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The Impact of Intraspecific Density on Garlic Mustard Sinigrin Concentration

Mercedes Harris

The Impact of Intraspecific Density on Garlic Mustard Sinigrin Concentration

A Thesis Presented

By

MERCEDES EDITH HARRIS

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MERCEDES EDITH HARRIS

Approved as to style and content by:

_____

Kristina Stinson, Chair

_____

Joseph Elkinton, Member

_____

Susannah Lerman, Member

_____

Tristram Seidler, Member

_____

Curt Griffin, Department Head,

Environmental Conservation

ABSTRACT

THE IMPACT OF INTRASPECIFIC DENSITY ON GARLIC MUSTARD SINIGRIN CONCENTRATION

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MERCEDES E. HARRIS, B.S., SALISBURY UNIVERSITY

M.S. UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Kristina Stinson

Garlic mustard (*Alliaria petiolata,* Brassicaceae) is a biennial herb that produces glucosinolates, a class of constituent secondary metabolites that defend against herbivores and pathogens allowing it to grow at high densities in invaded regions. The glucosinolate sinigrin is predominant in garlic mustard and aids in its competitiveness as an invasive species. In North America, garlic mustard can grow at high densities and form dense monocultures which may increase its apparency to herbivores and therefore increase its sinigrin production. I measured leaf sinigrin concentration in garlic mustard populations of different densities in the field and in greenhouse experiments to evaluate the response of sinigrin concentration and growth to density and light. Sinigrin concentrations of second-year plants were negatively correlated with growth metrics across all field densities; indicating a cost to sinigrin production. In the greenhouse density experiment with high and low rosette stem densities, sinigrin differed significantly by rosette density category. A factorial greenhouse experiment with light and density treatments discerned significant differences in sinigrin concentration by density. These findings suggest that sinigrin concentration may be influenced by intraspecific density across different light environments.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

Tradeoffs between primary compounds which aid in growth and secondary compounds which aid in chemical defense often affects a plants competitive ability and palatability to herbivores in invaded environments (Mitchell-Olds et al., 1996; Keane and Crawley 2002; Cipollni 2004; Crawley 2007; Keeler and Chew 2008; Stamp 2003; Ballhorn et al., 2014). While plants rely on surrounding resources for physiological processes such as photosynthesis, seed germination, and transportation of compounds, tradeoffs can occur among growth, reproduction, and defense (Herms and Mattson 1992; Feeny 1976). Garlic mustard (*Alliaria petiolata*), an invasive biennial brassica native to Europe, Asia, and Africa, uses invasion mechanisms that allow it to spread across regions and negatively disturb temperate forests in North America (Cipollini 2004; Lankau 2009; Pardini et al., 2009; Barto et al., 2010). Garlic mustard's invasion mechanisms include: the release of the large quantities of propagules entering the environment, fast-growing life stages, escaping natural herbivory, and exuding secondary compounds that are toxic to surrounding native species (Davis et al., 2000; Rai 2015).

Glucosinolates are secondary defense compounds found in garlic mustard and most related Brassicaceae. They are low weight, highly toxic to unspecialized herbivores and costly to produce (Coley 1987; Stamp 2003). Whether or not the expression of secondary defense compounds gives non-native plant species an advantage or disadvantage over surrounding native plant species has been proposed in different hypotheses such as the evolution of increased competitive ability (EICA) and the plant apparency (Callaway and Reidnour 2004; Cipollini 2004; Albuquerque & Lucena 2005).

The EICA predicts that in the absence of adapted specialists and generalist herbivores, invasive plant populations should lose costly traits that aid in herbivory resistance and allocate resources to primary functions that provide competitive advantages such as increasing in size or fecundity (Callaway and Ridenour 2004; Rai 2015). In the apparency hypothesis, apparent plants (e.g., woody trees) are species that are easily found by herbivores and tend to produce organic compounds low in toxicity but act as inhibitors to herbivore digestion, while non-apparent plants (e.g., herbaceous species) produce strongly bioactive organic compounds low in molecular weight and high in toxicity (Feeny 1976, Rhoades and Cates 1976, Albuquerque and Lucena 2005, Alencar et al., 2009).

According to the plant apparency hypothesis, the expression of defense either increases or decreases depending on ecological interactions with abiotic and biotic factors. In other words, plants produce defenses in direct proportion to their risk of detection from herbivores and in inverse proportion to the cost of defense. Such changes in defense may or may not benefit the plant species. For example, if a forest is newly invaded by a less dense invasive plant population compared the population to native species, the small invasive plant population is expected to escape discovery and have poor defense expression (Feeny 1977). The EICA and apparency hypotheses categorize secondary defense compounds as either qualitative or quantitative. Qualitative defenses are predicted to require small investments and can be highly toxic at low dosage (Fahey 2001; Stamp 2003). Conversely, quantitative defenses require large investments and interfere with the nutrient acquisition in herbivores. Quantitative defenses are also called digestibility reducing defenses because these compounds defend plant tissues through

increased mortality of generalist herbivores, reduced herbivore growth rates, and lowered fecundity once leaf tissue is consumed (Rhoades and Cates 1976). Glucosinolates are described as qualitative mobile defenses because they are often highly toxic at low doses and are continually being metabolized because of their high bioactivity (Coley et al., 1985; Feeny 1976; Fahey 2001).

Secondary metabolism in garlic mustard involves the biosynthesis, transportation, accumulation, and storage of glucosinolate by-products. Glucosinolates are a class of nitrogen- and-sulfur glucose containing anions whose hydrolyzed degradative products are released when plant cell vacuoles containing them are ruptured (Vaughn 1999). Glucosinolates are involved in numerous species interactions including specialist herbivore attraction, generalist herbivore resistance, and suppression of mycorrhizal fungi (Cipollini 2004; Stinson et al., 2006; Wolfe et al., 2008; Poon et al., 2015; Wheeler et al., 2016; Anthony et al., 2017). The glucosinolate sinigrin is the predominate glucosinolate found in garlic mustard leaves (Renwick and Lopez 1999; Cipollini 2004) and is the compound of interest for this study. In garlic mustard's invaded range, native Pierid butterflies are chemically attracted to oviposit on garlic mustard leaves because the secondary compounds in garlic mustard are similar to the secondary compounds found in the native Pierid host plant. Pupae of native Pierid butterflies consequently have difficulty completing the development cycle and an increase in mortality after ingesting garlic mustard leaves (Cipollini 2002; reviewed in Tallamy 2004; Keeler and Chew 2008; Davis et al., 2015). Evidence from these studies suggests that garlic mustard can alter secondary compound expression induced by herbivory, but the question remains as to

whether or not there are other ecological factors that cause changes in constitutive secondary compound expression.

Expression in constitutive secondary compound production may change in response to intraspecific competition from various plant densities. The biennial life cycle of garlic mustard requires investments of resources for primary functions of seed production, individual plant growth, and survivorship which affect its competitive ability (Blossey and Notzold 1995, Meekins and McCarthy 2001). Studies have recorded first-year vegetative rosette densities ranging from 20-2000 rosettes per m^2 and second-year adult densities from 10-150 plants per m^2 (Anderson et al., 1996; Meekins and McCarthy2001; Pardini 2009). Identifying additional influences on sinigrin concentration within garlic mustard populations could imply the need for changes to management applications.

Most garlic mustard management practices involve treating first-year vegetative rosettes with herbicide and second-year reproductive stems by manually hand-pulling to prevent the addition of new seeds to existing seed banks. These management practices can be costly and labor intensive because treatments must reoccur for several years to influence plant densities (Chapman et al., 2012). As described previously, populations that are not constantly managed form dense monocultures and decrease plant species diversity (Cipollini 2004). Increased intraspecific density within a population often reduce individual species survival or plant size and reduce seed production (Palmblad 1968; Ford 1975; Cannell et al., 1984). However, in garlic mustard intraspecific populations, plants may have a plastic response to density and adjust in size or defensive secondary compound expression instead of increased mortality as suggested in

Ford (1975), and Meekins and McCarthy (2002). Few data are available examining how garlic mustard sinigrin concentrations or individual plant size respond to intraspecific density. By examining intrapopulation sinigrin expression within the framework of invasion mechanism tradeoffs, it may be possible to predict future behaviors of populations in particular forests given the habitat characteristics and target crucial growth stages with effective management techniques (Meekins and McCarthy 2002; Pardini et al., 2009).

Changes in secondary compound concentration in herbaceous brassicas have been shown to vary in response to light, water, and surrounding native species (Kliebenstein et al., 2001, Brown et al., 2003; Cipollini 2004; Lankau et al., 2009; Lankau 2012; Frisch et al., 2014; Smith and Reynolds 2014), but there are few data showing interactions between garlic mustard growth and defense within intraspecific populations. Furthermore, the physiological tradeoff between primary and secondary functions constrains the evolutionary responses of plants as they interact with their biotic and abiotic environments (Herms and Mattson 1992). In the context of the EICA, plants that have escaped their native herbivores no longer need to continue to produce the defenses evolved to resist herbivory and should result in selection for the reduction of costly traits (Rai, 2015). Empirical evidence of the EICA would show a tradeoff in chemical concentration to benefit reproduction and growth of the invasive species outside of its native range. Cipollini (2002) observed that North American garlic mustard populations had lower chemical concentrations of total glucosinolates but greater specific leaf weight than populations in their native range in Europe. In contrast, empirical evidence of the plant apparency hypothesis would show variable associations between plant density and

chemical concentration based on the non-native species interactions with herbivores or the number of surrounding plants.

