vigor, growth rate, and direct effects on the pathogen (Colhoun 1973).

XANTHOMONAS ON GARLIC MUSTARD

In 2010, Cipollini had *X. campestris* identified by the Ohio State Plant Diagnostic Clinic from infected garlic mustard plants from Dayton, Ohio (D. Cipollini, pers. comm,) but did no further work on it at the time. Recently, Tancos and Frederick (2020) published the first official identification and sequencing of *X. campestris* infecting garlic mustard in the United States. Tancos and his lab at the USDA are investigating the host range for *X. campestris pv. campestris* and garlic mustard as a potential reservoir host. In the past year we have observed increases in the incidence of black rot on garlic mustard

in Ohio that was identified as *X. campestris* based on morphological characteristics. It appears that black rot has greater negative impacts on the fitness of garlic mustard than other pathogens that have been studied (Figure 1 and Figure 2) with plant populations declining significantly over the six-week observation when *X. campestris* was present in the field. Ciola and Cipollini (2011) found that though powdery mildew is well established on garlic mustard populations there was variation in disease incidence and susceptibility, with some populations with no signs of disease. Qualitative and quantitative variation in resistance to powdery mildew in garlic mustard have been studied (Ciola and Cipollini 2011, Cipollini et al. 2020), however the degree to which garlic mustard expresses qualitative and quantitative variation in resistance to *X. campestris* is unknown. While variation in susceptibility within and among populations is currently uncharacterized, it is plausible that garlic mustard is a more broadly suitable host for *X. campestris* than for powdery mildew.

Figure 1. Observations of *X. campestris* disease progression and plant response in a population of garlic mustard adjacent to the Wright State University Woods' over 6 weeks from July 5th to August 9th, 2021.

Figure 2. Observation of *X. campestris*'s impacts on garlic mustard population adjacent to the Wright State University Woods as of April 13, 2022, eight months after infection (See Figure 1).

BIO CONTROL RISK AND ASSESSMENT

Interest in *X. campestris* and its relationship with garlic mustard has led to a collaboration with Dr. Matthew Tancos of the Foreign Disease-Weed Science Lab of the USDA-ARS. Dr. Tancos and his lab are working to characterize *Xanthomonas* species in native and non-native cruciferous weeds and examine its potential as a biocontrol.

Although *X. campestris* had been detected years prior and the relationship between the pathogen and wild plant species (in this case) garlic mustard are being studied, the impacts the pathogen has specifically on garlic mustard are unknown as well as variation of susceptibility among garlic mustard populations (Ciola and Cipollini 2011, Enright and Cipollini 2009).

The objectives of my study were to explore the distribution, host range, and fitness effects of *X. campestris* on garlic mustard. My goals were to (i) examine variation in susceptibility and impacts of *X. campestris* on different garlic mustard populations in Ohio and Pennsylvania, (ii) determine the relative susceptibility of garlic mustard populations and several native species (two-leaf toothwort, cut leaf toothwort, and pale jewelweed) and agricultural species to predict potential reservoir hosts, (iii) examine the influence of environmental variables (light/shade availability and nutrients) on the susceptibility of garlic mustard to *X. campestris*, (iv) survey garlic mustard populations in the field for the presence of *X. campestris* to understand the distribution and incidence of this pathogen in Ohio, Kentucky, and Indiana, and (v) compare sequences of different strains of *X. campestris* from our collection to that of the USDA agricultural database.

II. METHODS

VARIATION IN SUSCEPTIBILITY TO *X. CAMPESTRIS* AMONG GARLIC MUSTARD POPULATIONS

To examine variation in susceptibility and impacts of *X. campestris* on different garlic mustard populations in Ohio and Pennsylvania, I opportunistic collected garlic mustard seeds from Ohio and Pennsylvania from multiple populations (Table 1) based on accessibility to roads, trails, and accessible land. Seeds were stored dry in the lab from June through Sept. 2020. I collected seeds from a minimum of 15 garlic mustard plants per population from each site to have representation of the variation of a population. Seeds were cold stratified in petri dishes lined with filter paper at 4 ◦C until germination (approximately 90 days) (Figure 3). Distilled water was added as needed to keep the seeds moist during this time. Ten seedlings of each garlic mustard population excluding BMSP (four seeds), EWP (six seeds), STN (nine seeds), TMP (five seeds), WPH (nine seeds), WSUBW1 (nine seeds), and YS (eight seeds) were transplanted in ProMix BX potting soil in 300 ml plastic pots. Plants were watered as needed with distilled water alternating with Nutriculture 20-20-20 soluble fertilizer every two weeks.

The experiment was conducted in a temperature-controlled lab on six tables, with four tables being 2 x 0.9 m and two tables being 1.3 x 0.7 m. Each table held four plant

trays (27 x 54 cm) that each held 18 plant pots (Figure 4). Trays were spaced 6 cm from each other. Fluorescent grow lights (white light 4100k) were placed on each table that produced a light intensity of 45± 5 µmol PAR/ m2/s at plant level on a 16hr light/8hr dark cycle. Plants from different populations were randomly allocated to both tray and table prior to treatment. Plants were grown for six weeks prior to inoculation.

Each plant was artificially inoculated using the scissors-clipping method by clipping the tips of each fully expanded leaf with sterile scissors dipped in diseased leaf inoculum (Peňázová et al. 2018). Controls were mock inoculated with sterile water (Tancos and Frederick 2020). Three cuts of 1.5 ± 0.25 cm per leaf for a total of nine cuts per plant were made per secondary vein near the leaf margins (Figure 5.) (Vicente et al. 2001). The suspension was made from a homogenate of visibly infected garlic mustard leaves grown in the laboratory or collected from the Wright State University Woods. The inoculum was made by homogenizing 5 g of infected leaves in 10 mL of DI water. This diseased leaf inoculum could have introduced other pathogens to the plants, however, I did not observe any disease symptoms other than those associated with *X. campestris*. Development of V-shaped yellow/necrotic lesions extending from the infection points (a characteristic symptom of infection by *X. campestris*) were documented every three days following inoculations (Tancos and Frederick 2020) by recording the area of the leaf diseased as a percentage. Disease progressions was followed for 30 days. I visually

assessed percent of disease for each leaf and took the average of the leaves measured on each plant to obtain plant average of percent of leaf area diseased.

The percentage of leaf area diseased was converted to proportion and arcsine square root transformed prior to statistical analysis. Repeated measures MANOVA using the Pillai's trace test statistic was used to examine variation in disease progression through time across population on day 9 and 30 as several sites had limited sample size. Univariate ANOVAs on disease data from each time point were used to analyze variation among populations in disease susceptibility using JASP [version 0.16]. Moran's I was calculated to determine if populations were spatially autocorrelated based on their susceptibility rates or were spatial outliers using R statistical software (v.4.1.3; R Core Team 2022). Controls were omitted since no symptoms developed. Significance was determined with an $\alpha = 0.05$.

Table 1.-Sites where garlic mustard seeds were collected.

Location Names	Abbreviation	Longitude (West)	Latitude (North)
Caldwell Park Preserve	CPP	-84.491928	39.201946
Wright State Cemetery-1	WSUC-1	-84.055727	39.785283
Wright State Brick Wall-1	WSUBW-1	-84.060025	39.779998
Anderson Township	ATS	-84.407193	39.085162
John Bryan State Park	JBBT	-83.859110	39.792038
West Price Hill	WPH	-84.582386	39.105863
Madisonville	MV-2	-84.395633	39.156104
Mt. Airy	MA	-84.560684	39.173612
Jacoby Rd	JR	-83.903496	39.764110
Yellow Springs	YS	-83.883565	39.804995
Alms Park	ALP	-84.428717	39.112573
Taylorville Metro Park	TMP	-84.164046	39.879627
Hyde Park Ault Park	HP	-84.416531	39.134495
Monfort Heights Woods	MHW	-84.599143	39.173113
Mt Echo Park (Price Hill)	MEP	-84.568723	39.095012
Carriage Hill Metro Park	CHMP	-84.08924	39.87609
Englewood Metro Park	EWP	-84.284942	39.884542
Clifton	CTN	-84.523146	39.156303
College Hill	CH	-84.527277	39.194914
Burnett Woods Clifton	BWC	-84.518822	39.138718
Muddycreek MSD Saylor Park	MSDMC	-84.684616	39.103376
St. Bernard	STB	-84.502671	39.157508
Barkcamp State Park	BCSP	-81.027319	40.046012
Black Moshannon State Park	BMSP	-78.064631	40.918100

Figure 3. Cold stratification of garlic mustard seeds in petri dishes lined with filter paper and 5 ml of DI water with seeds germinating after 90 days.

Figure 4. Layout and design of growth room.

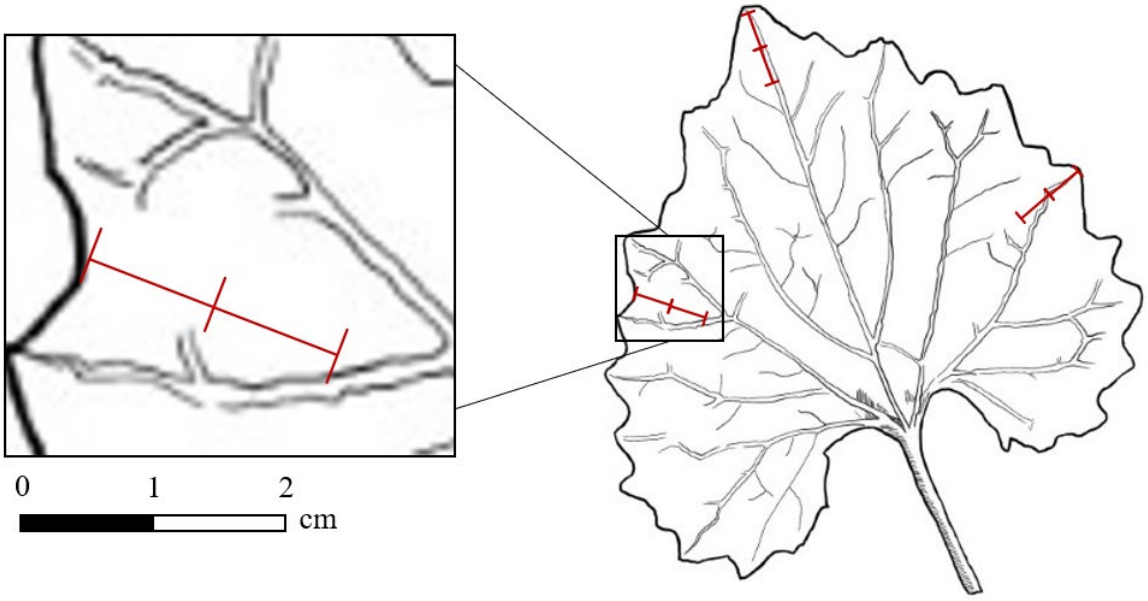

Figure 5. Illustration of garlic mustard receiving inoculation of *X. campestris* via the cutting technique using scissors dipped in a diseased leaf inoculum. Red marks are where cuttings occurred with a length of 1.5 ± 0.25 cm.

HOST RANGE OF THE GARLIC MUSTARD STRAIN OF *X. CAMPESTRIS* AND IMPACTS ON HOST PLANTS

To determine the relative susceptibility of several native species, I collected individuals of *Cardamine diphylla*, *Cardamine concatenata*, and *Impatiens pallida Nutt.* in natural areas where they grow alongside garlic mustard populations from March 2021 to May 2021. These species were chosen because they share similar environments as well as membership in the Brassicaceae (Cruciferae) excluding *Impatiens pallida* (Balsaminaceae). Similar species were examined in Ciola and Cipollini (2011) apart from jewelweed which Enright and Cipollini (2009) examined when looking at powdery

mildew as a proposed biocontrol for garlic mustard. *Cardamine diphylla* (two-leaved toothwort) is a native plant of eastern North America commonly found in riparian areas in forested habitats. It is a perennial forb with two leaf types (basal and stem) that flowers from April to May (Montaut et al. 2010). This species is the host plant for *Pieris virginiensis*, and other members of the Pieridae family. It is present in eastern Ohio, but not in the Wright State University Woods.

Cardamine concatenata (cutleaf toothwort), formerly known as *Dentaria lancinata*, is a perennial, spring ephemerals of forest understories, that blooms in March to May before going dormant by early summer (Rhoades and Block 2000). Cutleaf toothwort is a host for native butterflies in the Pieridae family. The species is commonly found in Ohio's woods, including the Wright State Woods (Rhoades and Block 2000). *Impatiens pallida Nutt. (Balsaminaceae)* (pale jewelweed) is an obligate annual plant (Gross et al. 1998) which can be found in deciduous forest of eastern North America (Schemske 1984). The species co-occurs with garlic mustard in the range where it has been introduced and is present in Wright State University woods (D. Cipollini, personal observation). It can produce two kinds of flowers: cleistogamous (automatic self-pollination) flowers and chasmogamous (cross-pollination) flowers (Gross et al. 1998, Schemske 1984).

