PLASTICITY AND EVOLUTIONARY POTENTIAL OF *ALLIARIA PETIOLATA*

LIFE HISTORY AND LEAF CHEMISTRY TRAITS IN DIFFERENT

COMPETITIVE ENVIRONMENTS

by

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Abstract

*Alliaria petiolata* is an invasive biennial herb in North America where it has detrimental impacts on flora and fauna. Allelopathy, the chemical inhibition of plant competitors, is one explanation for why *A. petiolata* is more successful in North America than in Eurasia, where it is native. There is strong laboratory evidence that *A. petiolata* is allelopathic, and recent evidence has suggested that natural selection acts on allelopathic compounds (i.e., allelochemicals). If selection is acting on allelochemicals, allelopathy should influence survival and reproduction; however, there is not strong evidence of this in field settings. To shed light on this discrepancy, I measured phenotypic selection, plasticity and heritability of putative allelochemicals and life history traits to determine if there is selection on allelochemicals, if allelochemicals can respond to selection, and if selection is due to a direct effect of allelopathy, or due to selection acting on a correlated trait. Using families from 23 *A. petiolata* populations, I measured glucosinolate, flavonoid and chlorophyll A expression in *A. petiolata* leaf tissue to estimate selection on these traits in inter- and intraspecific competition and without competition. A separate experiment was also conducted to test the effect of soil nutrients on glucosinolate, flavonoid and chlorophyll A plasticity using 3 different *A. petiolata* families. I found that *A. petiolata* exhibited strong plastic responses to both fertilizer application and competition, but most traits exhibited very low heritability. I also found that while selection appeared to act on glucosinolate expression during interspecific competition, there is more evidence to suggest that glucosinolate expression was under indirect selection due to direct selection acting on the correlated trait, chlorophyll A in response to light limitation. There was genetic variation for glucosinolate expression, but it was
mostly manifested in the ratio of glucosinolate: chlorophyll A expression, therefore, the heritability of glucosinolate expression is likely to vary among environments and to be higher in nutrient-rich environments. These results highlight the need to account for plasticity and heritability and measure other important traits to account for indirect selection when assessing evolutionary potential.
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List of Abbreviations

AMF Arbuscular mycorrhizal fungi
ERCA Evolution of increased competitive ability hypothesis
ERH Enemy release hypothesis
MAM Minimum adequate model
NWH Novel weapons hypothesis
PC1 Principal component one
PC2 Principal component two
PC3 Principal component three
PCA Principal component analysis
PRC Partial regression coefficient
QUBS Queen’s University Biological Station
RFI Relative flavonoid investment
RGI Relative glucosinolate investment
RGR1 Relative growth rate one
TLA Total leaf area
TNP Total number of pixels
Chapter 1

Introduction

Most organisms have range limits and have evolved for millennia to both the biotic and abiotic aspects of the environment. With human activity breaking down the physical range limits that once restricted the spread of species, many are now transported across the globe to a variety of new habitats (Pimentel et al. 2005). Most introduced species are poorly adapted to the environments into which they are introduced and perish in the process. Other species are pre-adapted to the environmental conditions in which they are introduced and become naturalized, but do not disrupt the ecosystem (Divíšek et al. 2018; van Kleunen et al. 2018). However, a few species not only become established in the new environment but also become more successful here than where they are native.

Many hypotheses explain the disparity in population size and density between invasive species in their native and invasive ranges. Greater frequency and density of propagule introduction into a new range can increase the likelihood of overcoming stochastic local extinctions, increase fitness through Allee effects, and provide more opportunity for adaptation to novel selection pressures. However, propagule pressure alone cannot fully explain why certain species become invasive (Colautti et al. 2006). Darwin (1859) was perhaps the first to propose an explanation for the enigma of invasive species and explained the phenomenon as a natural product of evolution. He posited that over evolutionary time, species occupy more and more specific niches due to selective pressures exerted by competition. This increased niche specificity may allow a foreign species to occupy an unexploited niche by chance, giving it a competitive advantage over other species and allow for its rapid dissemination (Cadotte et al. 2018).Introduced
species need to be somewhat pre-adapted to the environmental conditions of the new range to be successful in establishing (e.g., a deciduous tree introduced into a desert would not be). However, phylogenetic and morphological differences observed between the introduced species and native flora are associated with invasion success, which supports Darwin’s Naturalization Hypothesis 150 years later (Fridley and Sax 2014; Divišek et al. 2018).

Other invasion hypotheses have been proposed but many are derivatives of Darwin’s original formulation. For example, the enemy release hypothesis (ERH) implicitly follows Darwin’s idea that invasive species occupy unexploited niches in the environments to which they are introduced. Plants co-evolve defense compounds to defend themselves against the herbivores and pathogens that exploit them, and these pests in turn co-evolve mechanisms to overcome these defenses. The ERH posits that upon introduction, species may escape their natural enemies in the invasive range because they have evolved defense chemicals to which native herbivores and pathogens are not adapted. In other words, invaders evolve unique defenses which allow them to occupy a niche bereft of enemies. There is evidence that species can escape their natural enemies when introduced into a new range, reducing top-down population control and facilitating invasion (Keane and Crawley 2002; Liu and Stiling 2006). Furthermore, release from top-down control may relax selection on defenses and increase investment in growth and reproduction, further increasing the likelihood of invasion (Blossey and Notzold 1995; Müller-Schärer et al. 2004; Rotter and Holeski 2018).

Darwin’s logic surrounding species invasions is also observed in hypotheses that invoke allelopathy as a mechanism facilitating species invasions. Instead of evolution
molding unique defenses or resource acquisition strategies, species may evolve unique “offenses” which allow them to occupy a niche bereft of competitors (Lau and Schultheis 2015). Allelopathy (in botanical terms) is the chemical inhibition of one plant by another. Toxic compounds (“allelochemicals”), generated in either the leaves or roots of a plant, can be released into the soil through exudation or decomposition to inhibit competitors (Gomes et al. 2017). Callaway & Ridenour (2004) introduced the “novel weapons hypothesis” (NWH), which formalized the idea that allelopathy can facilitate species invasions. They posited that invasive species release compounds into the soil that are inert to the flora of its native range, because the flora there have evolved an immunity to the compounds. In contrast, the flora of the introduced range are evolutionarily naïve and thus susceptible to the compounds. The NWH has motivated many papers that attribute the success of invaders to their allelopathic effects on the susceptible flora in the introduced range (Inderjit et al. 2008; Cipollini and Cipollini 2016; Gomes et al. 2017).