The primary objective of this study is to determine how intraspecific competition and light influence leaf sinigrin concentration and growth. Here, I address the following questions. First, is there a tradeoff between leaf sinigrin concentration and primary functions toward the growth? Second, does density influence leaf sinigrin concentration? Third, does varied light levels affect sinigrin concentration of first-year rosettes during growth? I hypothesize that plants are more susceptible to discovery at high densities, thus second-year garlic mustard growing at high densities will invest more energy into defense than growth, resulting in higher sinigrin concentrations and stunted growth. I expect to observe a significant effect of light on sinigrin concentration because light influences chemical reactions, and light energy from photosynthesized products could be used towards growth or secondary compound production. Considering density and light, plants in low light environments are expected to forgo defense for growth (Herms and Mattson 1992), but high light levels may offset the tradeoff in growth and defense allowing garlic mustard to do both as observed in related brassica species (Siemens et al., 2002)

To observe possible growth tradeoffs in response to sinigrin, I (1) compared correlations in growth metrics of garlic mustard to leaf sinigrin concentration. To test the effect of density on leaf sinigrin concentration responses among second-year garlic mustard populations, I (2) examined whether leaf concentration varied within field observations across a density gradient and in greenhouse experiments of garlic mustard growing in different density treatments. Since previous studies have shown sinigrin concentrations to vary across a light gradient in the field (Smith 2015), measuring light

source will help indicate another possible factor that influences leaf sinigrin concentration. To test the effect of light on leaf sinigrin concentration, I (3) compared sinigrin concentration in the field and in full sun and shade treatments in a greenhouse setting of garlic mustard growing at different densities. Assessing garlic mustard leaf sinigrin concentration in the field allows for comparison of factors that aid in the primary functioning of growth and leaf sinigrin expression under natural environmental conditions, while the greenhouse experiments help pinpoint which abiotic and biotic factors aid in the expression of leaf sinigrin concentration during growth.

CHAPTER 2

METHODS

2.1 Study species

Garlic mustard is a non-native invasive, highly competitive, self-compatible, biennial herb that is widespread in deciduous forest understories and along forest edges throughout North America (Cavers et al., 1979; Meyers and Anderson 2003). The first recorded appearance of garlic mustard in North America was in 1868 in New York (Nuzzo 1993). Garlic mustard can form dense, virtually monotypic, stands that cover vast square meters and displace native species in invaded habitats (Cavers et al., 1979; Nuzzo 1991; Yost et al., 1991; McCarthy 1997; Meekins and McCarthy 2002). It occurs in habitats with irradiance levels varying from deep shade— in forest understories, to nearly full sun— along forest edges (Carvers 1979; Meyers and Anderson 2003). Its biennial life history consists of a basal rosette during the first-year of growth, which overwinters, bolts primary stems and bears flowers and fruits in the spring of the second growth year (Nuzzo 1991). Siliques house the seeds that exhibit a dormancy period, which can last from 8-10 months or until cold stratification occurs— initiating germination (Baskin and Baskin 1992; Anderson et al., 1996). Garlic mustard can produce up to 150 siliques, with up to 22 seeds per pod (Anderson 2012). Garlic mustard germinates from late February through April, overwinters as rosettes transitioning into the second-year, from which stems sprout and flowers late-spring, followed by a release of seeds from July to October (Cavers 1979) then plant senescence of the flowering plant in the late fall of the second growth year (Anderson et al., 1996).

2.2 Study areas

In April 2017, field sites were chosen in secondary northern hardwood forests and a floodplain forest in three Western MA towns near the Connecticut river valley (Amherst, Northampton, and Longmeadow, MA). The Amherst site included hardwood forests around the University of Massachusetts Amherst which consisted of Sylvan Woods, Brooks Wood, and Mill River Woods on the Northwest end of the University (42 º 24.036' N, 072 º 31.355'W). The Northampton site consisted of a single hardwood forest near the Mill River Reservoir that has a garlic mustard invasion extending ~100 m along the entrance to a man-made trail (42 º 18'46.37" N, 72 º 39' 19.74" W). The Longmeadow Conservation Area is an old growth floodplain forest (42 º 2'37.51''N, 72 º 35' 43.81'' W). All forests sites had preexisting garlic mustard invasions at varying densities and were adjacent to agricultural land. Seedlings for the greenhouse experiments were collected in South Hadley, MA (42º 15' 16.79'' N, 72 º 35' 07.10'' W) in an oak dominated hardwood forest adjacent Stony Brook and east of the Connecticut river.

2.3 Field density observations

In the three towns Amherst, Northampton, and Longmeadow MA, 1x1m quadrats were placed systematically at least 10m apart along multiple transects all within a 200m sample area. To assess garlic mustards relative field density each individual stem of second-year adults within the 1 x 1m quadrat was counted (Wilson 2007). Vegetative cover within each quadrat was visually estimated by two observers and later categorized into range cover classes (Daubenmire 1959). Most of the sampled plots consisted of

species other than garlic mustard which were counted along with the number of garlic mustard rosettes to assess total species count within each quadrat. Other species were Jewelweed (*Impatiens capensis*), Jack in the pulpit *(Arisaema triphyllum)*, and Ground Ivy (*Glechoma hederacea*) which was only found in Mill River Woods. Quadrats were established in April 2017 and sampled plants were harvested in June 2017.

2.4 Environmental Factors

To account for abiotic and biotic factors, within each plot soil temperature (Traceable® thermometer) and light as photosynthetic photon flux density (PPFD) (Li-Cor 250A Photometer) were measured weekly from April 2017- June 2017 once quadrats were established. Soil temperatures were taken at a depth of ~7cm and PPFD μmol/m^2/s was measured ± 3h to solar noon at center of each 1 x 1m quadrat with a Li-190R quantum sensor attached to the 250A Photometer. Four soil cores were systematically taken from each side around the center of the quadrat with an auger at the depth of 15cm, homogenized, ground, and oven dried at 23 º C prior to processing. Soil samples were processed at the University of Massachusetts Soil and Plant Nutrient Testing Laboratory Amherst, MA, for standard soil fertility tests measuring macro & micro nutrient availability, pH, extractable nutrients, cation exchange capacity and percent base saturation. Soil characteristics were analyzed in a MANOVA with garlic mustard second-year stem density as the independent variable.

2.5 Growth assessment in the field

In each 1 x1m quadrat, the garlic mustard second-year stem closest to the center of the plot was tagged and sampled for growth metrics and leaf concentration. Growth metrics included, the maximum height of the selected garlic mustard plant, relative chlorophyll content of the sampled leaf using SPAD-502 meter (Konica-Mintola, Japan), and the number of branches to assess the plants photosynthetic ability, and vigor. The number of siliques and, total and averaged seed mass were assessed as a measure of plant fitness along with oven-dried above-ground and belowground biomass after harvest in June 2017 just before senescence. The values from the SPAD-502 meter typically range between 0.0-50.0 as a proportion to the amount of chlorophyll present in leaves indication photosynthetic capacity (Uddling et al., 2007). Analysis in R Studio, software version 3.4.0 (R Development Core Team 2017) involved correlation analysis to assess tradeoffs between growth characteristics and sinigrin concentration of second-year garlic mustard stems. To examine the effect of density on leaf sinigrin concentration, I fit a multiple linear regression with log scaled sinigrin concentration as the dependent variable and light, town, and garlic mustard stem count as factors. Variance inflation factors were calculated to detect multicollinearity and factors with VIF values above four were not included in correlation analysis. Model selection based on AICc comparison from the candidate model global model which included sinigrin as the response, town, rosette count, 2^{nd} year stem count, and light interactions. Analysis was only conducted for the Amherst and Northampton towns since most of the Longmeadow site flooded. Growth traits of maximum height, shoot, root, total biomass, and sinigrin concentration data were log transformed to meet model assumptions. Numbers from three plots of garlic mustard

stem density count in the Amherst town were excluded in all analysis after evaluating those points as outliers in model fitting tests.

2.6 Density experiment

To assess the effect of density on leaf sinigrin concentration in a greenhouse setting, a completely randomized planter pot study design was set up at the College of Natural Science and Education Greenhouse at the University of Massachusetts, Amherst. Garlic mustard cotyledon-staged seedlings were collected from an oak dominated forest in South Hadley, MA adjacent Stony and the Longmeadow Conservation Area. Collected seedlings were transplanted during the week of May 24, 2017, into 10 cm plastic nursery pots at two densities: low-density, with one seedling per pot, and high-density, with five seedlings per pot There were 61 replicates (individual pots) of each density treatment (N=122). For each density treatment, thirty-four of the sixty-one were randomly selected (N=68) and the tallest stem of the centermost rosette was tagged and sampled for leaf sinigrin concentration and fresh leaf surface area measurements in August 2017. All pots were filled with ~3 L (0.14 cu ft.) of ProMix HP mycorrhizal soil mixed with 1/3 of Pavestone natural sand. Seedlings of the high-density 5 rosettes per pot treatment were planted equidistant of each other with one seedling in the center of the pot. Seedlings grew on a bench in the greenhouse under controlled day lengths and temperatures programmed at 16 h, 21ºC daylight; 8 h, 18ºC night. Growth was assessed as specific leaf area, leaf mass ratio, and total biomass once harvested on November 5, 2017 and oven-dried after twenty-two weeks in the greenhouse. Statistical analyses were conducted in R, software version 3.4.0 (R Development Core Team 2017). [1]To examine the effect of

density on sinigrin concentration data were analyzed in a one-way ANOVA with density category (high and low) as the main effect. [2]To assess tradeoffs in growth or sinigrin concentration, correlation analysis of sinigrin and the growth metrics describes above were performed. Only data from plants that remained alive during the entire greenhouse experiment were used in analyses.