Cardamine concatenata and *Impatiens pallida* were collected from the Wright State University Woods, a 220-acre natural woodland on the campus of Wright State

University (Dayton, 39.783316, -84.057704). *Cardamine diphylla* was collected from a private property in Hocking Country (39.526394, -82.654027). For examinations of susceptibility of these species to black rot under controlled conditions, I collected 10 wild individuals of the three native species from the field. One leaf per plant was detached and placed individually into a petri dish lined with filter paper. An incubator bioassay technique was optimized using detached leaves of garlic mustard which developed classic symptoms associated with black rot disease when inoculated and placed in incubator (Figure 6). Distilled water was added to the dishes as needed to keep the leaves turgid during this time. Leaves were randomly inoculated receiving either a diseased leaf inoculum or mock inoculated with sterile DI water using same procedure as in the first study. Each leaf received six cuts and dishes were kept at 25◦ C with 16hrs light/8hrs dark cycle. Development of V-shaped yellow/necrotic lesions and leaf condition (color deformities) at the infection point were documented daily for two weeks assessing the area of the leaf diseased as a percentage using same process as first study. Symptoms of *X. campestris* occurred more rapid in bioassay technique than when inoculated on a whole plant.

Percent leaf area diseased was converted to proportion and arcsine square root transformed to meet assumptions of ANOVA. MANOVA using Pillai's trace test statistic was used to examine variation in disease progression through time across the species. A univariate ANOVA on each time point was used to examine variation among the three

species in disease susceptibility. Values of zero were changed to 0.001, 0.002, or 0.003 and values of 100s were changed to 95-100 to allow analysis to run due to lack of variation. Value significance was determined with an $\alpha = 0.05$.

Figure 6. Detached leaf bioassay of susceptibility of garlic mustard to *X. campestris*. Photo is garlic mustard 11 days after inoculating using scissors cutting method.

SCREENING SUSCEPTIBILITY OF OHIO AGRICULTURAL SPECIES AND CULTIVARS

The susceptibility of a variety of commercially available agricultural species and cultivars to the strain of *X. campestris* infecting garlic mustard were examined under controlled condition as in experiment 1. Ten seeds from each species were each planted in ProMix-BX potting soil in 300 ml pots and were watered with distilled water on an as needed basis in the laboratory under growth lights (fluorescent light cool white 4100k) on 16-hr light/ 8-hr dark cycles. Agricultural species and varieties included were: Corn - *Zea mays*; Cabbage - *Brassica oleracea var. capitata*. Cairo Hybrid and cv. Everlast Hybrid;

Turnip - *Brassica rapa var. Rapa Hakurei*; Tomato - *Solanum lycopersicum*; Mung bean - *Vigna radiata*; Wheat – *Triticum*; Kale - *Brassica oleracea var. sabellica*; Cherry belle radish - *Raphanus sativus*; Long Island improved Brussel sprouts - *Brassica oleracea var. gemmifera*; Green wave mustard - *Brassica juncea*; Green sprouting calabrese Broccoli – *Brassica oleracea*; Bok choy - *Brassica rapa subsp. Chinensis* - Shanghai green; and Kai choi – *Brassica juncea* - Hirayama. Stock (*Matthiola incana*) was included since studies have shown (Vicente et al. 2001) *X. campestris* pv. *incanae* attacking it. This species is an annual, biennial, or perennial herb belonging to the Brassicaceae family (Miceli et al. 2019) and is native to southern Europe in the Mediterranean region where olive trees are grown and cultivated (Hickman 1993, Miceli et al. 2019). An isolate of *X. campestris* that was detected infecting garlic mustard in Maryland (Tancos and Fredericks 2020) aligned closely with a strain that attacks stock (*Matthiola incana*).

Seeds for all species were acquired from commercial sources (Carolina, Hudson Valley Seeds, Burpees 2021). Inoculations of *X. campestris* were performed using same procedure as experiment 1 with 10 plants of each species or cultivar being inoculated with a diseased leaf inoculum and 3 mock inoculated with DI. Controls for stock were not conducted due to limited seed availability. Plants were watered with distilled water as needed. Disease severity was recorded every three days for 30 days using same criteria as the first study. The percentage of leaf area diseased was converted to proportion and

arcsine square root transformed prior to analysis. MANOVA using Pillai's trace test on days 6, 15, 21, and 30 and ANOVA analysis on leaf area diseased using JASP software to determine if there were any variations among the species in disease susceptibility. Values of zero were changed to 0.001, 0.002, or 0.003 to allow analysis to run. Significance was determined with an $\alpha = 0.05$.

ENVIRONMENTAL EFFECTS ON DISEASE SUSCEPTIBILITY AND IMPACT ON GARLIC MUSTARD

To examine the influence of environmental variables on the susceptibility of garlic mustard to *X. campestris*, I conducted a factorial light and nutrient study outside the WSU greenhouse from May to June 2021. A treatment plot was set up next to the greenhouse. The plot was 2m x 3m and contained six 1 X 1 m plots to test the effect of light and nutrient availability on disease susceptibility (Figure 7). The six plots were separated by 0.3m to ensure shade was not cast on full light plots. Shade enclosures were made of PVC pipes and measured 0.46m (h) x 0.76 m (w). Shade cloths were draped 0.23 cm over the sides of the enclosure, leaving unshaded portion on bottom to allow for air circulation. I collected forty uninfected first year garlic mustard plants from Wright State University Woods on May 10, 2021, transplanted them in ProMix PX potting soil in 1.67L plastic pots in the greenhouse, and left them to acclimate for one week. Plants were watered with distilled water as needed. I then moved the plants outside to acclimate to

outdoor conditions for an additional week under shade cloths. Prior to treatment, I recorded petiole length and leaf width of leaves that were inoculated to follow disease progression and decline in leaves through time.

Ten garlic mustard plants were randomly assigned to each of the four treatments combinations (high light and high nutrients: HLHN, high light and low nutrient: HLLN, low light and high nutrient: LLHN, and low light and low nutrient: LLLN). Light treatment consisted of garlic mustards plants receiving either full light exposure (high) in three of the 1X1 m plots or placed under a shade cloth of black polyethylene that blocked 80% of ambient sunlight (low) in the three other plots. Shaded plots were equipped with shade cloth mounted on a square frame constructed with PVC pipes (~0.46m h) (figure 7a and 7b). The nutrient treatment consisted of half of the garlic mustards in each light treatment receiving Nutriculture 20-20-20 fertilizer at either 8.00g per 1 L (high) or 1.83g per 1 L (low) every two weeks. I artificially inoculated each plant using the scissors clipping method using a diseased leaf inoculum. Three cuts per expanded leaf for a total of nine cuts per plant were made on secondary veins near the leaf margins (Vicente et al. 2001). I made the suspension from infected garlic mustard grown in the laboratory or collected from the Wright State University woods. Development of V-shaped chlorotic/necrotic lesions at the infection point were documented every five days following inoculations (Tancos and Frederick 2020) assessing the area of the leaf diseased as a percentage. Plants that died throughout were assigned a 100% to indicate

completely diseased and dead. Combination treatments were implemented over 40 days. Garlic mustard controls that were mock inoculated never developed symptoms and were not included in this study. Data was converted from percentage to proportion and arcsine square root transformed prior to analysis. MANOVA using Pillai's trace test, and ANOVA were done on leaf area diseased using JASP software to determine if there were any independent or interactive effects of light and nutrient availability on disease progression.

I measured petiole length (cm) and leaf width (cm) on fully expanded leaves at three points: before inoculation (day 0), midpoint (day 26), and last day (day 40) of treatment. Petiole length of inoculated leaves was measured from base of the stem to the base of the leaf blade. Leaf width and petiole length of inoculated leaved on dead garlic mustards were given measurements of 0 and were included in analyses. Survival analysis was done on plants in the experiment using log rank test via the Astasta online calculator utilizing the R package 'survival'. A log rank test with $\rho=0$ was performed to compare survival rates of the four treatments of light and nutrient availability influence on disease susceptibility. MANOVA using Pillai's trace test and ANOVA were done on petiole length and leaf width to determine if influence of inoculations had impacts on plant's growth. Analysis significance was determined with an $\alpha = 0.05$.

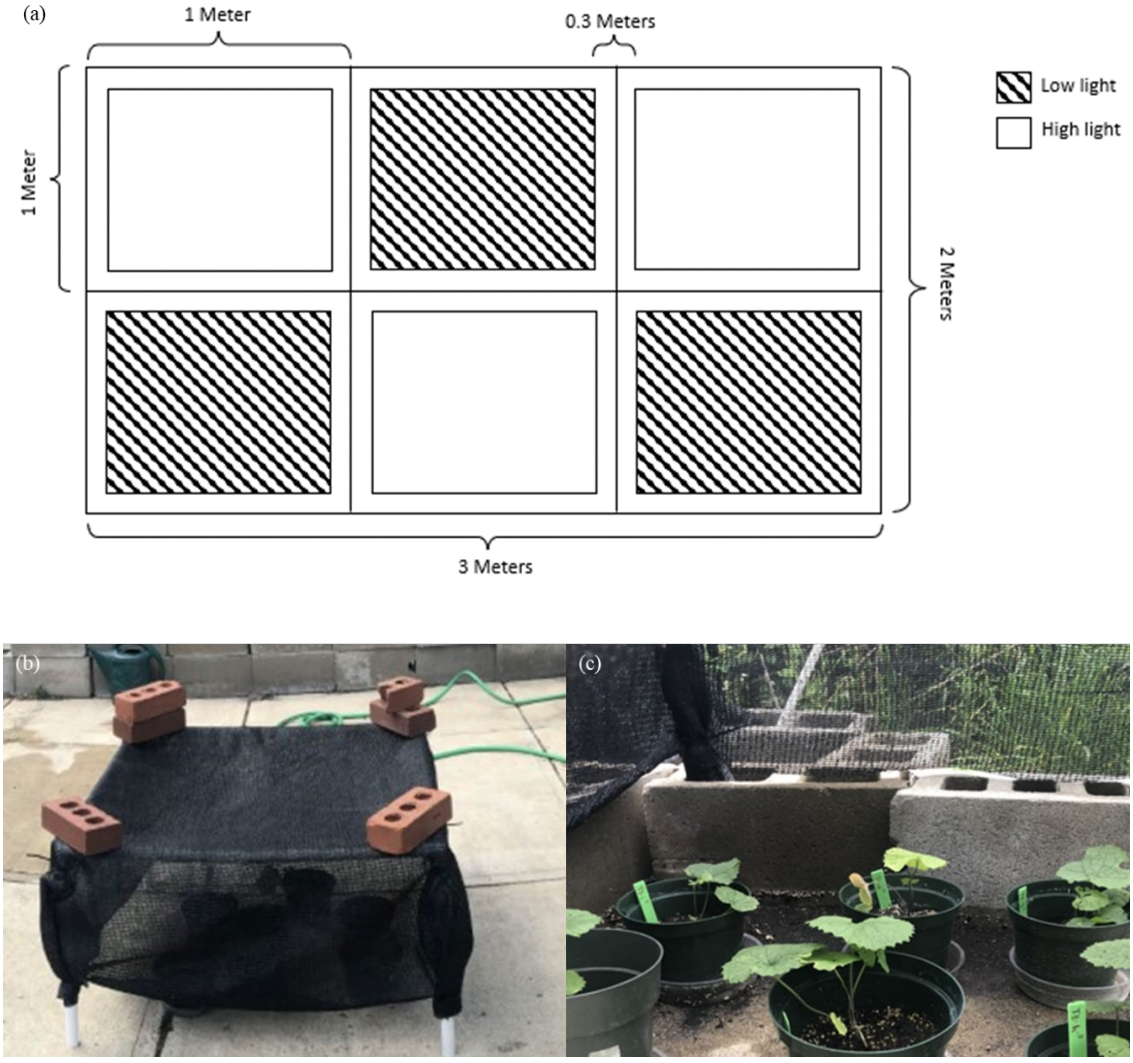

Figure 7. Layout and dimensions of treatment plot with blocks of each of the two light treatments: (a) plot layout; (b) photo of one of the shade enclosures adjacent to the Wright State University greenhouse with shade cloth draping 0.23 m down the sides which allowed circulation of air; (c) photo taken inside the shade enclosure with garlic mustard plants.