The NWH provides a new spin on allelopathy, a field of research with a rich and controversial history. While the concept of allelopathy is often credited to Augustin Pyramus de Candolle in 1832, the concept has been around much earlier. Perhaps the first to contemplate allelopathy was the Athenian, Solon (638-559 BC), who ordered fig and olive trees be planted specific distances apart because the plants may harm each other through the “effluvia” that they release (Willis 2008a). However, a modern acknowledgement of allelopathy wouldn’t be until the 1700s when the discovery of root excretions prompted the Dutch botanist, Justinus Brugmans and his student, Julius Vitringra Coulon, to ascribe the poor growth of contiguous plant pairs in crop rotations to the toxic effects of root exudates. An editor of The Monthly Review wrote of their paper
(Anonymous 1789): “They [M. Coulon, and Professor Brugmans] assert that the grain, instead of being deprived of nutrition, by continuing long in the same soil, ejects, from its roots, a fluid which is pernicious to its own growth, and to the growth of some other vegetables.”. Interestingly, the ideas of both individuals were brought to the English speaking world by Charles Darwin’s grandfather, Erasmus Darwin (Willis 2008b).

Over a century later, the Austrian physicist Hans Molisch termed the phrase allelopathy in 1937. By this time, there was enough research surrounding allelopathy for a literature review, which found little support for allelopathy and instead emphasized nitrogen limitation (Loehwing 1937). Nonetheless, a steady interest in the subject was maintained and over the following years and many more reviews were published on the topic (for a list, see Rice, 1979). It wasn’t until 1964, when C.H. Muller described the bare zones surrounding the desert shrub, *Salvia leucophylla* to be caused by chemical inhibition that allelopathy gained widespread interest (Muller et al. 1964). This paper made the cover of science magazine, attracting both praise and criticism surrounding the work (Halsey 2004). The proposed method of chemical transmission was the evaporation of toxic compounds from leaves into the atmosphere, which then condense as a dew to poison neighboring plants. Despite the criticisms, praise from influential ecologists (i.e. Whittaker & Feeny, 1971) invigorated the field of allelopathy. Publication rates on the subject tripled in the following year (Williamson 1990), an influential book describing the current evidence for allelopathy was published (Rice 1974), the National Academy of Sciences sponsored an allelopathy conference, and in 1974, the Journal of Chemical Ecology was founded (Williamson 1990). While this article brought fame to the field of allelopathy, it should have brough infamy.
Unconvinced by the work of Muller in 1964, Bruce Bartholomew investigated the bare zones surrounding *S. leucophylla* in the desert. Bartholomew (1970) used an herbivory exclusion technique to demonstrate that the bare zones supposedly caused by allelopathy were caused by the rodents, rabbits and birds that used the bush for cover and grazed on the surrounding vegetation. In spite of this evidence, the support from Whittaker and Feeny in 1971 was enough for the field to thrive. It wasn’t until John Harper gave a disparaging review of Rice’s book, Allelopathy, criticizing it for praising flawed experimental designs and ignoring contrary evidence, that the field was divided (Harper 1975). Shortly after, Stowe (1979) demonstrated that the outcome of allelopathy experiments depends on the method used to assess it - a phenomenon that has been demonstrated again recently by Parepa and Bossdorf (2016) - and an air of skepticism surrounded the field.

For the next 20 years, allelopathy fell out of favor until the hypothesis began to be implicated in species invasions. *Centurea maculosa* is a problematic invader in the US, that grows to very high densities and covers large ranges. While 13 biological control agents have been introduced to control the spread of the species, none have worked (Müller-Schärer & Schroeder, 1993). In an article published in Science, Bais et al. (2003) demonstrated that *C. maculosa* inhibits two grasses native to North America by releasing the compound catechin, triggering a reactive oxygen species cascade that ultimately results in the death of the root system in *Arabidopsis thaliana* and *Centaurea diffusa*. This influential article inspired an article of praise entitled “Making allelopathy respectable” (Fitter 2003) and contributed to the formalization of the NWH (Callaway and Ridenour 2004). Unlike the studies of the 60’s and 70’s, this modern approach to
allelopathy purported to avoid the pitfalls of previous work by demonstrating the entire causal pathway of allelopathy, from chemical donor to recipient. However, the high-profile experiment demonstrating allelopathy received further scrutiny. In the experiment, catechin was reportedly extracted in hexane; yet as a hydrophilic compound, catechin is insoluble in hexane. Using an appropriate solvent, Blair et al. (2005) could not detect catechin in soil from *C. maculosa* and found that concentrations 10X higher than that used in Bais et al. (2003) are required to cause a slight inhibition of a putatively susceptible species, *Festuca idahoensis*. These concentrations are far above levels that could occur in nature, making catechin-mediated allelopathy very unlikely (Blair et al. 2006; Blair et al. 2009).

There has always been difficulty in proving allelopathy no matter which approach is taken. Paracelcus wrote in 1538: “What is there that is not poison? All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison.” (Grandjean 2016). This observation underlies the difficulties associated with allelopathy experiments because all compounds are toxic at a high enough dose. To test causation, a putative allelochemical is applied to a plant, but the dose that is released into soil by the donor species, transported and absorbed by the target species in natural soils is often unknown. Allelochemicals undergo natural and biological degradation by microbes in soil and plant roots harbor rich communities of bacteria, fungi and protists, in what is known as the rhizosphere and the rhizoplane (the latter refers to microbes growing directly on plant roots) (Philippot et al. 2013). This reduces the quantity and may alter type of allelochemicals available for uptake by the recipient plant (Cantor et al. 2011; Parepa and Bossdorf 2016). The matter is complicated by the fact that compounds
invoked as potential allelopathic compounds have some other role in the plant, from anti-herbivore defenses, to frost tolerance, pigmentation, UV-light protection and more (Hopkins and Hüner 2009).