2.7 Density and light experiment

To assess the effects of light on leaf sinigrin concentration in addition to density, I employed a separate experiment at the College of Natural Science Greenhouse in Amherst, MA. In a 2x2 factorial design, seventy-two pots were arranged on a single bench with a light treatment of a 50% aluminet knitted nylon shade cloth draped over half of the bench shading thirty-six of the seventy-two pots with garlic mustard seedling density treatment levels low and high, as describe above for the density experiment, evenly distributed among the 50% shade and full sunlight treatments. Sub-sampling involved using a random number generator to select nine high-density and nine low-density treatment pots from both sun and shade treatment sides of the bench. A total of thirty-six pots (N=36) were sub sampled and measured as described in the density experiment above. Cotyledon-staged seedlings grew with a photoperiod set to the following;16 h, 21ºC day; 8 h, 15ºC night in the summer. In addition to leaf samples for sinigrin concentration measurements, growth was measured by fresh leaf surface area, and oven-dried specific leaf area, leaf mass ratio, and total biomass once harvested on November 6, 2017 after twenty-two weeks of growth in the greenhouse. [1]To examine the effect of density and light treatments on sinigrin concentration, data were analyzed in a

two-way ANOVA with density and light treatments as main effects and an interaction term. [2]Interaction plots with sinigrin concentration, specific leaf area, and total biomass as response variables to density and light treatments examined the effect of density treatment and light on the growth of rosettes. Only data from plants that remained alive during the entire greenhouse experiment were used in these analyses.

2.8 Leaf tissue collection

The collection of leaf tissue sampled to determine sinigrin concentration by HPLC analysis consisted of taking the same number of 1 cm hole punches from the leaf midrib and immediately placing the leaf sample into a 2mL vial of 99% menthol for the field samples and 95% for the greenhouse samples, to inactivate the degradative enzyme myrosinase (Lankau 2001; Keiddle et al., 2001; Kliebenstein 2001). In the field, the second leaf node down from the tip of the flowering stem was sampled and in the greenhouse the leaf of the tallest stem from the center rosette was sampled to assess singrin concentration. After each leaf sample collection, the hole-puncher was cleansed with 70% isopropanol to remove any residual phytochemicals left behind on the hole puncher. From the field observations, leaf tissue sampling took place in June 2017, just after the initiation of flowering. In both greenhouse experiments, leaf tissue was collected from the leaf of a randomly selected pot and tagged center rosette. Leaf tissue was collected mid-way through both greenhouse experiments, 11 weeks after transplanting and 11 weeks before harvesting. Leaf tissue samples were and stored in the reaction vials filled with methanol for a minimum of thirty days until HPLC analysis. Collected leaf samples were oven dried before weighing to determine dry mass.

2.9 Glucosinolate extraction and HPLC analysis

To prepare the plant material for HPLC analysis, leaf tissue samples were extracted through filter columns packed with QAE Sepahex A-25 (Sigma-Aldrich, St. Louis, MO, USA). The columns were pre-washed with sodium acetate buffer before the addition of 800µL of aqueous plant tissue material. The columns were washed sequentially 2 x with 750uL of 70% MeOH, 2 x with 750µL of ddH_2O, 1 x with 750µL of 20mM NaOAc, and 2 x with ddH_2O to create optimal conditions for 30µL of sulfatase from *Helix pomatia* type-H1 (Grosser and van Dam 2017). Desulfoglucosinolates were eluted the next day with 150µL of ultra-pure water (18.2 mΩ) purified with a Milli-Q water purification system (Millipore, Molsheim, France). The desulphated glucosinolates were analyzed on the Alliance 2695 dual-wave UV HPLC instrument using a reserved-phased Symmetry C18 (150mm x 4.6mm i.d., 5µ particle size) column at 40ºC and a VanGuard precolumn and cartridge holder (Waters, Milford, MA). All sample extracts were injected at 20µL and individual glucosinolates were detected by a diode-array detector at a UV wavelength of 229 nm. The linear gradient elution consisted of (A) HPLC grade water and (B) acetonitrile mobile phase at a flow rate of 1 mL/min with the following program set: 1.5% of B from 0 to 5 min; 2.5% B 6 to 7 min; 5.0% B from 8 to 14 min; 18% B from 15 to 16 min; increased to 46% B from 17 to 23 min; then 92% B from 23-24 min, then re-equilibrated to initial conditions at 25 to 29 min. Standards were run on a simplified gradient elution as described in Grosser and van Dam 2017. Following the procedure from Grosser and van Dam 2017, an external calibration curve of sinigrin monohydrate was analyzed with analyte samples to identify sinigrin. By comparing the retention time to a pure sinigrin monohydrate standard (LKT Labs, St.

Paul, MN, USA) specific compound concentrations can be quantified (Fahey et al., 1997; Kiddle et al., 2001; Kliebenstein et al., 2001; Yang and Quiros 2010; Prasad et al., 2015, Grosser and van Dam 2017). Sample peaks were compared to a calibration curve standard of sinigrin monohydrate and integrated using QuanLynx from MassLynx 4.1 Software (Waters, Milford, MA) and quantified using the linear calibration methods (Prasad 2015 and Grosser and van Dam 2017). The 2.11mM sinigrin monohydrate stock concentration was used as a multiplier in the calibration QuanLynx methods. Glucosinolate concentration excreted into field soil was not measured because of their little to no detectability (Cipollini 2004; Cantor et al., 2011). Due to their volatile nature and short half-lives, the presence of glucosinolates can dissipate in as little as three to twelve hours once excreted into the soil (Barto and Cipollini 2009; Cantor et al., 2011).

CHAPTER 3

RESULTS

3.1 Field density observations

Second-year garlic mustard density ranged from 3-83 stems across all 1 x 1m plots and rosette density ranged from 0-198 (Appendix table 1). A MANOVA of soil properties response to second-year stem count yielded no significant effects. Sinigrin concentration was negatively correlated with adult stem height (r = -0.65, p = 0.0021), shoot mass (r = -0.63, P = 0.0094), and root mass (r = -0.49, P = 0.052) (Figure 1). The best fitting model from the candidate global model included sinigrin concentration as the predictor to the response factors town and second-year density. In univariate ANOVA with second-year stem density as the response to sinigrin concentration, a significant effect of sinigrin concentration on stem height was observed $F_{(1,16)=}15.3$, P = 0.0012 (Table 1). In a one-way ANOVA a significant effect of adult stem density on sinigrin concentration was observed in the Amherst sites $F_{(1,10)=}$ 7.34, P = 0.022 (Table 2). Correlation analysis between second-year stem density and sinigrin concentration resulted in a positive relationship for the Amherst sites r = 0.69, P = 0.006 (Figure 2) and negative for the Northampton site (Appendix figure 1).

3.2 Density experiment

In a one-way ANOVA with singrin concentration as the predictor and density treatment as the response factor, a significant density effect of sinigrin concentration was observed, $F_{(1,42)}$= 5.128, P = 0.0290 (Table 3). Mean singrin concentration differed by category (Figure 4). Pearson's correlations were negative between shoot mass (r = -0.34,

P = 0.024), root mass (r = -0.38, P = 0.013), total biomass (r = -0.41, P = 0.0054), and sinigrin concentration (Figure 3).

3.3 Density and light experiment

In a two-way ANOVA with sinigrin as the response to rosette stem density and shade and sun light treatments, a significant main effect of density on sinigrin concentration was observed $F_{(1,24)}$=6.786, P = 0.015, but there's no significant main effect of light treatment or an interaction between density and light (Table 4). No significant interactions between specific leaf area and density and light treatments or total biomass and density and light treatments were observed (Figure 5).

3.4 HPLC

Sinigrin concentration from the field density observations on the natural scale ranged between 0.184-5.99 µM/g dry weight in high-density plots and from 0.3711-2.487 µM/g dry weight in low-density plots. Field samples consistently had retention times between 5.2- 5.11 minutes in comparison to the commercial sinigrin monohydrate standard retention time of 5.0 (supplemental figure 2). The greenhouse leaf samples consistently had retention times between 5.10-5.18 minutes in comparison to the commercial standard retention time of 5.0 minutes. Sinigrin concentrations ranged from 0.011-0.786 µM/g dry weight in the density experiment and 0.0293-1.482 µM/g dry weight in the density and light experiment (Appendix table 3 and 4).

CHAPTER 4

DISCUSSION

The life history and secondary compound expression of garlic mustard are mechanisms that allow it to persist as an invasive species in North American forests. Garlic mustard leaf phenology and population dynamics have been shown to vary due to changes in native species composition, intra- and interspecific density, and light (Pardini 2009; Myers and Anderson 2003; Smith and Reynolds 2015). Variation in garlic mustard's inducible secondary defenses based on herbivore interactions is documented (Cipollini 2002), but constitutive chemical concentration may vary more so by other external factors. This study investigated whether garlic mustard intraspecific density is an external factor that influence leaf sinigrin concentration, whether a non-apparent species by definition could act as an apparent species by altering sinigrin concentration, and whether evidence of the EICA is observed from tradeoffs in sinigrin expression and growth. Field observations allowed for the evaluation of the effect of density on garlic mustard's leaf sinigrin concentration in natural conditions, while greenhouse experiments allowed for evaluation of density and light on leaf sinigrin concentration during growth. Since garlic mustard grows at variable densities, alterations to the expression of sinigrin is expected with regards to resource availability, defense metabolism cost, and competition (Coley 1985).

Plants generally have constraints to support both growth and production of secondary compounds. As such, ecological tradeoffs are often detected (Herms and Mattson 1992). Here, I observed negative relationships between sinigrin concentration

and growth characteristics of garlic mustard second-year stems. Although the relationships in this study were not all significantly correlated, Figure 1 demonstrates that growth traits of maximum height, shoot and root mass of field populations have a strong negative relationship to sinigrin concentration. These negative correlations between constitutive defense and competitive ability were similarly observed in Ballhorn et al., (2014) where they found that plants with low investments in secondary compound expression were able to allocate resources to seed production and growth. Although the sinigrin concentration of field populations did not have a significant effect on seed production, the maximum height of second-year stems did. This supports findings reviewed in Herms and Mattson (1992) that plants are often unable to acquire resources needed for both primary and secondary compound processes to grow and defense within and among plant populations thus observable tradeoffs in chemical concentration and primary functions result.