DISTRIBUTION OF GARLIC MUSTARD WITH *X. CAMPESTRIS* DISEASE SYMPTOMS

I determined the distribution and disease incidence of *X. campestris* on garlic mustard populations in Ohio, Indiana, and Kentucky by surveying garlic mustard plants in 30 natural areas for symptoms consistent with *X. campestris*. Disease incidence is defined as the number of plants with symptoms of disease out of the total population surveyed. Surveys were conducted during from June 30 to August 12 of 2021, when visible symptoms of disease on susceptible host plants are most evident (Ruissen et al 1993). Surveys were conducted in 30 natural areas within a 120 km radial distance of Dayton, Ohio (Table 2). Natural areas were chosen based on public accessibility and trail availability. Surveys of garlic mustard populations for the presence of *X. campestris* were done using the same rapid sampling technique as Ciola and Cipollini (2011). I focused on populations of garlic mustard along trails and forest edges and recorded canopy cover at each survey site except WSUCW and HH. Canopy cover was determined by estimating how much of the canopy was open to the sky where full sun exposure was 100%, partial shade was 50%, and full shade at 25%. At each survey site, patches of first year garlic mustard were identified that were a minimum distance of 50m from other patches surveyed. Disease incidence of selected patches of garlic mustard were calculated as Ciola and Cipollini (2011), by dividing the number of first-year individuals showing visual symptoms such as necrotic V-shaped lesions in a patch by the total number of first-

year individuals in that patch. In most cases, I was able to recognize symptoms consistent with *X. campestris*, but some symptoms could have been the result of other factors. Plants that were entirely killed by *X. campestris* by the time of surveys could have been missed since *X. campestris* favors high temperature and humidity with symptoms most evident at 25-30 °C (Cook et al 1952; Ruissen et al. 1993, Van der Vossen and Kocks 1993). This makes estimates of disease incidence rates conservative. Measurements occurred on trails, and I walked until a patch was found. A patch of plants with a minimum of 30 individuals and at least three quantifiable patches had to be present to be considered a population. From there, I moved a minimum of 50m from the surveyed patch until another patch was found and quantified. Disease incidence of each patch within each population surveyed was averaged to determine the average disease incidence at each surveyed site.

Diseased incidence for each population was arcsine square root transformed. ANOVA was done to determine if there was any variation in disease incidence among the populations. I used Pearson's correlation analysis to determine if there was a relationship between disease incidence and canopy cover. Canopy cover (%) was converted to proportion and arcsine square root transformed prior to analysis. Values of zero were changed to 0.001, 0.002, or 0.003 to allow analysis to run for canopy cover. I calculated Moran's I to determine if disease incidence rates were spatially autocorrelated or were

spatial outliers using R statistical software (v.4.1.3; R Core Team 2022). Significance was determined with an $\alpha = 0.05$.

Table 2. Natural areas surveyed for the incidence of garlic mustard with symptoms of *Xanthomonas campestris* infection.

Park	Abbreviation	Longitude (West)	Latitude (North)
Buck Creek State Park	BCSP	-83.967254	39.967254
Scioto Auduban Metro Park	SAMP	-83.009118	39.948804
Kiser Lake State Park	KLSP	-83.972683	40.194751
Faurot Park	FP	-84.128441	40.731901
Tawawa Park	TAP	-84.131056	40.28862
Richwood Lake Park	RWLP	-83.298777	40.421643
Carriage Hill Metro Park	CHMP	-84.089282	29.873527
Wright State University Woods	WSUW	-84.058476	39.788230
Englewood Metro Park	EWP	-84.284942	39.884542
Summit Lake State Park	SMLSP	-85.304554	40.026697
Lake Loramie State Park	LLSP	-84.358477	40.360938
Ouabache State Park	OSP	-85.102426	40.721604
Winton Woods	WW	-84.512091	39.258558
Greenville City Park	GCP	-84.630618	40.110065
Springwood Park	SWP	-84.901102	39.853745
Whitewater Memorial State Park	WWMSP	-84.963805	39.605889
Hueston Woods State Park	HWSP	-84.736727	39.576517
Rentschler Forest Metro Park	RFMP	-84.498939	39.421195
Covered Bridge Park	CBP	-84.611725	38.994235
Versailles State Park	VSP	-85.225519	39.077790
Ault Park	AP	-84.415063	39.133440
Brum Woods	BW	-85.234035	39.292589
Mount Airy Forest	MTAW	-84.565137	39.171004
Caesar Creek State Park	CCSP	-84.018899	39.519486
Battelle Darby Creek Metro Park	BDCMP	-83.206705	39.918349
Pike Lake State Park	PLSP	-83.209106	39.156753
Tar Hallow State Park	THSP	-82.740926	39.387026
East Fork State Park	EFSP	-84.139271	39.005666
Rocky Fork State Park	RFSP	-83.531102	39.185267
Stonelick State Park	SLSP	-84.073815	39.216490
Hocking Hills State Park Forest	HHSPF	-85.536418	39.420965

COLLECTION AND COMPARISON OF SEQUENCES OF *X. CAMPESTRIS* STRAINS

To compare sequences of different strains of *X. campestris* from our collection of infected garlic mustard to that of the USDA agricultural database, sequencing and identification of our bacterial strain was accomplished via our collaborators at USDA and were compared to other known strains of *X. campestris*. Infected garlic mustard leaves from the Wright State Woods were collected and sent to the USDA: Foreign Disease-Weed Science Research. Putative Xanthomonads were isolated and cultured from the leaf samples and were sequenced and pathogenicity was verified via inoculations. DNA was isolated from the isolated bacteria and the 16S rRNA gene and eight conserved loci (atpD, dnaK, efP, glnA, gyrB, rpoD, tpiA, and fyuA) were sequenced and analyzed for pathovar determination as in Tancos and Frederick (2020).

III. RESULTS

VARIATION IN SUSCEPTIBILITY OF *X. CAMPESTRIS* AMONG GARLIC MUSTARD POPULATIONS

All garlic mustard populations were at least partially susceptible *X. campestris* and disease symptoms increased through time (Table 3, Figure 8). MANOVA revealed

disease susceptibility variation among populations ($F_{23,153} = 5.121$, $P < 0.001$). ANOVA showed there was significant variation among populations in disease susceptibility on day 9 ($F_{23,153} = 4.571$, $p < 0.001$), day 21 ($F_{23,153} = 7.00$, $p < 0.001$), day 24 ($F_{23,153} = 9.521$, $p < 0.001$), day 27 ($F_{23,153} = 13.354$, $p < 0.001$), and day 30 ($F_{23,153} = 15.569$, $p < 0.001$). Day 1, day 3, and day 6 were not analyzed because some symptoms were present, but most populations had not yet developed symptoms.

By day 30, BMSP exhibited the lowest susceptibility (5.3%) compared to CTN, who exhibited the highest susceptibility (90.7%). CTN, CPP, MSDMC, MEP, ATS, and WPH exhibited high levels of mean disease leaf area (>75%) by day 30 whereas WSUBW, BMSP, YS, TMP, and EWP exhibited the lowest levels of mean disease leaf area (<12.2%) (Figure 8). I acknowledge low disease rate may be due to the inoculum being less virulent or concentrated at this inoculation since I know the WSUBW population is indeed highly susceptible to *X. campestris*. WSUBW, BMSP, YS, TMP, and EWP all shared a similar inoculum preparation and date of inoculation. Low sample size due to seedling limitations for populations BMSP, EWP, and TMP as well as a much later time of inoculation may have resulted in an underestimate of their susceptibility to the disease. Overall, symptoms of disease were evident by day 9 in all populations with a mean leaf area diseased of 5.7% and by day 40 plants exhibited a mean of 47.0% leaf area diseased. Symptoms began appearing in ATS, MHW, and STB on day 3 and BWC by day 6. The appearance of symptoms increased steadily throughout the 40 days. Susceptibility tended to be positively spatially autocorrelated with high disease

susceptibility populations clustered spatially near other high sites and low disease susceptible populations clustered near other low disease incidence sites ($I = 0.118$, $p = 0.075$) (Figure 9a and 9b).

Table 3. Percentage of leaf area diseased with visible *X. campestris* disease symptoms of the 24 garlic mustard populations over the 30 days. Controls for all populations were omitted since no symptoms developed. Day 1 was omitted since no symptoms appeared and day 18 no data was recorded. - = no data recorded. N = 10 plants per population excluding: BMSP (N = 4), EWP (N = 6), STN (N = 9), TMP (N = 5), WPH (N = 9), WSUBW1 (N= 9), and YS (N = 8). See Table 1 for site abbreviations. Standard deviations were omitted to allow for accessible visualizations.

Populations	Day 3	Day 6	Day 9	Day 12	Day 15	Day 21	Day 24	Day 27	Day 30
CPP	0.0	0.0	3.2	12.3	28.6	51.3	70.9	74.3	86.9
WSUC-1	0.0	0.0	1.8	3.8	10.5	36.1	47.0	54.0	55.6
WSUBW-1	0.0	0.0	0.3	0.7	1.0	3.9	5.0	7.6	7.6
ATS	0.5	1.0	3.8	-	18.8	40.3	52.3	72.3	77.6
JBBT	0.0	0.0	1.4	4.0	8.3	23.8	29.8	40.8	46.9
WPH	0.0	0.0	4.4	12.1	21.4	48.6	68.7	70.5	75.8
MV-2	0.0	0.0	0.5	3.0	6.4	20.3	27.9	35.8	39.4
MA	0.0	0.0	0.5	1.1	3.4	10.1	13.0	17.4	21.1
JR	0.0	0.0	1.8	3.4	11.5	24.4	34.9	43.4	47.3
YS	0.0	0.0	0.2	0.5	3.2	7.0	8.7	10.7	12.2
ALP	0.0	0.0	2.0	8.0	9.9	30.6	44.0	53.1	47.5
TMP	0.0	0.0	0.0	0.0	4.5	6.8	9.0	9.5	11.5
HP	0.0	0.0	2.4	11.3	17.0	41.4	52.4	60.1	64.4
MHW	0.4	0.8	2.3	-	8.6	26.5	31.3	44.1	64.6
MEP	0.0	0.0	3.6	15.9	26.9	45.6	66.9	74.9	78.0
CHMP	0.0	0.0	0.6	1.9	5.5	15.5	19.1	24.1	12.4
EWP	0.0	0.0	0.2	0.8	2.2	5.0	6.4	8.8	9.4
CTN	0.0	0.0	2.6	15.6	28.4	53.4	65.9	85.6	90.7
CH	0.0	0.0	1.3	3.3	5.1	15.6	21.8	27.9	30.5
BWC	0.0	0.6	2.6	-	10.0	28.0	30.6	41.8	57.5
MSDMC	0.0	0.0	3.1	9.9	26.9	48.4	63.4	76.0	81.4
STB	0.3	0.5	2.5	-	10.5	23.3	27.0	32.4	33.5
BCSP	0.0	0.0	1.3	6.4	14.6	36.8	49.9	65.6	70.0
BMSP	0.0	0.0	0.7	0.7	1.0	2.0	3.7	4.7	5.3

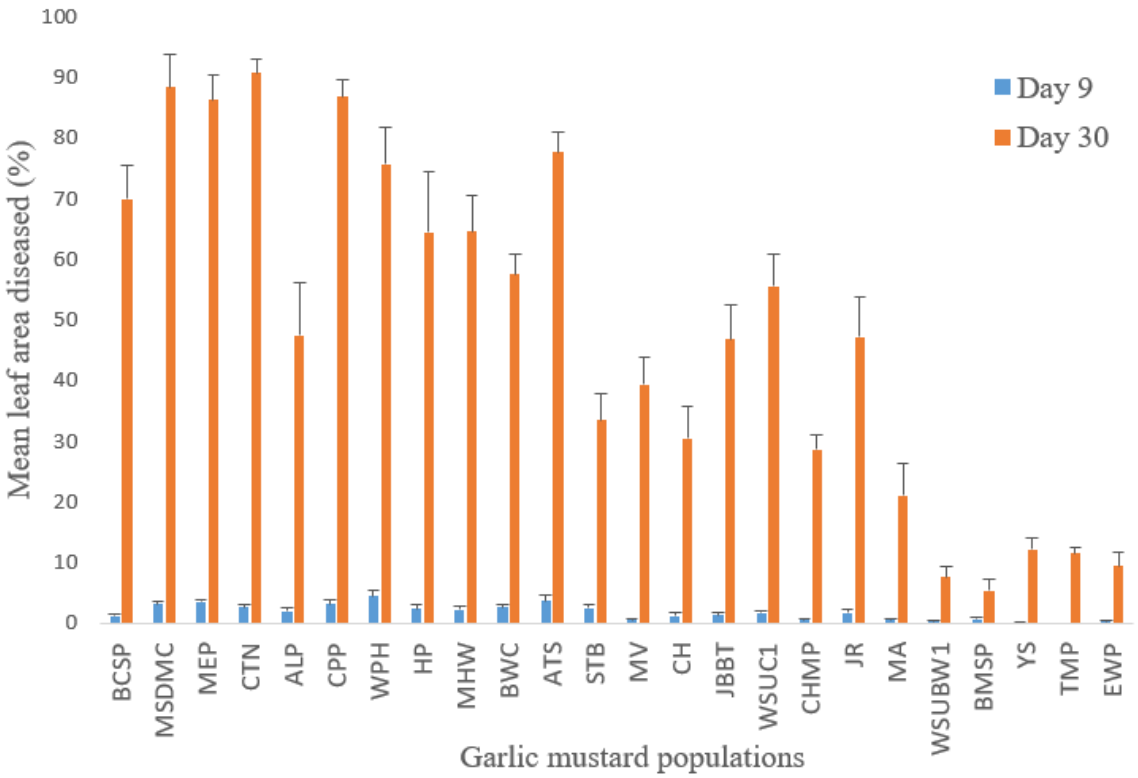

Figure 8. Mean (+1SE) percentage of leaf area diseased with visible *Xanthomonas campestris* disease symptoms of 24 garlic mustard (*Alliaria petiolata*) populations on day 9 and day 30 after inoculation. Day 9 was when development of symptoms was first observed across the 24 populations. See Table 1 for site abbreviations.