*Alliaria petiolata* has become a popular study system for allelopathy research due to its reported inhibition of symbiotic root fungi known as mycorrhizae. *Alliaria petiolata* produces secondary metabolites, glucosinolates and flavonoids in the leaves and roots, which can enter the soil through decomposition or root exudation (Barto and Cipollini 2009; Cantor et al. 2011). Glucosinolates are a class of compound unique to the Brassicaceae, which are degraded by the enzyme myrosinase to produce toxic products including substituted isothiocyanates, thiocyanides, nitriles and oxazolidinethiones (Vaughn and Berhow 1999; Roberts and Anderson 2001). Myrosinase is kept spatially separate from glucosinolates in the cell so that glucosinolates are only degraded upon receiving tissue damage. However, myrosinase has also been detected in soils in which *A. petiolata* was grown, where it may react with glucosinolates to form toxic compounds (Al-Turki and Dick 2003). Flavonoids are another class of potentially allelopathic compounds found in *A. petiolata* (Callaway et al. 2008). There are a wide variety of flavonoids (over 4500 known) and they are found throughout the plant kingdom and in every plant tissue type. They vary in function from pigmentation and pathogen protection to signaling symbiotic rhizobia (Hopkins and Hüner 2009; Panche et al. 2016).

Glucosinolates and flavonoids have been implicated to have toxic effects on arbuscular mycorrhizal fungi (AMF). AMF are obligate plant-root symbionts that greatly increase host performance by increasing water absorption, nutrient acquisition and disease resistance, and over 80% of land plants associate with them (Bonfante and Genre
2010; Denison and Kiers 2011). However, *A. petiolata* does not associate with AMF and has been shown to inhibit mycorrhizal spores and reduce mycorrhizal colonization in native plants in lab and field studies, which in turn may reduce interspecific competition (Roberts and Anderson 2001; Stinson et al. 2006; Barto et al. 2011). In addition, North American AMF have been demonstrated to be susceptible to *A. petiolata* extracts compared to more tolerant European AMF, suggesting that AMF can evolve resistance to the toxic effects of *A. petiolata* (Callaway et al. 2008). Yet in spite of this, studies often find that *A. petiolata* competitive ability, and thus allelopathy, plays little role in regulating population dynamics in nature. (Davis et al. 2014; Kalisz et al. 2014; Anderson et al. 2019). Instead, the success of *A. petiolata* may be attributable to early germination, high fecundity, high plasticity, self-fertilization, and selective grazing by white-tailed deer (Anderson et al. 1996; Cipollini et al. 2005; Kalisz et al. 2014; Smith 2015). Despite these results, a few studies have found evidence that natural selection acts on allelochemical production in natural populations (Lankau et al. 2009; Evans et al. 2016), which should not occur unless allelopathy conveys a benefit to *A. petiolata* and affects survival and/or reproduction.

*Alliaria petiolata* faces interspecific competition when it first colonizes a new environment; however, after some time, populations can grow to very high densities and experience intense intraspecific competition (Davis et al. 2014). If allelopathy in *A. petiolata* is mediated through the toxic effects of glucosinolates and/or flavonoids on AMF, then inhibition could only occur during interspecific competition, when competition is mainly against mycorrhizal hosts. In contrast, when facing intraspecific competition, competition is mainly against non-mycorrhizal hosts, and allelochemical
production would only incur a cost. For this reason, natural selection for allelochemicals may occur in colonizing *A. petiolata* populations, and not in older populations that face mainly intraspecific competition. Evans et al. (2016) sought to test this hypothesis by measuring selection on the glucosinolate compound, sinigrin under inter- and intraspecific competition with the herbaceous species, *Arisaema triphyllum*. They found selection for sinigrin production during interspecific competition, and selection against sinigrin production during intraspecific competition, consistent with allelopathy mediating selection for sinigrin. However, heritability and plasticity were not calculated for sinigrin, therefore, variation in trait values across treatments and the ability of the trait to respond to selection is unknown.

Other papers report evolution of glucosinolates, but also share some of the same limitations. Using one individual from each of 44 populations to obtain a population-level estimate of glucosinolate expression, Lankau et al. (2009) found that glucosinolate production was higher in younger *A. petiolata* populations, where competition was mainly interspecific, and was lower in older *A. petiolata* populations, where competition was mainly intraspecific. They posited that selection was acting on glucosinolate production to increase production in young populations and decrease production in old populations. Later, Lankau (2011) used the same 44 populations with up to five replicates per population to calculate an average glucosinolate trait value for each population. This time, however, a relationship between mycorrhizal inhibition and population age was not found. More recently, work by Huang et al. (2018) used 8 populations and demonstrated that sinigrin production decreased with age but interspecific competitive ability in *A. petiolata* actually increased, casting doubt that sinigrin-mediated allelopathy plays a role
during interspecific competition at all. Importantly, one commonality of the aforementioned studies is that none of these studies calculated the heritability or plasticity of sinigrin, which are important parameters for predicting evolution in response to selection.

*Alliaria petiolata* reproduces asexually, allowing a single individual to colonize a new habitat and establish a population. Indeed, a paucity of genetic variation has been observed within introduced populations of *A. petiolata* (Durka et al. 2005). While correlations between a trait and fitness (i.e., a selection differential) demonstrate natural selection, a population cannot evolve in response to selection on traits with limited genetic variation. To my knowledge, genetic variation for glucosinolate, growth and fitness have not been confirmed in studies of *A. petiolata* measuring selection on glucosinolate expression. Furthermore, it remains a mystery whether selection acting on glucosinolate production is direct or indirect. Direct selection occurs when a trait directly confers a benefit or detriment to the organism, whereas indirect selection occurs when a correlated trait affects fitness. Therefore, it is important to consider multiple biologically relevant traits. This can be done by estimating selection gradients, which measure direct and indirect selection on traits (Lande and Arnold 1983; Arnold and Wade 1984).