Many observations of garlic mustard growing at high field densities across North American forests have been recorded (Pardini 2009). Based on the variation of previously recorded garlic mustard densities, changes in garlic mustard's sinigrin expression were expected here. My observations of sinigrin concentration across a density gradient did not show garlic mustard conforming to the apparency hypothesis of having varied chemical expression based on the species detectability and relative densities to surrounding plant species. Plants growing at low densities are considered non-apparent and should not have large investments in defense, while the opposite is stated for apparent plants (Coley 1987). Across the stem density ranges observed here, there were no major variations in sinigrin concentrations across the density gradient, nonetheless stem density overall did

influence leaf sinigrin concentration. Variation in sinigrin concentration based on the number of stems would have been an indicator of garlic mustard adhering to the apparency hypothesis. In the greenhouse experiments, sinigrin concentration differed by stem density. Many studies have observed chemical concentration of secondary compounds to be at their highest during the early stages of seedling growth (Herms and Mattson 1992; Kliebenstein et al., 2001; Meyers and Anderson 2003), but intraspecific density and defense comparisons are few.

The apparency of garlic mustard defenses is most likely variable due to an array of factors. Here, only the constitutive secondary compound sinigrin found in leaves were evaluated but flavonoids and inducible compounds not found in leaves however, likely play a role in defense and have been shown to vary in other garlic mustard plant tissues such as roots and seeds by external stressors such as competition and herbivory (Cipollini 2002; Brown et al., 2003; van Geem et al., 2016). By assessing sinigrin concentrations in response to density in the field and greenhouse experiments, conclusions on influential biotic and abiotic factors on constitutive sinigrin may be drawn. Coley (2002), found that total glucosinolate levels did not vary significantly across garlic mustard field populations but soil characteristics did. Although belowground soil interactions which likely affect forest ecology were not measured here, the apparency of garlic mustard above-ground defenses suggests that leaf tissue may be poorly defended within garlic mustard populations in comparison to other plant tissues. The insignificant results of soil nutrient concentrations revealed that soil characteristics did not play a detectable role in above-ground defense responses under the conditions I examined. The expression of constitutive defenses in other plant tissues of garlic mustard may have greater effects on

resource accumulation and chemical distribution to plants surrounding garlic mustard populations. Results here suggest future studies focus on the differences in chemical concentration in above and belowground plant tissues within populations that have similar habitat qualities and populations with different habitat qualities. Inferring how different habitat qualities may affect singrin concentrations, invasion history of field sites need consideration (Lankau 2009).

The slope relationships between sinigrin concentration and garlic mustard stem count in the field did not signal significance when all sites were analyzed together but a difference in slope direction by town was clear with Amherst positively correlated. Amongst the density ranges observed in previous studies, the ranges observed here varied in rosette and adult density by site. Of the field populations, the Amherst forest site Sylvan Woods, appeared to be newly invaded by garlic mustard since this site had few abundances of second-year stems and very little presence of garlic mustard plants extending past the established study plots. The Sylvan Woods forest is mainly absent of garlic mustard stems but abundant in first-year rosettes. As with many of the field populations in Amherst, rosette count was higher than expected. Plots in the Northampton were nearly free of garlic mustard rosettes. This difference in first-year rosette density is a plausible explanation for the differing slope relationship in sinigrin concentration by second-year adult stem density. The difference in the density ranges of first and second-year garlic mustard stem count did fit within the observations discussed in (Meekins and McCarthy 2001; Pardini 2009; and van Geem et al., 2016) but not as extreme in the high-density ranges. Coley (1987) classified plant strategies to defend and noted that variation in chemical defense is most likely due to habitat quality. In this study, characteristics of

soil temperature, pH, micro and macronutrients and cation exchange capacity were measured and did not vary significantly across forests sites. The differing slopes by town is an indicator of changes in chemical expression driven by forest characteristics other than soil composition and light since no significant effect of light was observed in the field. Conversely, previous studies have observed physiological constraints to growth of garlic mustard because of light intensity (Smith and Reynolds 2014; Stinson and Seidler 2014), but the effect of light intensity on leaf sinigrin concentration by stem density appears adjustable.

Despite previous studies documenting significant changes in leaf glucosinolate expression of related brassica species during growth and different light levels (Kliebenstein 2001, Smith 2015), I found little evidence of light significantly effecting leaf sinigrin concentration. While these findings did not follow my prediction that light would cause a significant effect in leaf sinigrin concentration, other studies have observed trends of light effects on primary functions. For example, Myers and Anderson (2003) observed a plastic response of photosynthetic rates changing to different irradiance levels because of changes in forest canopy cover. Since light levels effect the rate of photosynthesis and light energy is used for growth or defense, a clear pattern of light effects on defense or growth was expected (Donaldson et al., 2006). Although an interaction between the light and density treatments were observed, this interaction was not significant. Even though higher light did yield increased specific leaf area growth, this difference was not significant across shade and sun treatments. Sinigrin's contrary response to light and density observed in the greenhouse experiment is interpreted as leaf sinigrin concentration having plastic responses to light with minimal differences in

concentration levels based on density. In an experiment of high and low light effects on garlic mustard rosettes, Smith (2015) observed a slight trend in leaf sinigrin concentration with concentration decreasing as light levels increase from 5% to 90% full sun. Here, the results show that the 50% shade treatment had no significant impact on rosette leaf sinigrin concentration, instead sinigrin concentration inversely responded to light and density.

CHAPTER 5

CONCLUSION

I conclude that the empirical evidence of trade-offs in growth and sinigrin concentration do not provide strong support for the EICA hypothesis. According to the EICA, tradeoffs in defense, growth and fitness are expected of non-native plants growing outside of their native range. Since tradeoffs in sinigrin concentration and fitness measured as seed production, were not significant for all growth metrics, second-year garlic mustard appears to be limited in abilities to continually defend and compete for space to grow. As Herms and Mattson (1992) suggested, all plants are limited to either grow or defend because large amounts of energy and resources are required for both functions. Long term research may yield results that mirror the observations discerned in a related brassica species, *Brassica rapa* where neither nutrient availability or competition influenced its defense and growth among native plants (Siemens et al., 2002). Siemens et al., (2002) concluded that glucosinolates were multifunctional, in the context of allelopathy, allowing the species to defend and grow.

The observation of garlic mustard sinigrin concentration across density gradients in the field and density treatments in the greenhouse reveal that stem density is an influential factor on leaf sinigrin concentration. In the field observations within Amherst, sinigrin concentrations were significantly positively associated with stem density and in both greenhouse experiments, rosette stem density resulted in a significant main effect on leaf sinigrin concentration. Although there are not many studies to which this finding compares, there are multiple studies that report variation in garlic mustard stem density

previously mentioned above. It's probable that greater densities may yield greater leaf concentration variation in natural environments.

From leaf sinigrin concentration response to light levels in the density and light experiment, I conclude that garlic mustard exhibits plasticity. The rosettes in the shade treatment altered sinigrin concentration and biomass inversely of the sun treatment, showing that this species exhibits plasticity to adjust in different light environments, even though this shift was not significantly different across density treatments. This is another feature known to enhance garlic mustard's competitiveness as it may invade new forests but persistence in low light conditions is likely constrained (Stinson and Seidler 2014). Here, it appears that plant density affects constitutive leaf sinigrin concentration such that as irradiance changes, above ground sinigrin concentration may change inversely thus influencing garlic mustard's invasion territory.

Although the leaves of garlic mustard plants growing at high densities in the field and greenhouse treatments were of higher sinigrin concentrations than the low-density category, the overall concentration of sinigrin found in the leaves appear minimal in comparison to leaf sinigrin concentration ranges observed in related brassica species by Kirkegaard and Sarwar (1998), where leaf sinigrin concentrations ranged between 0.1—26 μM/g of dry mass. The minimal sinigrin concentration levels observed here reveal that leaves are not of high risks, according to the plant apparency hypothesis. The fast-growing biennial life cycle of garlic mustard may be the reason for the minimal defense of leaves. As much as stem density changes the detectability of garlic mustard leaves, ecological interactions influence physiological processes which influence plant tissue compound expression (Poorter et al., 2012). Future work on assessing long term changes

in concentrations found within different plant tissues may help further identify the impact of light and density on sinigrin expression in garlic mustard and its effects on surrounding species.

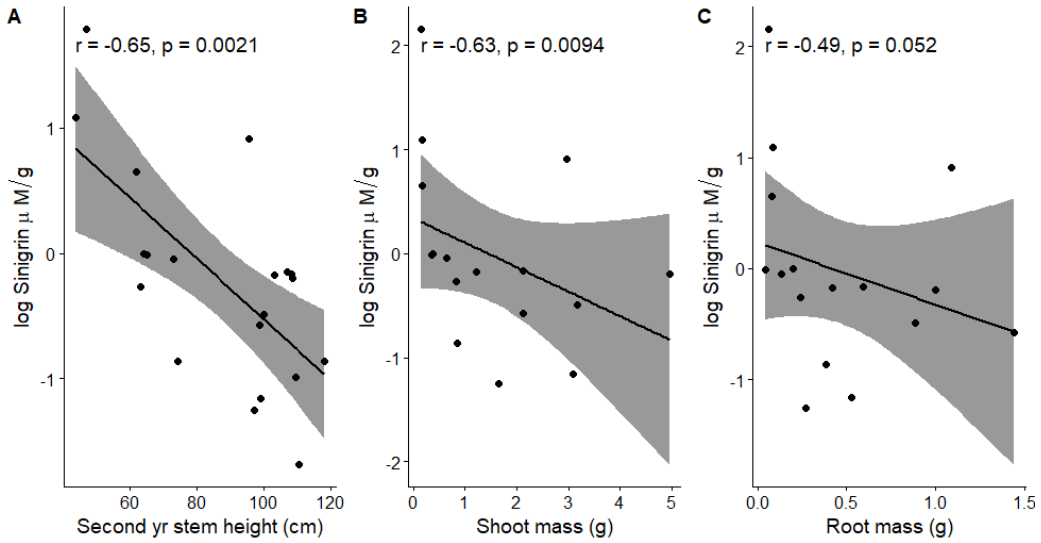


Figure 1. Field density correlation analysis of second-year stems. The relationship strength between growth metrics and sinigrin concentration indicated by Pearson's coefficient with 95% confidence intervals of significance for factors (a) second-year adult stem height, (b) above ground shoot mass, and (c) root mass.