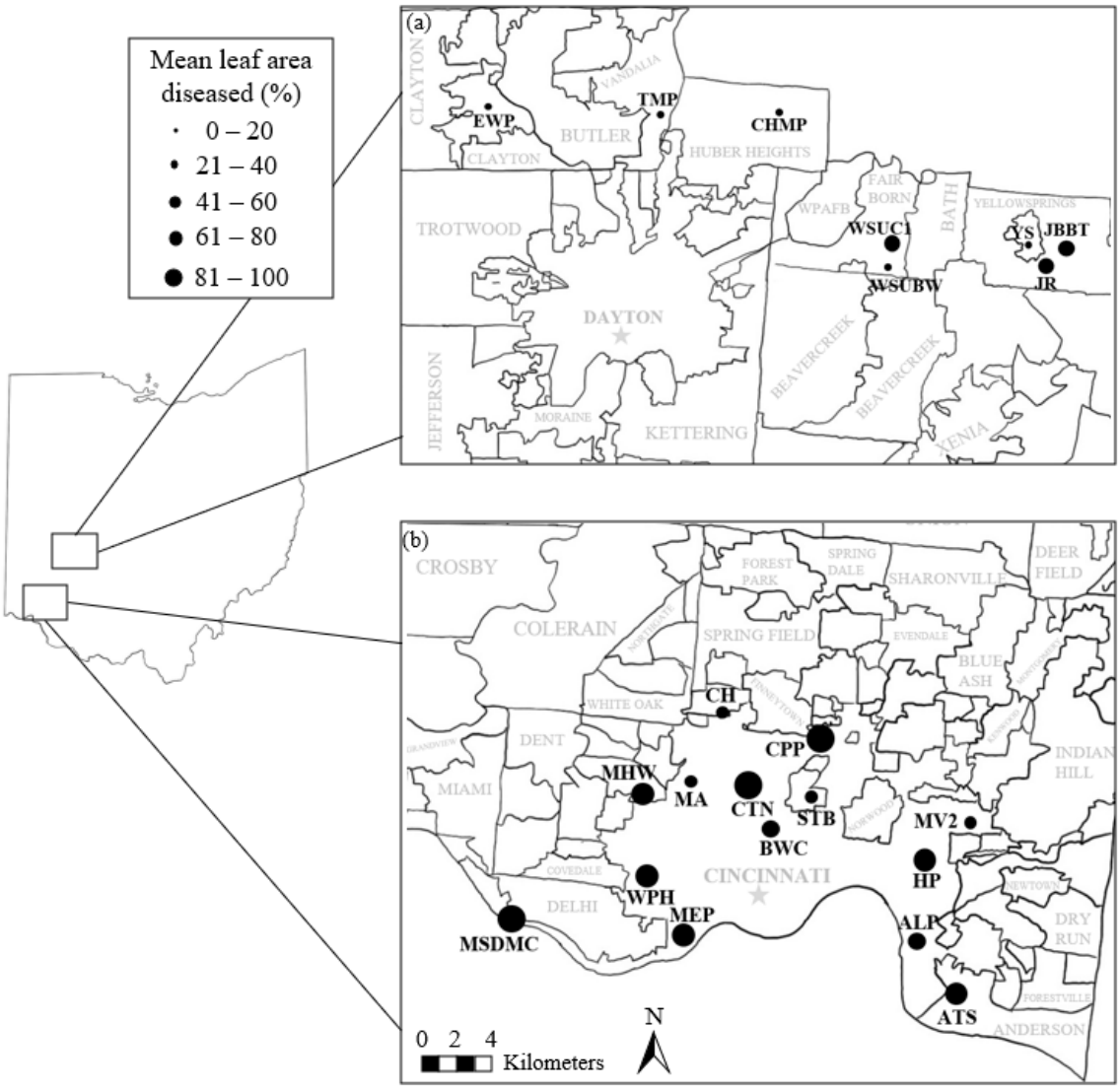

Figure 9. Mean percentage of leaf area diseased with visible *Xanthomonas campestris* disease symptoms on the 24 garlic mustard (*Alliaria petiolata*) populations collected in Ohio from (a) Dayton and (b) Cincinnati. Population BCSP and BMSP are not marked on the map. See Table 1 for site abbreviations.

IDENTIFICATION OF HOST RANGE OF THE GARLIC MUSTARD STRAIN OF *X. CAMPESTRIS* AND IMPACTS ON HOST PLANTS

Cutleaf toothwort and twoleaf toothwort were susceptible to *X. campestris* with disease symptoms increasing over time (Table 4, Figure 10, Figure 11). Pale jewelweed was not susceptible to *X. campestris* and not included in analyses. Between cutleaf toothwort and twoleaf toothwort, MANOVA showed significant variation of disease susceptibility. Cutleaf toothwort exhibited greater susceptibility to *X. campestris* than twoleaf toothwort on days 6,7,8,11,12,13, and 14 ($F_{1,14} = 228.329$, $p < 0.001$). There was significant variation in disease susceptibility on day 6 ($F_{1,14} = 730.312$, $p < 0.001$), day 7 ($F_{1,14} = 1619.485$, $p < 0.001$), day 8 ($F_{1,14} = 1921.688$, $p < 0.001$), day 9 ($F_{1,14} = 680.212$, $p < 0.001$), day 10 ($F_{1,14} = 321.964$, $p < 0.001$), day 11 ($F_{1,14} = 229.227$, $p < 0.001$), day 12 ($F_{1,14} = 114.024$, $p < 0.001$), day 13 ($F_{1,14} = 60.666$, $p < 0.001$), and day 14 ($F_{1,14} = 58.545$, $p < 0.001$). Day 1 through five were not analyzed since variance in twoleaf toothwort was zero.

Responses induced by *X. campestris* in cutleaf included the presence of necrotic lesions at sites of inoculations, which differs from the typical morphological symptoms associated with *X. campestris*. The necrosis spread throughout the leaf on day 3 with further expansion on day 4 ranging with 85-100% leaf area diseased. Further expansion of necrosis continued to day 4 with no typical V-shaped lesions associated with *X.*

campestris. By day 9, all cutleaf plants had browned completely to 100% leaf area, while controls remained green and healthy in appearance. Twoleaf toothwort showed the typical V-shaped necrotic lesions and chlorosis at sites of inoculation on beginning on day 6 with a mean of 1.1% leaf area diseased and finished on day 14 with mean of 40.6% leaf area diseased. Pale jewelweed had no responses to *X. campestris* inoculations.

Table 4. Mean percentage of leaf area diseased with visible *X. campestris* disease symptoms of pale jewelweed, cutleaf toothwort, and twoleaf toothwort over the 30 days. Controls for all populations were omitted because no symptoms developed. Day 1 and 2 were omitted since no symptoms appeared. N = 10. Standard deviations were omitted to allow for accessible visualizations.

Time (days)	Species		
	Pale jewelweed	Cutleaf toothwort	Twoleaf toothwort
3	0	21.9	0
4	0	55.3	0
5	0	92.5	0
6	0	93.8	1.38
7	0	95.3	5.38
8	0	97.1	9.88
9	0	100	14.8
10	0	100	23.8
11	0	100	31.6
12	0	100	39.5
13	0	100	46.9
14	0	100	50.8

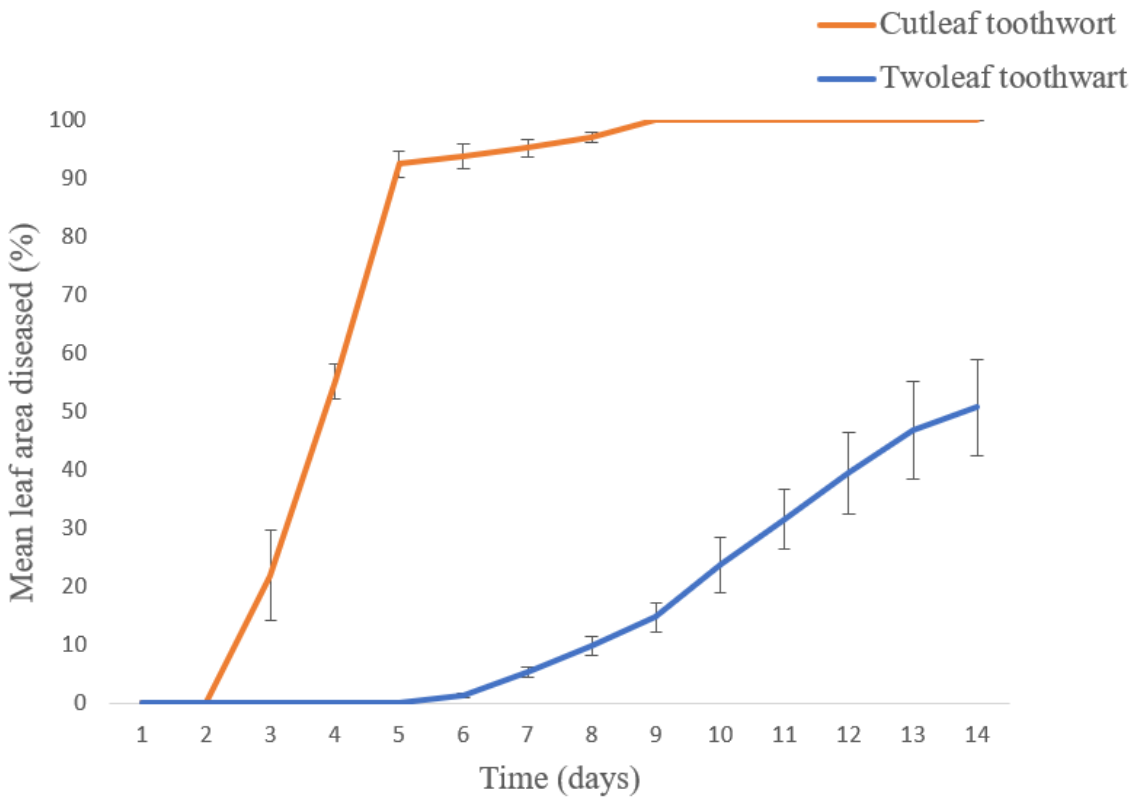

Figure 10. Mean (1±SE) percentage of leaf area diseased with visible *X. campestris* disease symptoms cutleaf toothwort and twoleaf toothwort over 14 days. Controls for all 3 species were omitted since no symptoms developed. Pale jewelweed is omitted since no symptoms appeared. N = 10 plants per population.