In addition to selection gradients and heritable genetic variation, it is important to consider trait plasticity in order to reconstruct evolutionary processes occurring in natural populations of invasive species. Plasticity is an important aspect of species invasions, as plastic responses to environmental stress can promote survival and reproduction in novel environments - especially for species with little genetic variation. For example, Hiatt & Flory (2020) demonstrated that invasive grasses in the genus *Imperata* are more plastic
than all six native species involved in the study, suggesting that plasticity may contribute to the invasiveness of these species. Furthermore, Davidson et al. (2011) analyzed 75 different species and found that invaders on average are more plastic than non-invasive species. Plasticity may be an important aspect of *A. petiolata* invasions because of its high selfing rate and because secondary compound production has been demonstrated to vary significantly with water, light and nutrients within natural populations (Cipollini et al. 2005; Hillstrom and Cipollini 2011; Smith 2015). Aside from the importance of plasticity in species invasions, plasticity can also influence the heritability of traits.

Heritability is an estimate of the proportion of trait variation explained by genetic differences among genotypes or groups of relatives. Environmental effects on phenotype therefore reduce heritability. This means that the heritability of a trait depends on the environment in which the organisms were grown. For example, heritability estimates are often higher when resources are readily available, which may cause greenhouse experiments to overestimate the potential response to selection relative to nutrient-limited natural populations (Charmantier and Garant 2005). In line with this, the more plastic a trait is, the more it responds to environmental variation, which limits heritability and thus evolution in response to selection (Hendry 2016). In other words, plasticity masks the effects of adaptive alleles and impedes adaptive evolution.

The effect of environment on heritability can make predicting responses to selection problematic in a highly plastic plant such as *A. petiolata*. Plastic trait variation could be mistaken for genetic variation and reduce the accuracy of population-level means. For example, Smith (2015) demonstrated significant variation in root glucosinolate expression in an *A. petiolata* population across a light gradient, but found
that none of the variation was attributable to genetic factors when plants were moved to a standardized greenhouse environment. Accounting for phenotypic plasticity is also necessary to obtain accurate population-level estimates of trait values. The more a trait is affected by environmental variation (e.g., greenhouse bench effects, competitor strength, etc.), the more difficult it is to obtain accurate estimates of population parameters.

The goal of my thesis was to estimate plasticity, heritability, and selection on leaf chemistry traits (i.e., glucosinolate, flavonoid and chlorophyll A) and life history traits in *A. petiolata* under intra- and interspecific competition. Specifically, my goals were to (1) determine if glucosinolate and flavonoid expression undergo direct or indirect selection when grown in three different competitive environments; (2) test whether traits under selection exhibit heritable variation and thus could evolve in response to selection; (3) identify correlated traits under selection during inter- and intraspecific competition; and (4) quantify the effects of inter- and intraspecific competition on *A. petiolata* fitness. The answers to these questions are necessary to inform whether or not allelopathy plays an important role during *A. petiolata* invasion.
Chapter 2
Methods

2.1 Study systems

*Alliaria petiolata* (Garlic Mustard) is an invasive plant in North America and was first noted in 1868, in Long Island, New York. *Alliaria petiolata* is native to Eurasia and is a member of the Brassicaceae family. Since its introduction, *A. petiolata* has spread across North America at an alarming rate of expansion, estimated at 6400km²/year as of 1991 (Rodgers 2008). It now occurs most densely along the East Coast of North America, but is also found in the Prairies and along the West Coast between Alaska and Oregon (Anderson 2012). *Alliaria petiolata* is an obligate biennial species, which emerges in early spring, overwinters as a rosette and then forms a flowering bolt in the spring of the following year (Anderson et al. 1996). It preferentially grows on forest edges of mesic deciduous forests, although, *A. petiolata* is shade tolerant and is capable of growing in the forest understory. *Alliaria petiolata* is often associated with high levels of nutrient availability and may alter nutrient cycling by depositing leaf litter high in nitrogen (Rodgers, Wolfe, et al. 2008; Anthony et al. 2017). In North America, it can invade the forest understory and quickly proliferate to form dense populations with detrimental effects on native flora and fauna (Rodgers, Stinson, et al. 2008). Here, *A. petiolata* undergoes significant predation from at least three different insects (Yates and Murphy 2008), and it undergoes natural infection by powdery mildew fungus (*Erysiphe cruciferarum*) (Ciola and Cipollini 2011).

*Alliaria petiolata* reproduces asexually over 80% of the time via self-fertilization (Durka et al. 2005), but can also be pollinated by syrphid flies, midges and bees (Cavers...
et al. 1979; Cruden et al. 1996). *Alliaria petiolata* reproduces only through seeds and exhibits a very high seed rain. A single plant can produce over 3500 seeds and seeds can persist in the soil for over 10 years (Rodgers, Stinson, et al. 2008). The asexual and fecund nature of *A. petiolata* allows a single individual to create new populations. It is therefore unsurprising that 80% of the genetic variation in the species lies among populations (Durka et al. 2005). Despite high inbreeding, the species does not suffer from inbreeding depression, possibly due to its hexaploid genome, which may reduce the likelihood of expressing deleterious recessive alleles (Mullarkey et al. 2013).

*Acer saccharum* (Sugar Maple) is an economically important species in Canada due to its wood and sweet sap. It is native to eastern North America and grows in cool moist regions with deep soils between the latitudes of approximately 35° and 50°. *Acer saccharum* is a shade tolerant species that associates with AMF and grows in over 25 different forest types including hardwood, oak and conifer-dominated forests (Ralph 1998). After 22 years of growth, *A. saccharum* is able to produce fruits in relatively small amounts. Older plants, however, can be very fecund with a seed rain that can exceed 2200 seeds/m². *Acer saccharum* seeds exhibit very high germination success (~95%), resulting in a forest understory with an abundance of *A. saccharum* seedlings, often exceeding 37 plants/m² (Godman et al. 1990). This density of seedlings holds potential for competition with an invading *A. petiolata* population because *A. petiolata* co-occurs with *A. saccharum* throughout the majority of *A. saccharum*’s range in Eastern North America.