Table 1 Analysis of Variance: Field observations of Garlic Mustard second year trait responses to sinigrin concentration, light, and stem density.

		Stem height		#of Siliques		Total seed weight		Avg. seed weight		Shoot mass		Root mass	
	Df	F	P	F	P	F	P	F	P	F	P	F	P
[Sinigrin]	1,16	15.3	0.001**	0.984	0.336	0.726	0.406	0.341	0.567	0.77	0.393	0.003	0.959
Light	1,16	3.28	0.089	1.586	0.226	0.152	0.701	0.305	0.588	0.069	0.795	1.622	0.221
Adult stem count	1,16	0.004	0.954	0.291	0.597	3.137	0.095	0.266	0.612	2.15	0.162	3.301	0.088

Significance codes: p<0.001***, p<0.01**, p<0.05*

Table 1. Univariate ANOVA results of field density observations. Factors of growth traits response to log scaled sinigrin concentration, light, and adult stem count predictors.

Table 2 Analysis of Variance: Sinigrin concentration response to field stem density and light

	Df	Mean SS	F	P
Total plots				
Adult stem count	1,16	1.4342	2.1031	0.1663
Rosette count	1,16	0.7004	1.027	0.3259
Light	1,16	0.6228	0.9132	0.3535
Amherst Region				
Adult stem count	1,10	2.6672	7.3419	0.0220*
Rosette count	1,10	0.156	0.4293	0.5271
Light	1,10	0.601	1.678	0.2243
Northampton Region				
Adult stem count	1,2	1.0798	0.7096	0.4883
Rosette count	1,2	0.1277	0.084	0.7992
Light	1,2	0.4034	0.2653	0.6578

Significance codes: p<0.001***, p<0.01**, p<0.05*

Table 2. ANOVA of field observations singrin response to density and light. Three separate liner regressions with factors adult stem count, rosette count, and light analyzed together and by town.

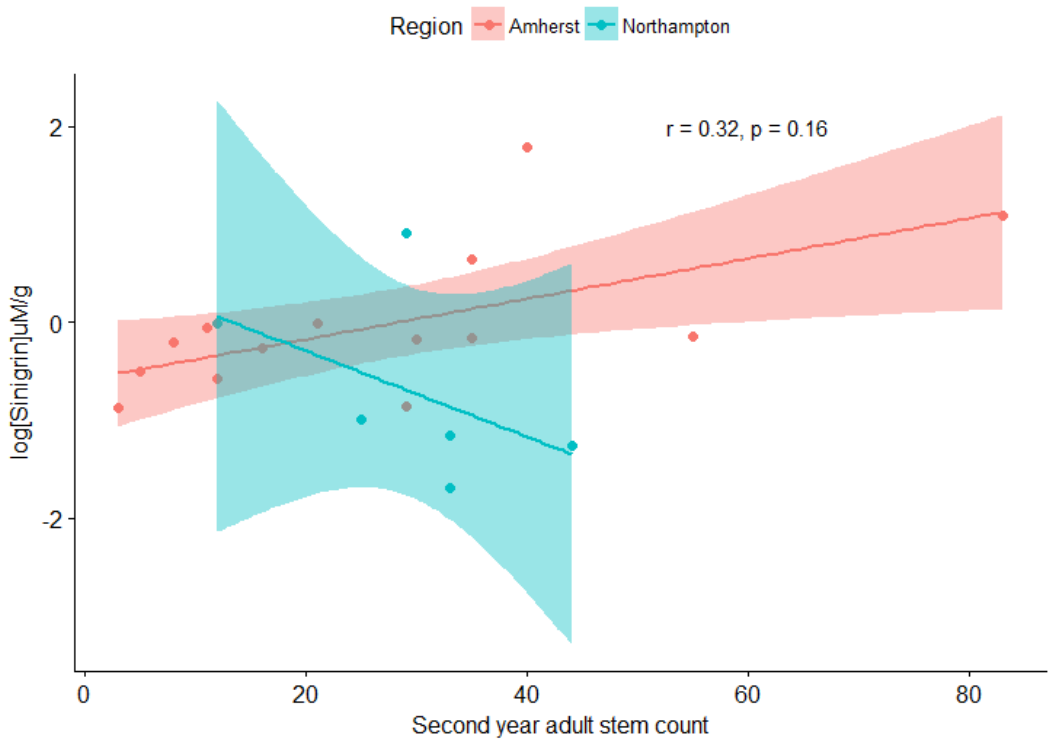


Figure 2. Field density correlation analysis within towns. The relationship and significance between sinigrin concentration and second-year adult stem count indicated by Pearson's coefficient and p value.

Table 3 Analysis of Variance: Density experiment [Sinigrin] response to stem density

X	Df	Mean.SS	F	P
Density treatment	1	6.298	5.128	0.0290*
Residuals	42	1.233		

Significance codes: p<0.001***, p<0.01**, p<0.05*

Table 3. Density effect in the density greenhouse experiment. Effect of rosette stem density on sinigrin concentration.

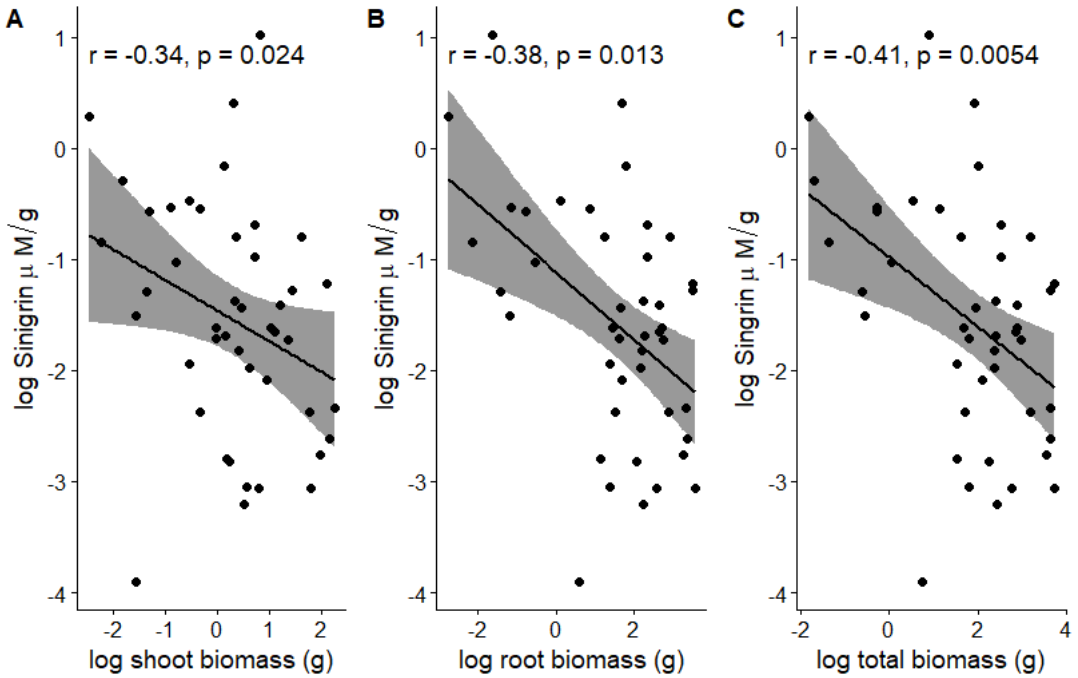


Figure 3. Correlations between sinigrin and rosettes biomass in the greenhouse. Factors of the density greenhouse experiment include (a) shoot mass (c) root mass (d) total biomass. Relationship strength and significance indicated by Pearson's coefficient and p value.

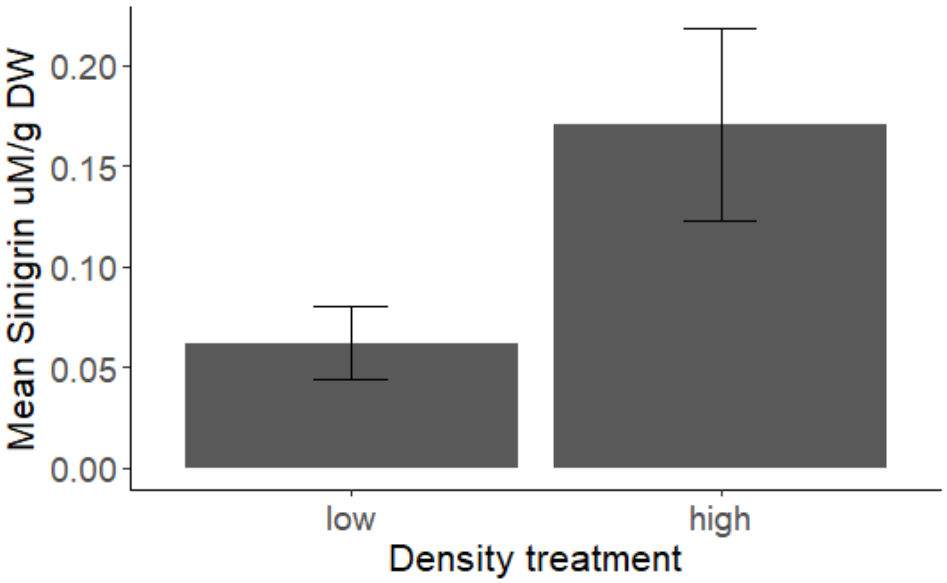


Figure 4. Greenhouse density experiment mean sinigrin and standard error.