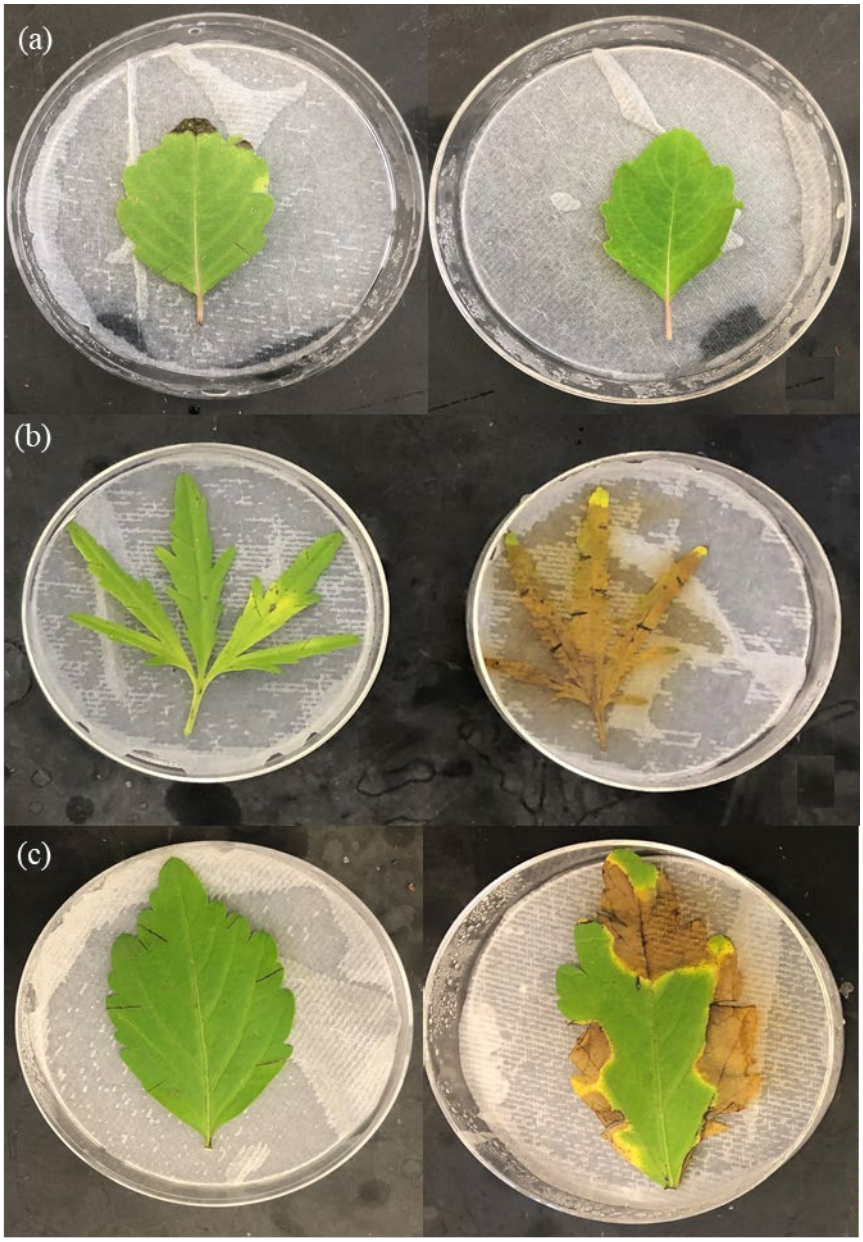

Figure 11. Responses induced by *X. campestris* on (a) pale jewelweed, (b) cutleaf toothwort and (c) twoleaf toothwort 14 days after inoculation. Controls are on the left and inoculated on the right.

Susceptibility to *X. campestris* varied among the 14 agricultural species by day 30 (Table 5). MANOVA revealed variation in disease susceptibility across the agricultural species ($F_{13,125} = 3.074$, $p < 0.001$). ANOVA revealed significant variation in disease susceptibility on day 9 ($F_{13,125} = 12.508$, $p < 0.001$), day 12 ($F_{13,125} = 18.327$, $p < 0.001$) day 15 ($F_{13,125} = 10.573$, $p < 0.001$), day 18 ($F_{13,125} = 5.117$, $p < 0.001$), day 21 ($F_{13,125} = 7.772$, $p < 0.001$), day 24 ($F_{13,125} = 11.283$, $p < 0.001$), and day 30 ($F_{13,125} = 19.561$, $p < 0.001$). Days 1 and 3 were not analyzed since plants showed no symptoms at that point.

Susceptibility of the 14 agricultural species varied with three species experiencing >45% leaf area diseased. Cherry belle radish experienced the highest leaf area diseased (75%) followed by cabbage (53.1%) and turnip (47%). Corn, tomato, wheat, broccoli, bok choy, and green mustard experienced minor leaf area diseased ranging between 0.4-12 %. Kale, brussels sprouts, and kai choi had the lowest leaf area diseased with no symptom development. Responses seen in corn, tomato, wheat, Bok choy, broccoli, and green mustard appeared to be reactions to the wound, as evidenced by lesions near site of inoculation, and were negligible. Stock and mung bean did not develop typical V-shaped lesions associated with infection by *X. campestris*. Stock exhibited yellowing of the inoculated leaves spreading in the direction of the stem over the 30 days (figure 12n). Mung bean exhibited a hypersensitive response with darkening of the veins and at the margins of the blade at inoculation site starting by day 9 (figure 12e). Darkening of the leaves appeared restricted to the margins and did not reach the midrib. Small purple spots

on inoculated leaves occurred on day 9 and were seen to occur on leaves that were not actively inoculated by day 30. Controls for all 14 species developed no symptoms.

Table 5. Mean percentage of leaf area showing response to inoculation of the 14 agricultural species over the 30 days. Controls for all populations were omitted since no symptoms developed. Day 1 was omitted since no symptoms appeared and day 27 no data was recorded. N = 13 plants per population excluding cabbage (N = 12).

Species	day 1	day 3	day 6	day 9	day 12	day 15	day 18	day 21	day 24	day 30
Stock	0	0	0	0	0	0	0	0	0	0
Corn	0	0	0	0	0.7	0.8	0.8	0.8	0.8	0.8
Tomato	0	0	0	0	0	0.4	0.4	0.4	0.4	0.4
Mung bean	0	0	0	0	0	0	0	0	0	0
Wheat	0	0	0	0	0.1	0.4	0.4	0.7	0.7	0.7
Kale	0	0	0	0	0	0	0	0	0	0
Cherry belle radish	0	0	0.1	1.8	4.3	10.7	29.8	53	64.5	75
Brussel sprouts	0	0	0	0	0	0	0	0	0	0
Turnip	0	0	0.1	2.7	8	8.5	30	38.5	46.5	47
Cabbage	0	0	0	0.11	1.33	5.11	24.6	43.7	52.9	53.1
Broccoli	0	0	0	0	0.8	1	1	1	1	1
Bok choy	0	0	0.1	0.5	1.2	2	7	8	12	12
Kai choi	0	0	0	0	0	0	0	0	0	0
Green mustard	0	0	0	0	0	0	0.3	0.4	0.6	0.6

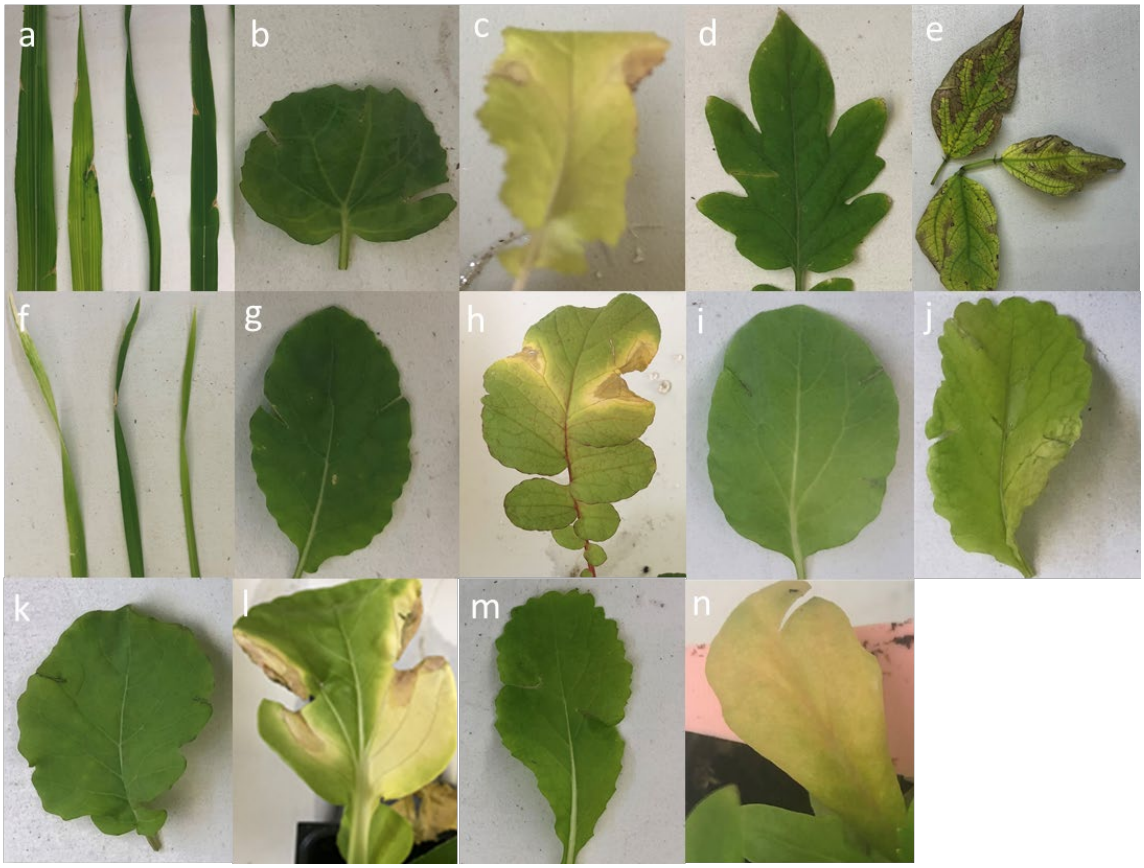

Figure 12. Responses to inoculations induced by *Xanthomonas campestris* on agricultural species and varieties (a) corn, (b) cabbage; (c) turnip; (d) tomato (e) mung bean; (f) wheat; (g) kale; (h) cherry belle radish; (i) brussel sprouts; (j) green wave mustard (k) broccoli; (l) bok choy; (m) kai choi-Hirayama; and (n) stock, an ornamental plant. Photos taken 33 days after inoculation.

EFFECTS OF ENVIRONMENTAL FACTORS ON GARLIC MUSTARD SUSCEPTIBILITY

In all four treatments combinations, disease occurred with several plant dying before the end of the 40-day period with leaf area diseased increasing steadily (Figure 13). MANOVA showed disease susceptibility and development were significantly higher in the fertilized plants and that there was no significant effect of light availability (Nutrients: $F_{1,36} = 2.796$, $p = 0.023$, Light: $F_{1,36} = 1.101$, $p = 0.388$). Nutrient availability had a significant effect on day 15 ($F_{1,36} = 4.638$, $p = 0.037$), day 20 ($F_{1,36} = 6.592$, $p = 0.015$), day 25 ($F_{1,36} = 7.215$, $p = 0.011$), and day 30 ($F_{1,36} = 7.334$, $p = 0.010$) though the effects did not persist to day 35 or day 40 ($p > 0.05$). Light availability only had significant impacts on day 20 ($F_{1,36} = 7.203$, $p = 0.011$) and day 30 ($F_{1,36} = 4.252$, $p = 0.046$), but its effects did not persist. By day 35 and 40, nutrient and light had no significant effect on disease susceptibility and development. Light and nutrient availability appeared to have an interaction effect however the interaction was not statistically significant ($F_{1,36} = 2.256$, $p = 0.057$).

There was no statistically significant difference in survival rates among the four treatment combinations of light and nutrient availability, $X^2 = 4.65$, $p = 0.199$. Each treatment combination had garlic mustard plants that survived to day 40 (Figure 14) with treatment LLLN having the most plants survive (N = 6), followed by HLLN and LLHN (N = 4), and HLHN with the lowest amount (N = 2).

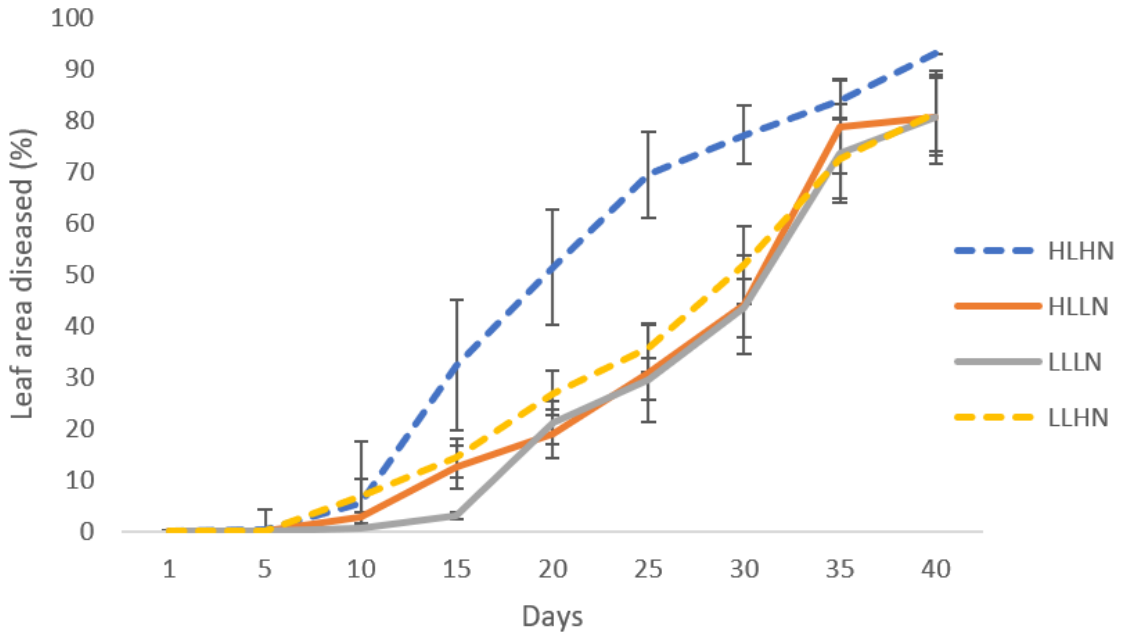

Figure 13. Mean (1±SE) of leaf area diseased (%) of garlic mustard plants with the four treatments of light and nutrient availability over 40 days. Treatments were HLHN (high light, high nutrient), HLLN (high light, low nutrient), LLLN (low light, low nutrient), and LLHN (low light, high nutrient). N = 10 plants per treatment.