*Acer saccharum* seedlings face predation from ungulates, hares and rabbits, which can facilitate the invasion of pest species such as ferns, grasses, beech and striped maple,
and may prevent *A. saccharum* seedlings from establishing (Ralph 1998). Interestingly, selective deer herbivory on native species has also been implicated in the success of *A. petiolata* in colonizing forest understories, providing one hypothesis as to why *A. petiolata* displaces *A. saccharum* in some areas (Kalisz et al. 2014; Anderson et al. 2019). Alternatively, *A. saccharum* has been demonstrated to be particularly susceptible to *A. petiolata* allelopathy in the greenhouse (Stinson et al. 2006), and in the field *A. saccharum* exhibits reduced mycorrhizal colonization where it co-occurs with *A. petiolata* (Barto et al. 2011).

### 2.2 Experimental design

#### 2.2.1 Overview of experiments

In answering my research questions, there is a trade-off between obtaining realistic environmental conditions and controlling for confounding variables. I chose a balanced approach and used a greenhouse to eliminate many confounding variables in the early stages of growth, but I used homogenized field soil and an interspecific competitor from a habitat that is susceptible to *A. petiolata* invasion. *Alliaria petiolata* needs to overwinter in order to flower, and overwinter survival is an important aspect of *A. petiolata* population dynamics in nature. Therefore, after the first growing season, all plants (which remained in their pots) were transplanted to a field site at the Queen’s University Biological Station in the fall to complete their life cycle in the field.

In the main experiment, I used 23 different *A. petiolata* seed families and grew at least 3 individuals from each family in each of 3 different competitive environments: interspecific competition with *A. saccharum*, intraspecific competition with a different *A. petiolata* seed family, and alone. I also grew 31 *A. saccharum* seedlings alone (i.e., the
“maple alone” treatment) as a control for *A. saccharum* growing in the interspecific treatment. In the first growing season, these plants grew in pots in a greenhouse at Queen’s University where I measured leaf chemistry traits, growth rate, rosette size, fern abundance (which grew in pots due to natural inoculation from field soil) and any damage that occurred on the plants as a result of pests.

In a second, smaller experiment, hereafter referred to as the “fertilizer experiment”, I used 3 different *A. petiolata* families and grew at least 10 individuals from each family in the same 3 treatments as above: interspecific competition, intraspecific competition, and alone. In contrast to the main experiment, there was no maple alone treatment and half of the pots in each treatment received a slow-release fertilizer. The goal of this experiment was to determine how *A. petiolata* responds to nutrient availability in each treatment. These plants were harvested after the first growing season and all measurements on these plants took place in the greenhouse. On these plants I measured leaf chemistry traits and rosette shoot mass.

2.2.2 Seed, sapling, and soil sources

*Alliaria petiolata* seeds were collected between 2009-2012 as part of the Global Garlic Mustard Field Survey (Colautti et al. 2014). I considered individuals within a population to represent an inbred line as *A. petiolata* exhibits high rates of inbreeding under natural conditions (> 80%) and 80% of genetic variation segregates among populations in this species (Durka et al. 2005). To eliminate within-population genetic variation, I used the progeny of a single individual in each population to represent a seed family. Four seed families are the first-generation offspring of parental plants in natural
populations (i.e., the families CBMCK1, MAVBEL2, SMAKC1 and RULEB1). Each of the other seed families represent the second-generation progeny an individual that was first grown to maturity in a common garden in 2017 at the Queen’s University Biological Station (QUBS; located in Elgin Ontario: 44.5675° N, 76.3245° W). In 2017, seeds were germinated on autoclaved sand: potting soil (4:1) in petri dishes sealed with parafilm. After germination in October, the plants were transplanted to the field in the summer of 2017 and seeds were harvested the following summer in 2018. Seeds were labeled according to the seed family and stored dry at room temperature until the start of my 2019 experiment. I continued the inbred line by using the offspring of a single individual from the 2017 experiment to represent a seed family. Seed families provide the basis for genetic variation in this experiment.

On May 8, 2019, *A. saccharum* saplings were collected from a natural population located in a forest abutting QUBS (44.5671° N, 76.3250° W). This section of the forest was not invaded by *A. petiolata*, but there was an expanding *A. petiolata* population as near as 25m away from the sampling area and the site seemed suitable for *A. petiolata* invasion. The saplings were extracted by removing a block of soil 12cm deep with an abundance of saplings. This block was transported back to the greenhouse where seedlings were placed into experimental pots, retaining as much field soil around the root system as possible to keep the root hairs and mycorrhizae intact. As *A. saccharum* seedlings were of naturally occurring ages, initial stem heights varied considerably: from 32mm to 145mm at the initiation of the greenhouse experiment. Field soil was obtained adjacent to the area where the saplings were collected. Debris and seedlings were scraped from the surface of the soil and the top layer (approximately 12cm deep) was collected.
Large roots and rocks were removed from the soil, which was then thoroughly mixed with shovels and rakes to homogenize the soil microbiota and nutrient profiles.

2.2.3 Greenhouse design

2.2.3.1 Seedling germination and transplantation

Seeds were stratified in the dark at 4°C in petri dishes (10 seeds per plate) containing unsterilized field soil in order to expose seedlings to the soil characteristics and microbiota at germination. Each plate was treated with 2mL of deionized water, as this volume moistened the soil without saturating it, and plates were then wrapped in parafilm. Seed germination took place between March 7th and May 7th of 2019. If seeds did not germinate before March 26, 2019, germination was forced via scarification and application of 2ml of 0.001M gibberellic acid directly into the petri dishes containing field soil (Sosnoskie and Cardina 2009). Once seedlings germinated, they were transferred into 10×20-cell plug trays filled with a 1:1 mixture of sand and Sun Grow Sunshine® Mix 2 potting soil, which does not contain added nutrients. These trays were kept in a growth chamber programmed to simulate early spring: constant 10°C, with a daylight period of 12 hours at half-light. Trays were watered as needed from above using deionized water.