Table 4 Analysis of Variance: Greenhouse density and light experiment sinigrin and growth response to treatments.

	[Sinigrin]			Specific Leaf Area			Total Biomass		
	Df	F	P	Df	F	P	Df	F	P
Density	1,23	7.362	0.012*	1,23	8.512	0.007**	1,23	6.672	0.016*
Light	1,23	1.476	0.236	1,23	0.301	0.593	1,23	0.242	0.627
Density:Light	1,23	0.138	0.714	1,23	0.103	0.752	1,23	0.378	0.544

Significance codes: p<0.001***, p<0.01**, p<0.05*

Table 4. Two-way ANOVA effects of density and light treatments. Factors include sinigrin concentration, specific leaf area, and total biomass in response to density categories (high and low), light treatments (shade and sun), and an interaction term.

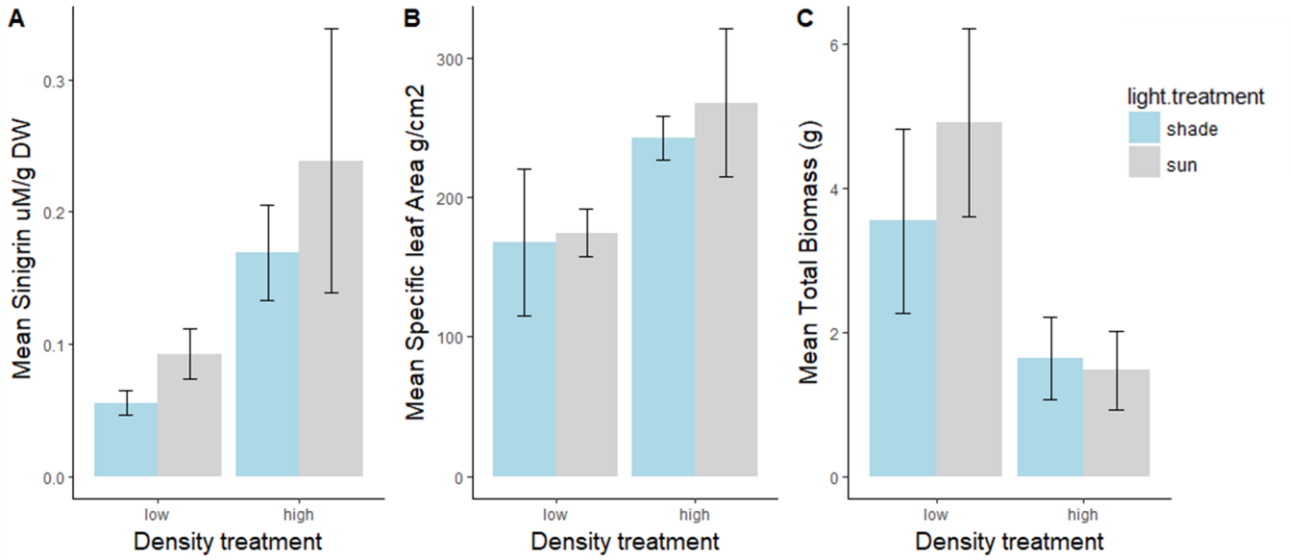


Figure 5. Density and light greenhouse experiment mean and standard error. Response factors include (a) mean sinigrin concentration, (b) mean specific leaf area, and (c) mean total biomass interaction with density and light treatments.

Table 5: Sinigrin concentration mean and standard errors by density and light treatmen

Treatments	Mean	Standard error
High-Shade	0.3003	0.135
High-Sun	0.238	0.099
Low-Shade	0.055	0.009
Low-Sun	0.093	0.019

Table 5. Density and light greenhouse experiment sinigrin mean standard error. Sinigrin concentration mean separated by treatments.

APPENDIX A

FIELD OBSERVATIONS AND GREENHOUSE EXPERIMENTS

Summary of Field Plant Density

Statistic	N	Mean	St. Dev.	Min	Max
Adult stem count	30	28.233	16.747	3	83
Rosette count	30	29.233	49.896	0	189
Garlic mustard total	30	57.067	51.296	6	221
Species	30	54.500	42.462	6	231
Relative density	30	52.422	26.377	11.832	97.753

Appendix Table 1. Summary statistics of field density observation. Statistics include the stem count of 2nd year adult garlic mustard, 1st year rosette, total count of garlic mustard combining 1st and 2nd year, species count as total plant count of garlic mustard stems and other plant species within quadrats, and relative density as the ratio garlic mustard species total in proportion to non-garlic mustard species.

Supplement table: Multiple regression of maximun stem height response to [sinigrin], light and density

Predictors	Estimate	Std.error	P
[Sinigrin]	-17.391	5.199	0.0044**
Light	0.0195	0.011	0.0882
Adult stem count	0.0545	0.223	0.8110
Rosette count	0.1124	0.115	0.3446

Significance codes: p<0.0001***, p<0.01**, p<0.5*

Appendix Table 2: Regression results of field density observations maximum adult stem height response to [sinigrin], light, and density. Model F value= 4.8, Adjusted R^2=0.45, Residual standard error= 17.17 on 15 degrees of freedom.

Summary of Greenhouse Density Experiment

Statistic	N	Mean	St. Dev.	Min	Max
Summer sample:					
Leaf surface area	44	78.068	60.642	1	203
Specific leaf area	44	168.274	83.911	1.006	449.612
[Sinigrin]	44	0.117	0.175	0.011	0.786

Appendix Table 3. Summary statistics of greenhouse density experiment. All mass is weighed in grams.

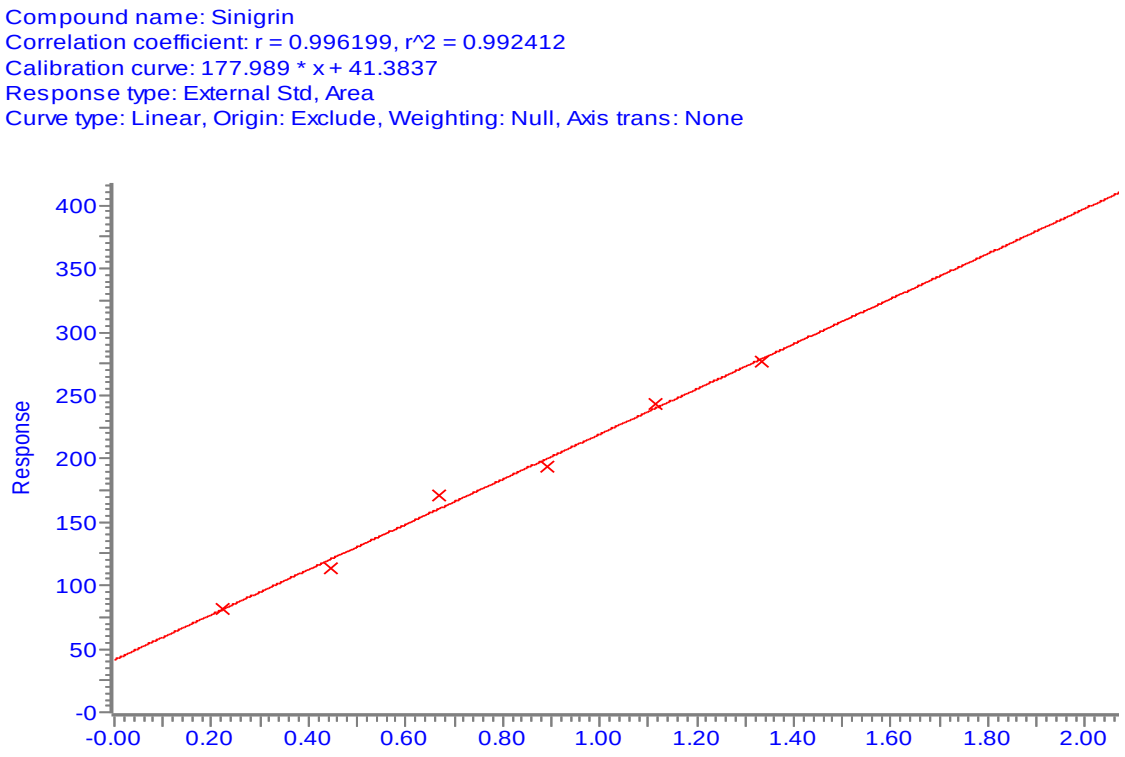
Summary of Greenhouse Density and Light Experiment

Statistic	N	Mean	St. Dev.	Min	Max
Leaf surface area	28	33.607	24.281	3	92
Specific leaf area	28	213.587	77.357	12.587	382.166
[Sinigrin]	28	0.182	0.279	0.029	1.482