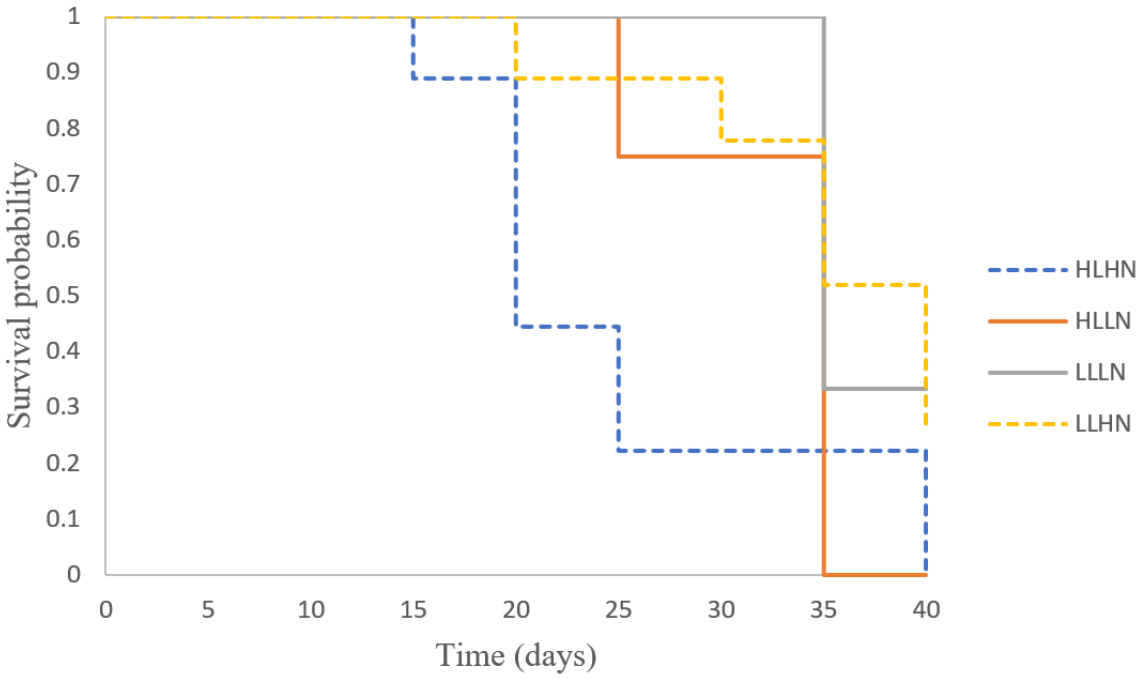

Figure 14. Kaplan-Meier survival curve of garlic mustard plants from the four treatment combinations over 40 days. Treatment combinations were HLHN (high light, high nutrient), HLLN (high light, low nutrient), LLLN (low light, low nutrient), and LLHN (low light, high nutrient). N = 10 for each treatment.

Light availability had a significant effect on petiole length over the 40 days, but nutrient availability had no effect ($F_{1,36} = 11.309$, $p < 0.001$ light, $F_{1,36} = 1.042$, $p = 0.386$ nutrient). Light had significant effects on petiole length at the midpoint (day 26) ($F_{1,36} = 33.087$, $p < 0.001$) and last day (day 40) ($F_{1,36} = 6.637$, $p = 0.014$), decreasing in length over time (Figure 15). Mean petiole length declined most in plants receiving high light with treatment SF by 62.1% followed by treatment Sf by 33.2%.

Light availability had a significant effect on leaf width over the 40 days with all combination treatments inducing a decline in leaf width (Figure 16), but nutrient availability had no effect (Light: $F_{1,36} = 8.256$, $p < 0.001$, $F_{1,36} = 0.422$, $p = 0.738$ nutrient). Light only had significant effects on plant leaf width at the midpoint of the experiment (day 26) ($F_{1,36} = 25.769$, $p < 0.001$). Plant width decreased most in treatment HLHN declining by 75.9% followed by LLHN declining by 40.2%. Combination treatment LLLN experienced the least decline in leaf width of 30.0% (Figure 16). Overall, plants in the high light, high fertilizer treatment showed the fastest disease progression, the highest mortality rate, and the largest decline in leaf sizes among survivors.

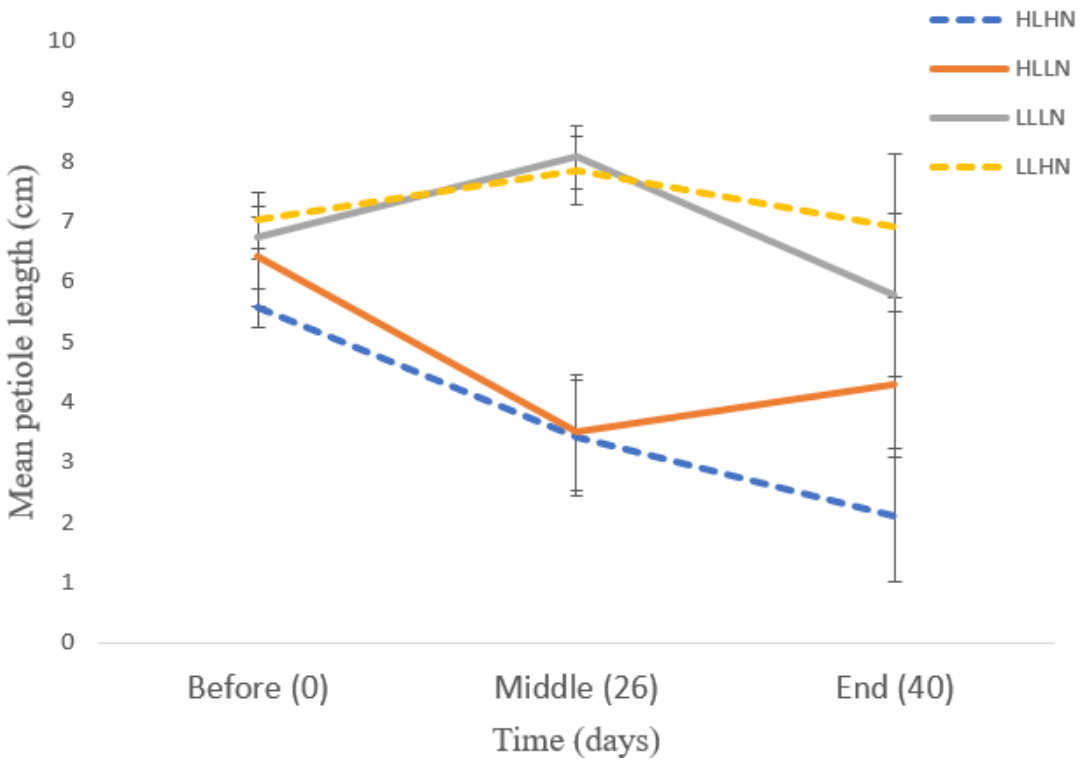

Figure 15. Light and nutrient availability measurement impacts of disease on mean (1±SE) petiole length of garlic mustard (cm) before, middle, and end of study. Treatments were HLHN (high light, high nutrient), HLLN (high light, low nutrient), LLLN (low light, low nutrient), and LLHN (low light, high nutrient). Petiole length was measured from stem's base to blade's base.

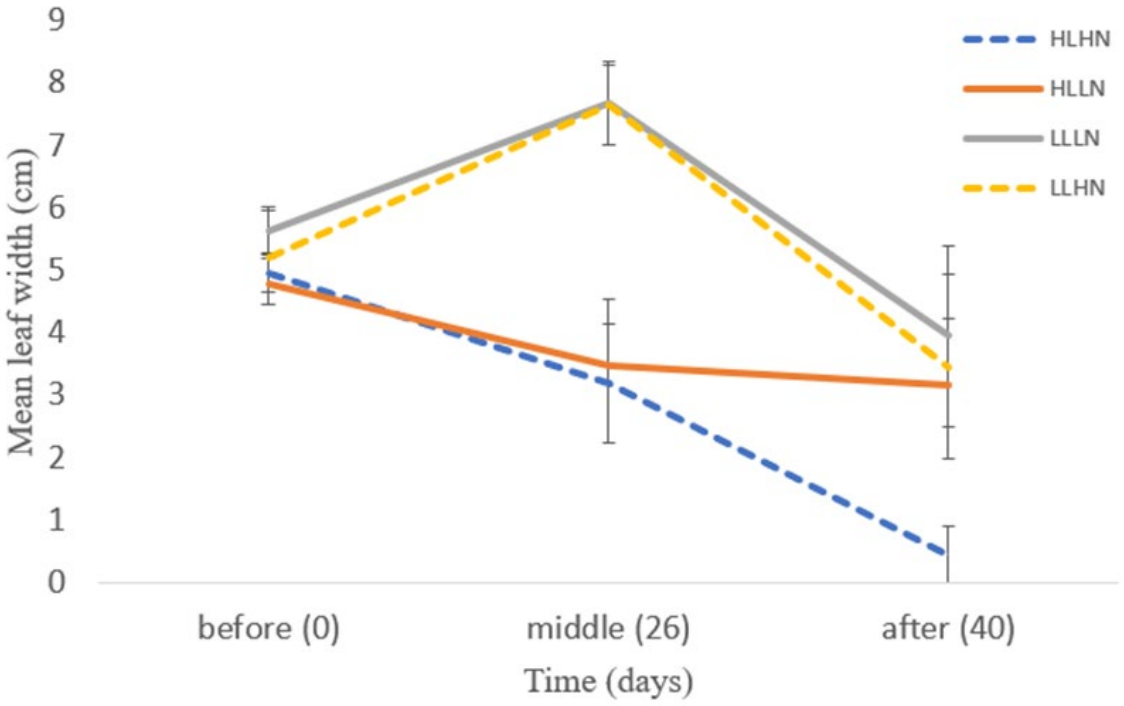

Figure 16. Light and nutrient availability measurement impacts of disease on mean plant leaf width of garlic mustard (1±SE) (cm) before, middle, and after end of study. Treatments were HHHN (high light, high nutrient), HLLN (high light, low nutrient), LLLN (low light, low nutrient), and LLHN (low light, high nutrient).

DISTRIBUTION OF GARLIC MUSTARD WITH *X. CAMPESTRIS* DISEASE SYMPTOMS

The incidence of garlic mustard with evidence of disease caused by *X. campestris* varied across sampled sites (Figure 17, figure 18). ANOVA revealed there was statistically significant variation among the 30 populations in disease incidence ($F_{28,144}$ = 3.555, $p < 0.001$) and canopy cover ($F_{29,146}$ = 9.905 , $p < 0.001$) across sampled sites. Mean disease incidences ranged from zero to about 36% in garlic mustard populations

exhibiting disease symptoms. BW and LLSP exhibiting the highest disease incidence (>30%) with TAP, THSP, AP, CCSP, RFMP, CBP, PLSP, SWP, MTAW, KLSP, RWLP, SMLSP, EFSP, OSP, SAMP, FP, SLSP, WWMSP, EWP, RFSP, BDCMP, and WW experienced disease incidence of less than 10%. TAP and THSP had the lowest mean disease incidence. Correlation analysis showed a significant negative relationship between disease incidence and canopy cover ($r = -0.204$, $p = 0.007$; figure 19). Incidence of disease across garlic mustard populations tended to be positively spatially autocorrelated with high disease incidence clustered near other high incidence sites and low disease incidence clustered near other low disease incidence sites ($I = 0.021$, $p = 0.078$).