On May 9-10, 2019, seedlings were transplanted into 4” round plastic pots (surface area = 81cm²; volume = 524.39 cm³) containing a 1:1 ratio of field soil: potting soil (Sun Grow Sunshine® Mix 2) and were moved into a greenhouse. Pot size was chosen to reflect the competition intensity of natural conditions while still maintaining high survival. In natural conditions, A. petiolata density can be as high as 18 plants/dm².
in April and dwindle to as little as 0.6 plants/dm$^2$ by June (Anderson et al. 1996). Density in this experiment was either 1.23 plants/dm$^2$ in the alone treatment or 2.46 plants/dm$^2$ in the intraspecific treatment. Plants were kept under natural light and were artificially shaded with shade cloth to simulate a forest understory. Plants were broadcast watered once or twice per day to maintain soil that was wet to the touch. All plants were tracked using an ID unique to each plant in the form of a barcode sticker, created with the barcodeR package in R, and attached to a plastic garden marker (Wu et al. 2018).

In total, I tried to germinate 3300 seeds from 66 families (50 seeds per family) which originated from 32 different populations. I transplanted into plug trays a total of 1350 seedlings that germinated before the cut-off day of May 7th, 2019. Variable germination and mortality among seed families limited the number of seed families available for experimentation. Seed families were excluded if they did not have enough seedlings available for appropriate replication (more details below). Following transplantation into experimental pots, there was a small amount of mortality. If any individual in a pot perished in the first growing season, all plants in the pot were excluded from the analysis.

2.2.3.2 Main experiment

The main experiment included 23 families and each family was replicated across three treatments: alone, interspecific competition and intraspecific competition (see Table A1 for replication and Fig. A4 for a visualization). To determine the competitor pairs to be used in the intraspecific treatment, I wrote an algorithm in R to randomly pair individuals from different seed families without repeating pair combinations. Pots were
randomized across the first four benches in the greenhouse and the location of each pot (i.e., greenhouse bench, column and row) were recorded.

Seed families were excluded from the main experiment if they did not consist of at least 9 individuals to allow 3 replicates of each family, in each of the 3 treatments. A small amount of mortality occurred after transplantation which reduced the replication to 8 for two families; but most families had higher replication (average N = 22 per family, range N = 8 to N = 31). In the main experiment, after removing 12 individuals that died in the first year, and 7 individuals with competitors that died, I was left with 506 individuals representing 23 genetic families (Table A1). These families originated from 22 populations located along the East Coast of North America and one population located in Northern Italy (Fig. 1). In the maple control treatment, 2 individuals died in year 1, reducing the sample size from 31 to 29 in this treatment. At the end of year 1, plants in the main experiment were moved to the field.
2.2.3.3 Fertilizer experiment

The fertilizer experiment was conducted to test the effect of soil nutrients on *A. petiolata* plasticity. This experiment involved only 3 seed families (i.e., KVEDG1, SMITH1, MSMID1), which originated from three of the same populations used in the main experiment but represent a different inbred line. For example, in the population KVEDG1, one inbred seed family was used in the main experiment, and another family was used in the fertilizer experiment. The 3 seed families were replicated across the same 3 competition treatments involving *A. petiolata* in the main experiment (i.e., the alone, interspecific and intraspecific competition treatments). However, half of the pots received one tablespoon of a slow-release fertilizer with micronutrients (12-4-8 Miracle-Gro® Shake 'n Feed® All Purpose Plant Food) at the beginning of the experiment. Like the main experiment, plants in the intraspecific treatment were paired with different seed
families, but unlike in the main experiment, competitor pairs were replicated due to the low number of unique combinations available with only three genotypes. Plants in this experiment were randomized across a single bench in the same greenhouse where the main experiment took place.

Replication in the fertilizer experiment differed from the main experiment as each family was to be replicated at least 5 times across competition and fertilizer treatments (i.e., 5 individuals × 3 competition treatments × 2 fertilizer treatments). After removing from the analysis dead individuals and individuals with competitors that died, the experiment consisted of 59 individuals representing 3 families replicated unevenly across competition treatments, which prevented a genetic analysis of these plants (Table A2). At the end of year 1, plants in the fertilizer experiment were harvested and were not moved into the field.

2.2.4 Field design

Plants from the main experiment were transported from the greenhouse into a fenced-in field at QUBS on October 5, 2019, to allow plants to acclimatize to cold temperatures before winter. In October 2019, air temperatures averaged 14°C in this region. In the field, I dug 49 square holes with dimensions of approximately 0.6×1.2 meters wide and 15cm deep and I arranged these holes in a grid to identify each plant’s position in the field. I randomly assigned between 7-16 pots to each hole, the number of pots in each hole reflects variation in the size of each hole, which were dug manually in field soil with debris and rocks. Each pot was spaced approximately 10cm apart from all adjacent pots. Plants from the experiment were kept in their original pots, placed in the
holes and buried up to the rim to prevent desiccation while maintaining an intact competitive environment.

2.3 Greenhouse (year 1) measurements

2.3.1 Body size measurements

To account for variable initial transplant size, each *A. petiolata* and *A. saccharum* plant were measured for length of the largest leaf, the number of true leaves, and (for *A. saccharum* only) stem height. This occurred on May 16\textsuperscript{th} - 17\textsuperscript{th} 2019, approximately one week after transplantation into experimental pots.

In the main experiment, I measured total leaf area (TLA) for each *A. petiolata* rosette, and I measured TLA and stem height for each *A. saccharum* plant at the end of year 1. I obtained TLA by manually measuring the length and width of each leaf using a caliper, multiplying the length and width of each leaf and summing the products. The TLA of rosettes at the end of year 1 is hereafter referred to as “rosette size” and was used to indicate performance. *Acer saccharum* TLA at the end of year 1 was used to indicate performance as most of the variation induced from this experiment was expressed in leaf area, not stem height. In the main experiment, these measurements took place between August 14\textsuperscript{th} - 21\textsuperscript{st}, 2019.