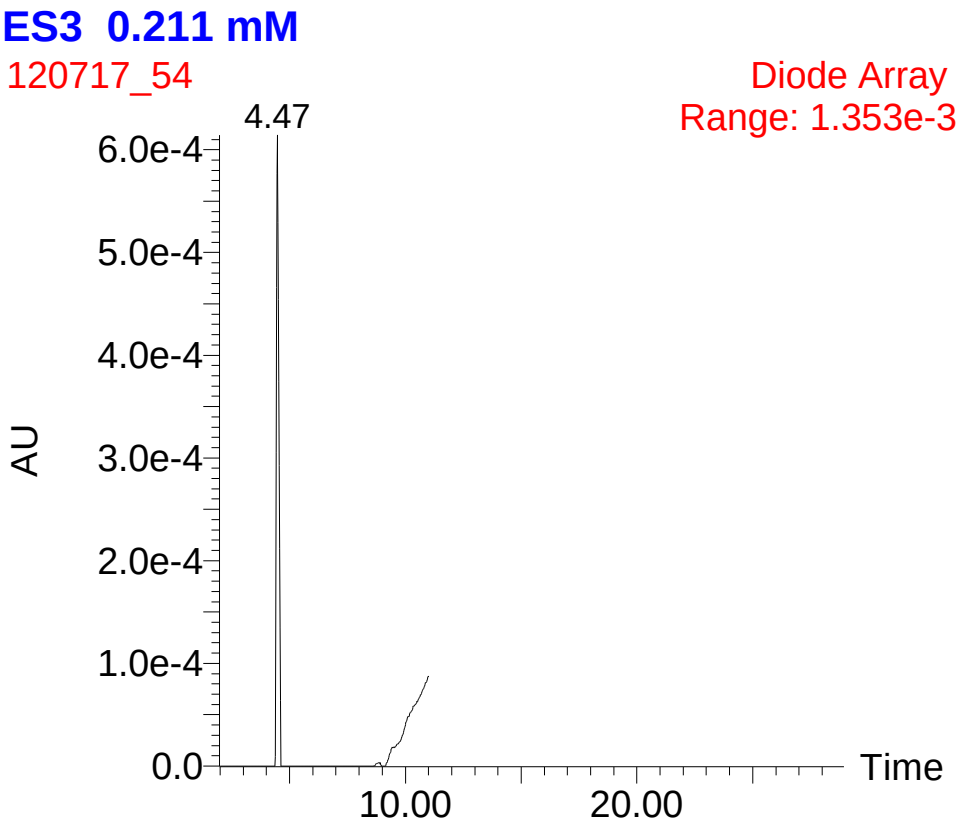
Appendix Table 4. Summary statistics of greenhouse density and light experiment. All mass is weighed in grams.

APPENDIX B

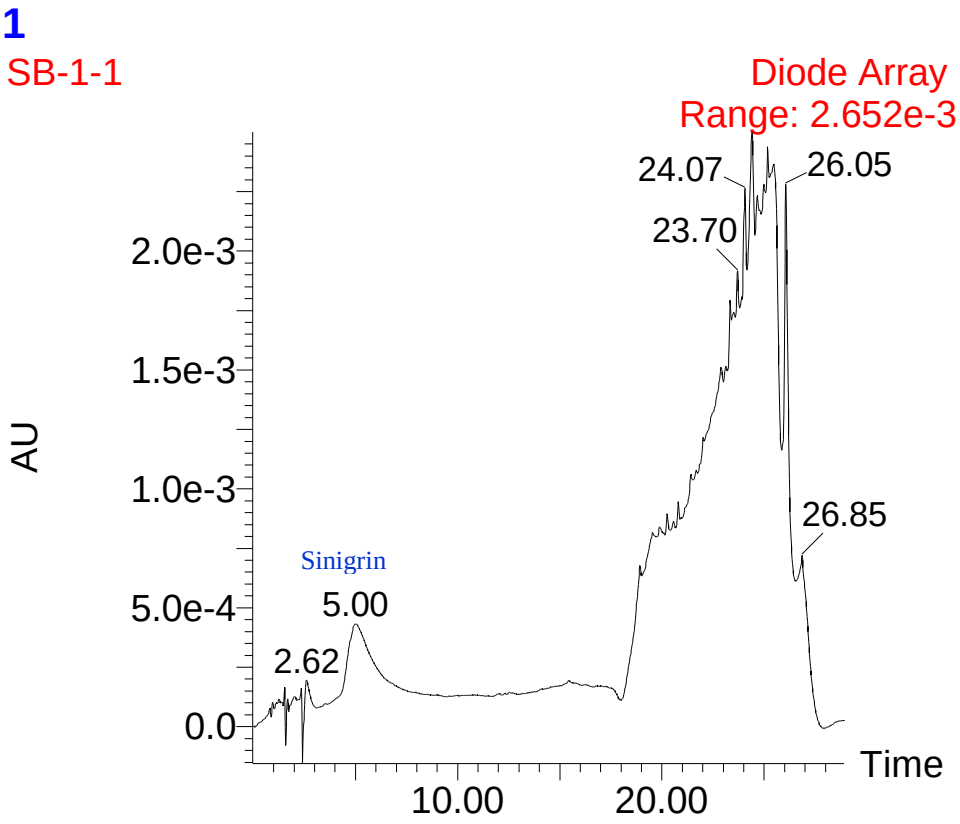
HPLC GRAPHS



Appendix Figure 1. HPLC standard calibration curve of sinigrin (monohydrate). Concentrations were made from 2.11mM stock with ranges similar to Grosser and van Dam 2017. Standard peak area absorbance responses are plotted against concentrations and fit to a straight line.



Appendix Figure 2. Representative chromatogram of the external standard sinigrin monohydrate concentrated at 0.211 mM and UV absorbance at 229nm.



Appendix Figure 3. Representative chromatograph of greenhouse low-density rosette leaf sample with the HPLC conditions set to 229nm UV absorbance with a diode array detector. Peak at 5.00 minutes identified as sinigrin based on external standard and retention time.

BIBLIOGRAPHY

Albuquerque, U.P. and R.F.P. Lucena. 2005. Can apparency affect the use of plants by local people in tropical forests? Interciencia 30: 506-510.

Alencar, N. L., de Sousa Araujo., Cavalcanti de Amorim, E. L., and U.P de Albuquerque. 2009. Can the Apparency Hypothesis explain the selection of medicinal plants in an area of *caatinga* vegetation? A chemical prespective. Scientific Note 910-911.

Anderson, H. 2012. Invasive Garlic Mustard (*Alliaria petiolata*) Best Management Practices in Ontatio. Ontario Invasive Plant Council (OIPC).

Anderson, R. C., S. S. Dhillion, and T. M. Kelley. 1996. Aspects of the ecology of an invasive plant, garlic mustard (*Alliaria petiolata*), in central Illinois. Restor. Ecol. 4:181–191.

Anthony, M. A., S.D. Frey, and K. A. Stinson. 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. Ecosphere 8(9):e01951.10.1002/ecs2.1951

Ballhorn, J. D., A. L. Goschalx, S.M. Smart, S. Kautz, and M. Schadler. 2014. Chemical defense lowers plant competitiveness. Oecologia 176:811-824.

Barto, K. E. and D. Cipollini. 2009. Half-lives and field concentrations of *Alliaria petiolata* secondary metabolites. Chemosphere. doi:10.1016/j.chemosphere.2009.02.020.

Barto, K. E., R. J. Powell, and D. Cipollini. 2010. How novel are the chemical weapons of garlic mustard in North American forest understories? Biol Invasions 12:3465-3471.

Baskin, J. M. and C.C. Baskin. 1992. Seed germination biology of the weedy biennial *Alliaria petiolata*. Nat. Areas J 12:191-197.

Bauer, J. T., R. C. Anderson., and M. R. Anderson. 2010. Competitive Interactions among First-Year and Second-Year Plants of Invasive, Biennial Garlic Mustard (*Alliaria petiolata*) and Native Ground Layer Vegetation.

Blossey, B., R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83: 887–889.

Blumenthal, D. 2005. Interrelated Causes of Plant Invasion. Science 310: 343-344.

Blumenthal, D. 2006. Interactions between resource availability and enemy release in plant invasion. Ecology Letters 9:887-895.

Bossdorf. O. 2013. Enemy release and evolution of increased competitive ability: At last, a smoking gun! New Phytologist 198:638-640.

Brown, P. D, J. G. Tokuhisa, M. Reichelt, J. Gershenzon. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry 62:471-481.

Callaway, M. R. and M. W. Ridenour. 2004. Novel Weapons: Invasive Success and the Evolution of Increased Competitive Ability. Frontiers in Ecology and the Environment. Vol. 2 No. pp.436-443.

Cannell, M. G. R., P. Rothery and E. D. Ford. 1984. Competition within stands of *Picea sitchensis* and *Pinus contorta*. Ann. Bot. (London) 53:349-362

Cantor, A., Hale. A. Aaron, J. Traw. M. B, and S. Kalisz. 2011. Low allelochemical concentration detected in garlic mustard invaded forest soils inhibit fungal growth and AMF spore germination. Springer Science 13:3015–3025.

Carvers, P. B., M. I. Heagvy, and R. F. Kokron. 1979. The biology of Canadian weeds 35. *Alliaria petiolata* (M. Bieb.) Cavara & Grande. Can. J. Plant Sci 59:217–229.

Chapman, J. I., P. D. Cantino, and B. C. McCarthy. 2012. Seed Production in Garlic Mustard (*Alliaria petiolata*) Prevented by Some Methods of Manual Removal. Natural Areas Journal 32:305-315.

Coley, P. D., J. P. Bryant, F. S. Chapin III. 1985. Recourse Availability and Plant Antiherbivore Defense. Science 230 (4728):895-899. [doi:10.1126/science.230.4728.895]

Coley, P. D. 1987. Interspecific Variation in Plant Anti-Herbivore Properties: The Role of Habitat Quality and Rate of Disturbance. New Phytol 106:251-263.

Crawley., J. M. Plant population dynamics. 2007. Pages 62-82 in R. M. May, editor. Theoretical Ecology Principles and Applications 3 (ed) University of Oxford Press, Oxford, UK.