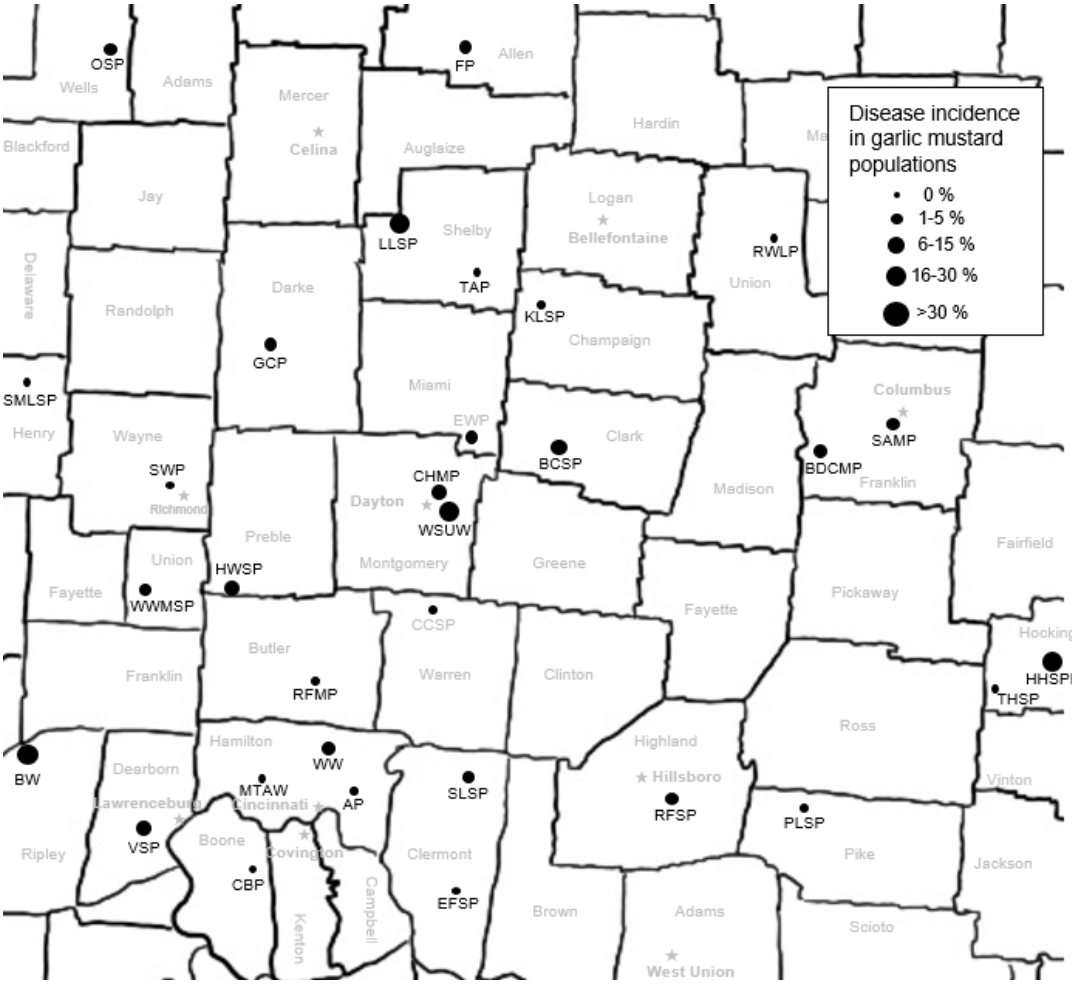

Figure 17. Spatial distribution of the incidence of *X. campestris* disease caused by *Xanthomonas campestris* on garlic mustard (*Alliaria petiolata*) populations within a 120 km radial distance of Dayton Ohio. Disease incidence represented as percentage of population showing evidence of disease. See Table 2 for site abbreviations.

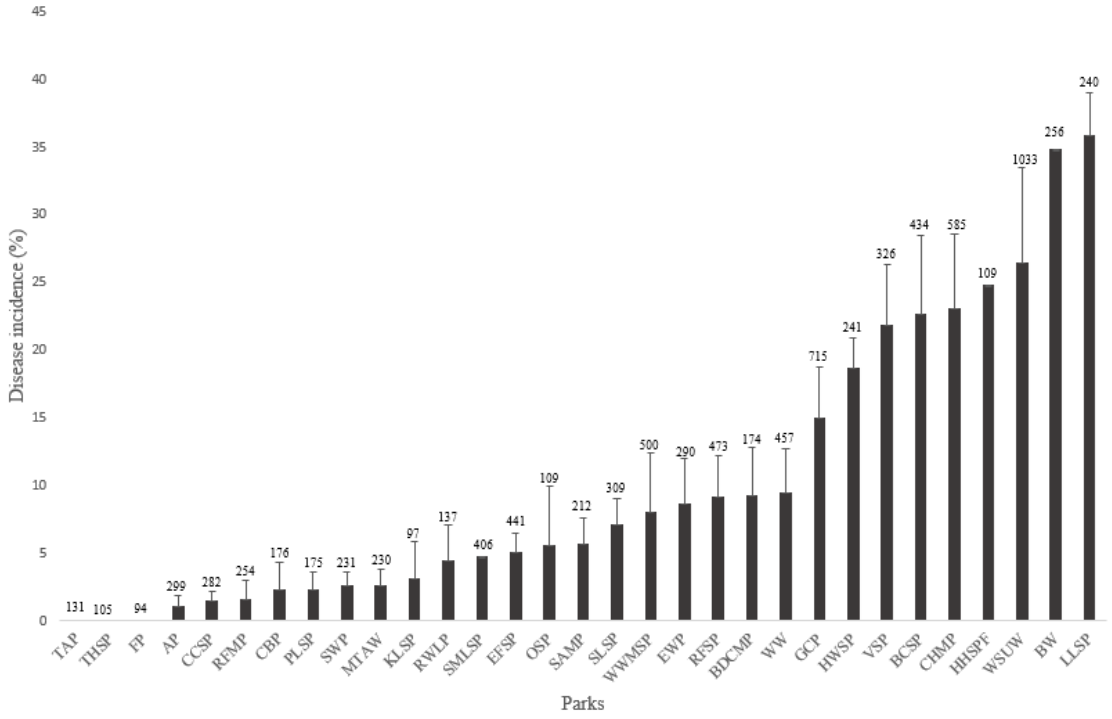

Figure 18. Mean (+1SE) incidence of *X. campestris* disease (%) caused by *Xanthomonas campestris* on garlic mustard (*Alliaria petiolata*) populations within 120 km radial distance of Dayton Ohio. See table 2 for site abbreviations. The number of garlic mustard populations sampled at each site is shown above each bar.

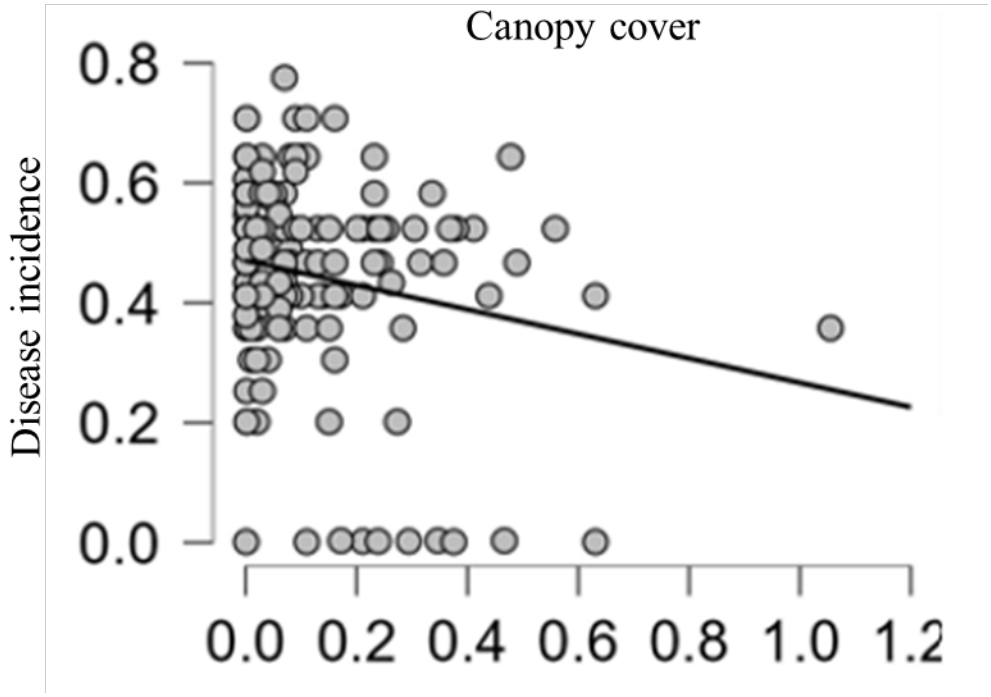

Figure 19. Relationship between disease incidence of *X. campestris* on garlic mustard and canopy cover.

COLLECTION AND COMPARISON OF SEQUENCES OF *X. CAMPESTRIS* STRAINS

Sequencing of our bacterial strain 21-021 collected from Wright State University was accomplished via our collaborators at USDA and resulting sequences were compared to other known strains of *X. campestris*. The bacterium isolated from garlic mustard was identified as *X. campestris pv. incanae*. This pathovar is not considered a widely prevalent pathovar. Strain 21-021 is distinct from Tancos' GM strain 18048. Both garlic mustard strains cluster with the Xci pathovar but are in different groups (Figure 20). Only

18048 and 21-021 originated from garlic mustard whereas the other strains shown originated from various cruciferous weeds and ornamentals (*Matthiola incana* and *Erysimum cheiri*). GM-18048 clusters with the Xci strains collected from cruciferous weeds while Strain 21-021 clusters with Xci strains collected from ornamental crucifers.

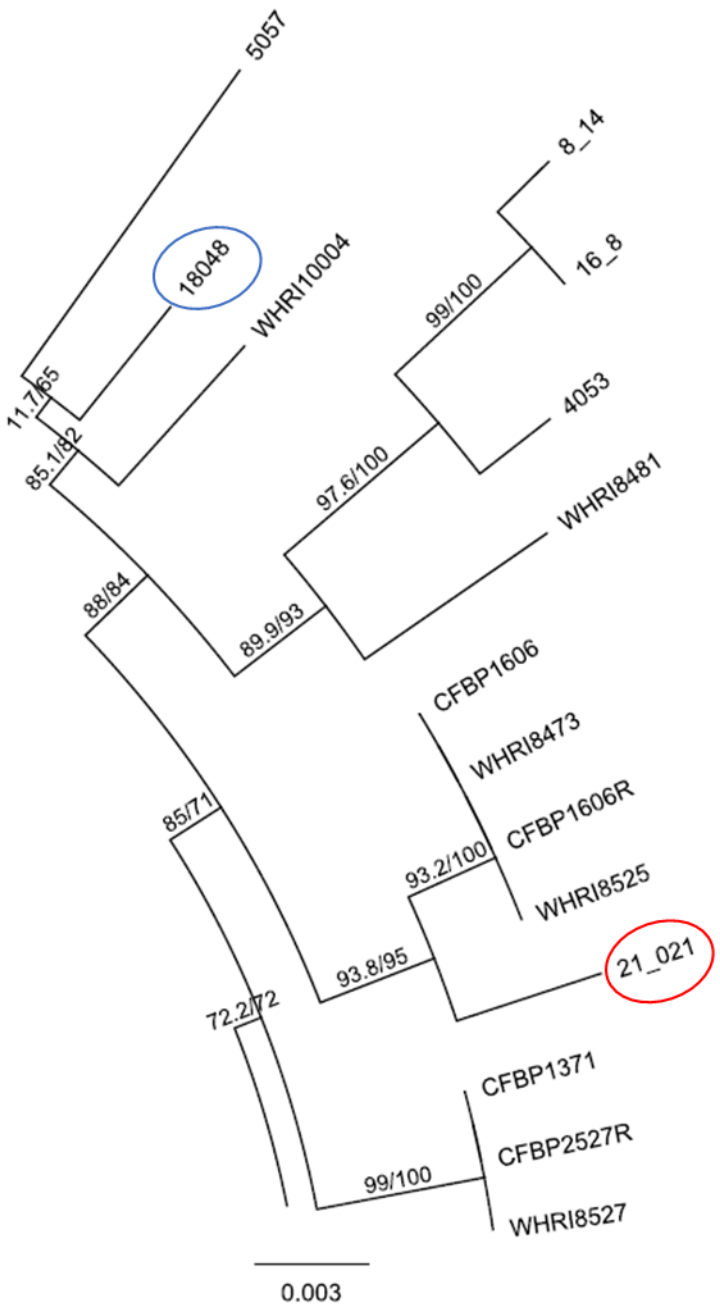

Figure 20. Bootstrap consensus tree from Maximum Likelihood tree analysis of *Xanthomonas campestris pv. incanae* clade with strain groupings. Bootstrap support

values are indicated at the nodes. Strain 21_021 is the Wright State University garlic mustard sample collected on 6/5/21 circled in red. Strain 18048 identified by Tancos's sample (Maryland) circled in blue collected from garlic mustard. Other strains are from crucifer ornamentals and weeds.