In the fertilizer experiment, a manual measurement of each leaf was not necessary as these plants were only grown for one season and could be harvested. A full harvest was done on plants in the fertilizer experiment between August 27\textsuperscript{th} - 28\textsuperscript{th}, 2019. The shoot system was cut where the stem entered the soil and was then dried to a constant weight before measuring shoot mass on a scale with resolution to 0.01g.
2.3.2 Relative growth rate

In the main experiment only, I measured the growth rate of *A. petiolata* in the greenhouse. To do this, every two weeks starting on May 27, 2019, the size of each *A. petiolata* rosette was obtained by measuring the total number of pixels (TNP) corresponding to the rosette in an image. TNP was measured by imaging the plants, storing the images and analyzing them later on. The top and side of each *A. petiolata* rosette were photographed using two webcams in a portable image-capture stand I constructed (Fig. A5). An image of the top and side of the rosette was needed because the rosettes tended to droop, causing parts of leaves to be hidden from the top camera. By capturing the photos at a constant distance, the area the plant occupies within the photos could be used to estimate the size of a rosette (Fig. A3).

Image analysis was performed using a machine learning algorithm written in Python using the packages OpenCV2 and PlantCV2 (Open CV Dev. Team 2013; Python Core Team 2015; Berry et al. 2018). I trained the computer to identify leaves based on the value of each pixel in the image (each pixel has 1 red, 1 green and 1 blue value which range from 0-255 to create a color). I used a subset of training images and sampled pixels that formed the leaves, and pixels that formed background in each image. This resulted in two sampling distributions, one of plant pixels and one of background pixels. An algorithm available in the PlantCV2 package used Bayesian statistics to assign new pixels as either plant or background based off what is more likely (Berry et al. 2018).

The TNP identified as rosette from the top and side photo were summed and used to estimate the size of each rosette. A subset of 76 plants were used to verify the method: TLA was calculated manually using a ruler (to obtain the length and width of each leaf).
and TNP was obtained on the same plants using computer vision. Without any
manipulations of the pixel data, there was a strong correlation between TNP and TLA
($p<2.2e-16$, $R^2=0.852$). This analysis was automated to analyze all 5659 photos. Each
photo that was analyzed produced an output image, outlining the parts identified as
rosette (i.e., Fig. A3). All of these output images were manually verified to ensure
appropriate identification of the plant and errors were corrected as necessary.

I created another program in python in order to operate the portable image capture
stand. This program automated the two webcams and barcode machine involved in the
photo apparatus. The algorithm reads the ID of the plant, displays webcam feeds, takes a
photo with each webcam, displays the photos to ensure quality, and stores the images
with the appropriate identification and the exact time that the photos were taken. Time
was denoted as seconds since Jan 1$^{st}$, 1970 (i.e., Unix time) – this time represents the
exact second when each photo was taken but is also a quantitative number that can be
used to calculate growth rates with high accuracy.

Relative growth rate (RGR) was calculated for each of the 4 intervals following
Hoffmann and Poorter (2002):

$$RGR = \frac{\ln(TNP_2) - \ln(TNP_1)}{t_2-t_1} \quad (2.1)$$

where $t$ denotes time and subscripts indicate the instance of measurement. Five different
growth rate measurements were made: May 27-28, June 11-12, June 24-25, July 11-12
and August 8-9, 2019. It took two days to image all of the plants in the main experiment.
The last measurements were taken a month apart as plants had observably slowed their
growth. The majority of growth occurred between the first and second measurement (Fig.
B2), therefore only the first relative growth rate measurement (RGR1) was used in analyses.

2.3.3 Leaf chemistry

Total glucosinolate, flavonoid, and chlorophyll A concentration were quantified in *A. petiolata* leaf tissue at the end of year 1 (i.e., August 14th - 21st, 2019 for plants in the main experiment and on August 27th -28th, 2019 for plants in the fertilizer experiment). I obtained two 8mm diameter holepunches from a single leaf and pooled these samples to give a composite sample of a leaf. I only sampled leaves that exceeded 30mm and had > 50% green tissue. Care was taken to sample undamaged areas of the leaves. I sampled a maximum of 2 leaves per plant; however, certain plants had only enough leaves meet the criteria to permit 1, or in some cases 0 samples. If a plant had more than two leaves which met the criteria, the smallest leaves over 30mm in length were sampled. I also recorded leaf area of the sampled leaf (length × width) using a caliper. Fresh tissue samples were flash frozen and stored at -80°C until further processing.

Frozen leaf tissue was pulverized in 1.5mL centrifuge tubes using a Next Advance Bullet Blender®. Two cycles of 15s on the highest power (12) were performed and samples were re-submerged in liquid N₂ after each cycle. Samples were kept frozen to inhibit enzymatic degradation of target compounds. Leaf tissue was then extracted in 0.55mL of 100% methanol, incubated for 1 hour at 25°C and 300rpm and were centrifuged for 2 minutes at 2500g (Eppendorf, 5427 R). Next, the pellet was discarded and 485μL of supernatant was stored at –80°C until further processing. The concentration of total glucosinolate, flavonoid, and chlorophyll A were determined using 96 well assays with spectrophotometric analysis and two technical replicates per sample. Chlorophyll A
was evaluated by transferring 20µL of extract into wells; the sample was then evaporated and resuspended in 50µL of 100% methanol immediately prior to reading. Chlorophyll A concentration was then quantified as in Ritchie (2006) using the following formula after subtracting all absorbance values by their absorbance at 750nm as a control:

\[
\text{Chlorophyll A (µg/ml)} = -8.0962 \times A_{652} + 16.5169 \times A_{665}
\] (2.2)

where \(A_{652}\) is the absorbance at 652nm and \(A_{665}\) is the absorbance at 665nm.