Cipollini, D. 2002. Variation in the expression of chemical defenses in *Alliaria petiolata* (Brassicaceae) in the field and greenhouse. American Journal of Botany 89:1422-1430.

Cipollini, D. 2004. Chemical defenses in garlic mustard (*Alliaria petiolata*) and their potential role in species interactions in forest understories. Proceedings, XV USDA Interagency Research Form on gypsy moth and other invasive species.

Daubenmire, R. 1959. A Canopy-Coverage Method of Vegetational Analysis. Northwest Science 33:43-64.

Davis, M. A., K. J. Wrage, P. B. Reich, M. G, Tjoelker, T, Schaeffer, C. Muermann. 1999. Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. Plant Ecology 145: 341-350.

Davis, S. L., Frisch, T., Bjarnholt, N., and D. Cipollini. 2015. How Does Garlic Mustard Lure and Kill the West Virginia Butterfly? Chem. Ecology. DOI: 10.1007/s10886-015-0633-3.

Dennis, B., R. A. Desharnais, J. M. Cushing, and R. F. Costantino. 1995. Nonlinear demographic dynamics: mathematical models, statistical methods, and biological experiments. Ecological Monographs 65:261–281.

.

Donaldson, J.R., E. L. Kruger., and R. L. Lindroth. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol 169:561-570.

Fahey, J.W., Y. Zhang., and P. Talalay, 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proceedings of the National Academy of Science of the USA 94, 10367±10372.

Feeny, P. 1976. Plant apparency and chemical defense. In Wallace JW, Nansel RL (Eds.) *Biological Interactions Between Plants and Insects*. Recent Advances in Phytochemistry 10. Plenum Press. New York, USA. pp. 1-40.

Feeny, P. 1977. Defense Ecology of the Cruciferae. Annals of the Missouri Botanical Garden. 64:221-234.

Ford, E. D. 1975. Competition and stand structure in some even-aged monocultures. J. Ecol., 63:311- 333.

Frisch, T., N. Agerbirk, S. Davis et al., 2014. Glucosinolate-related glucosides in *Alliaria petiolata*: sources of variation in the plant and different metabolism in an adapted specialist herbivore, *Pieris rapae*, Journal of Chemical Ecology 40(10)1063–1079.

Futuyma D. 1976. Food plant specialization and environmental predictability in Lepidoptera. American Naturalist 110:285–292.

Herms, D. A., W. T. Mattson. 1992. The Dilemma of Plants: To grow or defend. Quart Rev Biol 67:283-335.

Hastings, A., and L. W. Botsford. 2006. A simple persistence condition for structured populations. Ecology Letters 9:846–852.

Keane, R. M., and Crawley, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution, 17(4): 164-170.

Keeler, S. M., and F. S. Chew. 2008. Escaping an Evolutionary Trap: Preference and Performance of a Native Insect on an Exotic Invasive Host. Oecologia 156:559-568.

Kiddle, G., R. N. Bennet., N. P. Botting., N. E. Davidson., A. Avril., B. Robertson., and R. M. Wallsgrove. 2001. High-performance Liquid Chromatographic Separation of Natural and Synthetic Desulphoglucosinolates and their Chemical Validation by UV, NMR and Chemical Ionization-MS Methods. Phytochem. Anal. 12:226-242.

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

Kliebenstein, J. D., J. Kroymann, P. Brown, A. Figuth, D. Pedersen, J. Gershenzon, and T. Mitchell-Olds. 2001. Genetic Control of Natural Variation in Arabidopsis Glucosinolate Accumulation. Plant Physiology 126:811-825.

Lankau. A. R. 2008. A chemical trait creates a genetic trade-off between intra- and interspecific competitive ability. Ecology 89(5):1181–1187. doi: 10.1890/07-1541.1

Lankau, A. R., V. Nuzzo, G. Spyreas., and A. Davis. 2009. Evolutionary limits ameliorate the negative impact of an invasive plant. PNAS 106:15362-15367.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689-711.

Meekins, F. J., and B. C. McCarthy. 2002. Effect of Population Density on the Demography of an Invasive Plant (*Alliaria petiolata*, Brassicaceae) Population in a Southeastern Ohio Forest. Am. Midl. Nat. 147:256-278.

Mitchell-Olds, T., D, Siemens, and D. Pedersen. 1996. Physiology and costs of resistance to herbivory and disease in *Brassica*. Entomologia Experimentalis et Applicata 80:231–237.

Myers, C.V., and R. C. Anderson. 2003. Seasonal Variation in Photosynthetic Rates Influence Success of an Invasive Plant, Garlic Mustard (*Alliaria petiolata*). Am. Midl. Nat. 150:231–245.

Myers, C. V., R. C. Anderson, and D. L. Byers. 2005. Influence of shading on the growth and leaf photosynthesis of the invasive non-indigenous plant garlic mustard [*Alliaria petiolata* (M. Bieb) Cavara and Grande] grown under simulated late-winter to mid-spring conditions. J of Torrey Botanical Society 132:1-10.

Nuzzo, V. A. 1991. Experimental control of garlic mustard [*Alliaria petiolata* (Bieb.) Cavara and Grande] in Northern Illinois using fire, herbicide, and cutting. Nat. Areas J 11:158-167.

Nuzzo, V. A. 1993. Distribution and spread of the invasive biennial *Alliaria petiolata* (garlic mustard) in North America, p. 137-145. In: B. N. McKnight (ed.). Biological pollution: the control and impact of invasive exotic species. The Illinois Academy of Science, Indianapolis, IL.

Pardini, A. E., J. M. Drake., J. M. Chase., and T. M. Knight. 2009. Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). Ecological Applications 19:387-397.

Palmblad, I. G. 1968. Competition in experimental populations of weeds with emphasis on regulation of population size. Ecology, 49:26-34.

Poon, G. T., and H Maherali. 2015. Competitive interactions between a nonmycorrhizal invasive plant, *Alliaria petiolata* and a suite of mycorrhizal grassland, old field, and forest species. Peer J 3:e10900: DOI 10.7717/peerj.1090

Poorter, H. K. J. Niklas., P. B. Reich., J. Oleksyn., P. Poot., and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193:30-50.

R Development Core Team 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rai, K. P. 2015. What makes the plant invasion possible? Paradigm of invasion mechanisms, theories and attributes. Springer International. Environmental Monitoring Assessment 187: 759.

Renwick, J. A., and K. Lopez. 1999. Experience-based food consumption by larvae of *Pieris rapae*: addiction to glucosinolates? Entomologia Experimentalis et Applicata 91: 51–58.

Roberts, K. J., and R. C. Anderson. 2001. Effect of Garlic Mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi. Am. Midl. Nat. 146:146-152.

Rhoades, D. F., and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Pages 168– 213 in Recent Advances in Phytochemistry 10:168-213.

Siemens, D.H., Garner. S.H, Mitchell-Olds T, Callaway R. M. 2002**.** Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology 83: 505–517.

Smilanich, A. M., R. M. Fincher, and L. A. Dyer. 2016. Does plant apparency matter? Thirty years of data provide limited support but reveal clear patterns of the effect of plant chemistry on herbivores. New Phytol 210:1044-1057 doi:10.1111/nph.13785

Smith, M. L., H. L. Reynolds. 2014. Light, allelopathy, and post-mortem invasive impact on native understory forest species. Biol Invasions 16:1131–1144.

Smith, M. L. 2015. Garlic Mustard (*Alliaria petiolata*) Glucosinolate Content Varies Across a Natural Light Gradient. Chemical Ecology 41:486-492.

Stamp, N. 2003. Out of the quagmire of plant defense hypothesis. The Quaterly Review of Biology Vol. 78, 1 pp. 23-55.

Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4:e140.

Stinson, K.A., T.G. Seidler. 2014. Physiological constraints on the spread of *Alliaria petiolata* populations in Massachusetts. Ecosphere 5. doi:10.1890/es14-00164.1

Tallamy, D. W. 2004. Do Alien Plants Reduce Insect Biomass? Conservation Biology 16:1689-1692.

Uddling, J., J. Gelang-Alfredsson, K. Piikki, H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth Res 91:37–46.

van Geem, M., R. Gols., C. E. Raaijmakers., and J. A. Harvey. 2016. Effects of population-related variation in plant primary and secondary metabolites on aboveground and belowground and multitrophic interactions. Chemoecology 26:219-233.

Warnes. G.R., B. Bolker, L. Bonebakker R. Gentleman, W. Huber, A. Liaw, T. Lumley, M. Maechler, A. Magnusson, S. Moeller, M. Schwartz, B. Venables. 2017. ggplots: various R programming tools for plotting data. R package version 2.2.1.

Wheeler, J. A. S.D. Frey, K. A. Stinson. 2017. Tree seedling response to multiple environmental stress: Interactive effects of soil warming, nitrogen fertilization, and plant invasion. Forest Ecology and Management 403:44-51.

Whittle, A. J., S. Lenhart, and L. J. Gross. 2007. Optimal control for management of an invasive plant species. Mathematical Biosciences and Engineering 4:101–112.

Wilson, A. M. 2007. How to: Measuring vegetation characteristic per area. Organ State University. http://oregonstate.edu/instruct/bot440/wilsomar/Content/HTM-perarea.htm#Cover>. Accessed 12 Nov 2016.

Wolfe, B. E., Rodgers, V. L., Stinson, K. A. and Pringle, A. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology, 96: 777–783. doi:10.1111/j.1365-2745.2008.01389.x

Yang, B., C. F. Quiros. 2010. Survey of glucosinolate variation in leaves in *Brassica rapa* crops. Genet Resour Crop Evol 57:1079-1089.

Yost, S. E., S. Antenen and G. Hartvigsen. 1991. The vegetation of the Wave Hill natural area, Bronx, New York. Bull. Torrey Bot. Club 118:312-325.