IV. DISCUSSION

The objectives of my study were to explore the distribution, host range, and fitness effects of *X. campestris* on garlic mustard. Development of V-shaped lesions after inoculation with a pathogen-containing inoculum were recorded to assess variation in susceptibility across different garlic mustard populations, native wild plants, and agricultural species. The influence of light and nutrient availability on disease susceptibility and plant responses was assessed. The presence of disease symptoms consistent with *X. campestris* infection was surveyed in garlic mustard populations in the field to determine the distribution and incidence in Ohio, Kentucky, and Indiana. Lastly, strains of *X. campestris* collected from garlic mustard were isolated and sequenced for identification via collaboration with Dr. Matt Tancos of the USDA.

The 24 garlic mustard populations screened from across Ohio and one population in Pennsylvania appear to be universally susceptible to *X. campestris,* but severity of disease varied. Variation in severity may be due to factors such as strength of inoculation, spatial structure (population size and distribution) (Barrett et al. 2008, Barret et al. 2009),

genetic differences, and history of exposure to pathogen. Genetic polymorphisms and heterogeneity can suppress pathogens in the field (Jump, Marchant, and Peñuelas 2009). Zhu et al. (2000) looked at how rice genetic heterogeneity could suppress the effects of *Magnaporthe grisea*, the causal agent of blast disease. They found that rice monocultures exhibited much higher disease severity versus rice grown in populations with mixed susceptibility. Genetic polymorphisms in ribwort plantain revealed 16 phenotypes were resistant to powdery mildew strains where non-infected populations exhibited higher resistance than infected populations (Jump, Marchant, and Peñuelas 2009). Several populations were inoculated at later dates which may have impacted the severity observed. In a study on resistance variation in garlic mustard populations to *Erysiphe cruciferarum*, the causal agent of powdery mildew, Cipollini et al. (2020) found that among garlic mustard populations from across Europe and North America, there was qualitative variation in resistance to the pathogen. My study, however revealed only quantitative variation in resistance.

Xanthomonas campestris is the primary source for black rot disease in Brassicaceae species (Vicente and Holub 2012) with twoleaf toothwort and cutleaf toothwort being susceptible to the pathovar. Even though twoleaf toothwort and cutleaf toothwort are susceptible in the lab under conducive conditions, they showed no symptoms of disease in the field because not under conducive conditions. The spring ephemerals studied here have different phenology than *X. campestris*, which prefers warmer and humid conditions

experienced in the summer months. *Cardamine concatenata* flowers the earliest of the native plants and was observed flowering from March to May whereas *Cardamine diphylla* flowers from April to May (Rhoades and Block 2000, Montaut et al. 2010). The time from when *C*. c*oncatenata* and *C. diphylla* flowers and senesces to when garlic mustard begins to develop symptoms of *X. campestris* infection is about one month later in July. *Cardamine* c*oncatenata* and *C. diphylla* varied in disease susceptibility with *C*. c*oncatenata* quickly becoming overwhelmed. Low resistance to *X. campestris* could be due to trade-offs between growth and defenses (Karasov et al 2017). Since these native Brassicaceae species have different phonologies than *X. campestris*, they are able to escape this "summer" pathogen in the field. There is potential risk of disease for these Brassicaceae species due to the continuous presence of garlic mustard though differences in phenology could allow for continued escape. However, as climate changes so does the suitable conditions required for both pathogens and plants. Early warming with climate change could lead to loosened restrictions of *X. campestris's* environmental requirements allowing for the disease to occur earlier in the field and expose the native Brassicaceae species (Chaloner et al 2017). Warming temperatures can also change the phenology of the native Brassicaceae species by causing earlier flowering dates (Rafferty et al 2020) allowing for continued escape from *X. campestris* infection. Reports of wild native plants and weeds as alternate host of *X. campestris* vary with several weeds being confirmed to be alternate host in Brazil (Santos et al 2020) while cruciferous weeds in New York do not act as reservoir host (Lange, Tancos, and Smart 2022)

Cherry belle radish, turnip, cabbage, bok choy, mung bean, and stock were the only crop cultivars (excluding stock, an ornamental plant) species to become diseased by *X. campestris* in my study with varying severity. Several strains and pathovars of *X. campestris* can infect broccoli (Lee et al. 2006), turnip (Zhao et al. 2000), tomato (Ciardi et al. 2000), wheat (Kandel et al. 2012), kale (Obradović & Arsenijević, 1999), brussel sprouts (Mirik et al., 2008), bok choy (João et al., 2010), kai choi, and mustard (Vicente and Holub 2012, Cruz and Cruz 2017). However, in my study corn, tomato, wheat, kale, brussel sprouts, broccoli, kai, choi, and green mustard were not diseased. Yellowing of leaves is consistent with how *X. campestris* progresses in stock (Koike 2018). Infected stock would eventually wilt, but this was not observed since not enough time was given. Mung bean, the only non-Brassica plant, had a strong response to inoculum but it is uncertain if the reaction was a hyper-sensitive response (a strong defense response) or a symptom of infection. Further study is required. Lack of responses to inoculations in several of these species could be due high host specificity among *X. campestris* pathovars and strains (Kingsley et al. 1993, Fargier and Manceau 2007). A similar study conducted by Robinson et al. (2006) using different agricultural species looked at lettuce, tomato, pepper, parsley, cilantro, and beet to determine host range of *X. campestris pv. vitians* (causal agent of bacterial leaf spot in lettuce) and found that pepper was a potential host. Ssekiwoko et al (2006) did a similar study to determine the host range of *X. campestris pv. musacearum* (causal agent of banana Xanthomonas wilt) in wild banana relatives,

ornamental/weed intercrops, and known hosts. They found the pathogen could infect banana relatives, *Musa ornata* and *Musa acuminata 'Zebrina'*, and *Canna indica*, an ornamental wild weed intercrop. The isolate of *X. campestris* I studied is virulent on garlic mustard, but not very damaging to cultivated *Brassica* species or other more unrelated species, in general. Strains of *X. campestris* are increasingly being reported in previously unreported crops, such as in cauliflower in Turkey (Aksoy et al. 2018).

Nutrient and light availability had an influence on garlic mustard's susceptibility and severity to *X. campestris* as well as on plant growth. Though disease developed in all four treatments, nutrient availability had a significant effect on susceptibility and severity of disease. This may be due to plants and pathogens often sharing the same environmental factors that allows for optimal growth. Increases in nitrogen availability, a major nutrient found in fertilizer, has been seen to favor infection (Yarwood 1959). The addition of phosphorus can also increase symptoms so long as the plant is growing (Bawden and Kassanis 1950). This could explain why *X. campestris* disease was more severe in the plants receiving higher nutrients via use of fertilizer. While light did not have a significant effect on disease susceptibility and development, garlic mustard plants receiving treatments with high light did exhibit earlier and higher disease development in the beginning. This could be due to *X. campestris* preferring warmer temperatures in combination with garlic mustard preference for partial light. High light has been seen to negatively impact garlic mustard at early life stages but have positive impacts on growth

and seed production at later life stages (Phillips-Mao et al. 2014), which could explain why the effect of light did not persist through the 40 days.

Inoculation induced a decline in plant growth over the 40 days as measured by petiole length and leaf width. Light availability reduced petiole length which became harder to measure with stunted growth or death of the plant over the 40 days. This could be explained by *X. campestris* spreading to the vascular system and affecting all parts of the plant, resulting in stunted growth, necrosis, and death (Vicente and Holub 2012). In addition, optimal temperature for *X. campestris* is between 25-30°C (Esgalhado et al. 1995), which could cause growth decline in garlic mustard in warmer high light environments. Garlic mustard is a shade tolerant plant and though it can tolerate full sun, it performs best in partial shade (Lankau et al. 2009, Cavers et al. 1979, Byers and Quinn 1998). Though there was no variation in survival rates among the combination treatments, mortality, and severity of disease in garlic mustard, mortality and severity increased with little less than half of individuals in each treatment dead by day 40. Conditions of leaves followed petiole conditions with decline in size throughout the 40 days.

Surveys of the distribution of *X. campestris* on garlic mustard suggest that the disease is well established on garlic mustard populations within a 120 km radial distance of Dayton, Ohio. However, variation in disease incidence varies among the sites, with

three populations showing no evidence of disease symptoms. Variation in disease incidence could be due to factors such as genetic variation in resistance, environmental conditions, and dispersal limitations (Colhoun 1973). Natural areas were surveyed from June 30 to August 12 of 2021, when visible symptoms of disease are most evident in the field. *X. campestris* favors relatively high temperatures and humidity (Ruissen et al 1993) which are experienced during summer months in the Midwest, which could have led to the variation in disease incidence recorded. Infection with *X. campestris* often results in death of the plant so it is possible that plants in surveys at natural sites in August could had already been killed by *X. campestris* prior to being surveyed. This would suggest low or no disease incidence when that may not be the case. Environmental heterogeneity in canopy cover and other factors can assist and/or hinder the pathogen and plant host. *Xanthomonas gardneri*, causal agent of bacterial spot disease in tomatoes, promotes influx of water intake from the environment to allow for pathogen survival and dispersal (Velásquez et al. 2018). Variation in host density and dispersion across the sites could directly or indirectly influence disease incidence of *X. campestris* in garlic mustard populations. Variation in the number of available plants and distance between individuals could explain why some sites with high density population allow for easier dispersal of *X. campestris* (Burdon and Chilvers 1982). Incidence of disease across garlic mustard populations tended to be positively spatially autocorrelated with populations with low disease incidence near other sites of low disease incidence and vice versa. Plants in close proximity tend to share similar environmental conditions may be genetically similar

allowing for equivalent susceptibility to the disease thus allowing similar disease incidence (Pautasso 2017). Genetic variation may be the primary reason for the variation observed in garlic mustard's susceptibility and severity to *X. campestris*. Some populations of garlic mustard exhibited little to no disease incidence in the field but did exhibit disease in the lab. Severity of disease in garlic mustard can vary if in a conducive environment that favors the *X. campestris* pathogen compared to an environment that is not conducive for *X. campestris*. Due to limited disease dispersal could explain why *X. campestris* is not evident in the field for some populations though they are susceptible to the disease.

Canopy cover was also recorded at all but two survey sites, as light exposure is known to influence where garlic mustard can be found and its performance (Lankau et al. 2009, Cavers et al. 1979). There was a negative correlation between canopy cover and disease incidence. This relationship may be due to density dependence where *X. campestris* has higher disease incidence where there are greater host densities (Freckleton and Lewis 2006). Garlic mustard in dense strands, would enable dispersal of *X. campestris* among the individuals, however density was not recorded to address relationship between disease and density. The significance of canopy cover and disease incidence result is unknown; however, canopy cover can influence light availability and intensity, among other factors, that may affect disease incidence (García-Guzmán 2016).

Xanthomonas campestris pv. incanae is host specific and typically causes bacterial blight in ornamental crucifers (Fargier et al. 2011, Tang et al 2021). Various strains of *X. campestris pv. incanae* have been found to infect cruciferous weeds and ornamentals as well as garlic mustard (Tancos and Fredericks 2020, Lange, Tancos, and Smart 2022). The two strains collected from garlic mustard, 21_021 and 18048, belonged to different clades so it possible there are other strains throughout North America. Garlic mustard infected with *X. campestris* samples collected from Ohio, Kentucky, and Indiana have been sent to be isolated and sequenced to determine if strains are identical to the strain in Wright State University or if they vary.

V. CONCLUSION

In summary, I have demonstrated that, although most populations are susceptible to some degree, there is variation in susceptibility to *X. campestris* among garlic mustard populations in Ohio. Susceptible, native Brassicaceae species should be able to continue escape from infection in the field due to different phenologies. Environmental factors, such as nutrient and light (and temperature, humidity, soil pH, and others) will influence disease incidence in natural areas where garlic mustard can be found. Nutrient availability had a greater influence on disease susceptibility and severity of *X. campestris* on garlic mustard whereas high light availability caused earlier and more severe disease development. *Xanthomonas campestris* is present in garlic mustard populations

throughout Ohio, Kentucky, and Indiana with disease incidence tending to cluster near similar values. I expect *X. campestris* to spread throughout the Midwest and Northeast. Garlic mustard populations in Maryland are also known to host *X. campestris*. Variation of pathogen strains, genetic variation in susceptibility, and host densities could influence the rate of spread and severity. The often-severe impacts induced by *X. campestris pv. incanae,* on garlic mustard could moderate the effects of garlic mustard on native plant communities, with moderate risk to native and agricultural Brassicaceae species in Ohio. This strain could be considered as a potential biocontrol as it is already established in the field. More information is needed on what strains are present in infected garlic mustard populations to determine if the strains are identical or not. If the strain is isolated to the Midwest, studies on the host range of states of wild native Brassicaceae plants and important agricultural species will need to be conducted.

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