Total glucosinolate and flavonoid concentration was evaluated by rinsing 465µL of extract with 650µL of hexane to remove other pigments or secondary compounds that may interfere with analysis. After rinsing, samples were centrifuged at 2500g for 10 minutes at 4°C. I then transferred 100µL of the defatted sample to wells, left it to evaporate and resuspended it in 100µL of 100% methanol immediately prior to reading. Total flavonoid content was assessed with reference to “Procedure 1” in Pękal and Pyrzynska (2014), in which AlCl₃ is applied to the sample to bind with flavonoids and form a yellow-colored flavonoid-aluminum complex. This complex absorbs UV light at a higher range than other phenol compounds, allowing flavonoids to be quantified. I modified the procedure by using 50µL of 2% (w/v) aluminum chloride (AlCl₃) solution and 50µL of 1M acetic acid to make it appropriate for a 96 well plate (Granato et al. 2016). Rutin was used as the standard to construct a standard curve due to its similarity to the most abundant flavonoid in *A. petiolata*, isovitexin 6-O-B-d-glucopyranoside (see Fig. A2 for standard curve; Haribal and Renwick 2001).

Glucosinolates were quantified with reference to Ishida et al. (2011) and Ishida et al. (2012); in which, the molecule palladium forms a yellowish-brown-colored complex with glucosinolates which shifts their UV absorption to a unique range to allow
quantification. I modified the procedure by using 100µL of 3.529mM of sodium tetrachloropalladate (NaCl₄Pd) to make the method appropriate for a 96 well plate (Mawlong et al. 2017). Sinigrin was used as a standard as it is one of the most abundant glucosinolates in A. petiolata and is suspected to have allelopathic effects (see Fig. A2 for standard curve; Vaughn and Berhow 1999). Both assays were read at 425nm before and after reagents were added. Initial absorbance values were divided by 2 and subtracted from the final absorbance values to control for variability in initial color of extract. Dividing by 2 was necessary as solutions were twice as concentrated before reagents were added; this can be demonstrated using the following formula, which was derived from the \( C_1V_1 = C_2V_2 \) equation:

\[
A_{after} = \frac{A_{prior} \times V_{prior}}{V_{after}}
\]  

where \( A_{prior} \) is the absorbance of the sample before reagents were added, \( V_{prior} \) is the volume before reagents were added and \( V_{after} \) is the volume after reagents were added. The variable \( A_{after} \) reflects the absorbance in the sample that cannot be attributable to the target compound; it is the theoretical absorbance of the sample had the tissue extract been simply diluted instead of reacted with reagents.

A pooled extract, composed of many different A. petiolata leaves, was extracted in 100% methanol and stored with the rest of the extracted samples. Glucosinolate, flavonoid and chlorophyll A content in the pooled extract were determined in each run performed. This allowed each plate to be verified for its accuracy. If the pooled sample deviated more than two standard deviations from the mean, data from the entire plate were discarded, which occurred in 3/56 plate reads. The end result, after accounting for plants that could not be sampled, or plants where only one leaf could be sampled, was
317/506 plants with samples from two leaves, 99/506 plants with samples from one leaf, and 90/506 plants with no samples.

2.3.4 Biotic interactions

When measuring rosette size in the main experiment and harvesting *A. petiolata* in the fertilizer experiment, I also measured pathogen damage on leaves. The same leaves that were chosen for secondary compound analysis were also chosen for pathogen damage measurements on the plant. To measure pathogen damage, a photograph was taken of the sampled leaves. Using these photos, I quantified thrip damage (genus *Thysanoptera*) by counting the number of white spots on each leaf, and I quantified black pathogen abundance by counting the number of black spots on each leaf. Lastly, I recorded whether or not each leaf had a presence of powdery mildew infection (*Erysiphe cruciferarum*); which has a distinct cloudy-white appearance (see Fig. A1 for an example of pathogen identification). The black pathogen was not identified but was similar in presentation to ring spot fungus (black blight; *Mycosphaerella brassicicola*), which is known to infect Brassicaceae species. All plants were considered inoculated with each pathogen as pathogens likely came from the field soil in which the plants were grown, infection was dispersed throughout the greenhouse, and plants were touched by the same cloth bi-weekly for imaging. I quantified herbivore damage on *A. saccharum* by recording the number of leaves with and without tissue damage (defined as a hole in the leaf observable to the naked eye). This data was then used to give a percentage of leaves with herbivore damage to be used in analyses. *Acer saccharum* damage was likely also caused by thrips.
In addition to pathogens, I also noticed that many of the pots had ferns growing in them. On October 1, 2019, I counted the number of ferns in each pot. A maximum of 15 individual fern plants were counted in each pot as a fern abundance of > 15 fully saturated a 4” pot. Since field soil was thoroughly mixed prior to the experiment, each pot received the same inoculation potential for ferns. While this was an unexpected competitor in our experiment, ferns represent an aspect of the environment which *A. petiolata* would invade and thus provided a growing environment more reflective of natural conditions.

2.4 Field (year 2) measurements

On May 13th, 2020, overwinter survivorship, bolt size and field location (row and column) were recorded for each plant. Bolt size was measured as shoot length, number of leaves, and the length and width of the largest leaf for each *A. petiolata* plant that survived. These measurements were then used in a principal component analysis (PCA) to obtain a single composite measurement of bolt size. Because bolts were not yet fully developed, bolt size variation is primarily a function of emergence time and growth rate. I recorded fecundity at the end of year 2 as a measure of reproductive fitness. Between July 8th-9th of 2020, siliques from all remaining *A. petiolata* plants and the shoots of all remaining *A. saccharum* plants were harvested. Plant material was then dried and the dry mass of *A. petiolata* siliques were used to indicate reproductive output (i.e., fecundity) and the shoot mass of *A. saccharum* was used to indicate performance at the end of year 2.
2.5 Statistical analyses

2.5.1 Data transformations

Independent variables were log-transformed as needed for non-normal data and all dependent and independent continuous variables were standardized by subtracting the mean and dividing by the standard deviation. This standardization results in a mean centered around zero and data expressed in units of standard deviation. This is commonly done to fitness data because the predicted response to selection is a function of relative variation in fitness. For example, if a 1 unit increase in glucosinolate production results in a 1g increase in fecundity, this may seem like a lot but may in fact only represent a 0.01 standard deviation increase in fecundity and be relatively unimportant. It also facilitates the comparison of variables with different units or scales. For example, this can be useful to determine if interspecific competition affected *A. petiolata* fecundity or *A. saccharum* shoot mass more.

Standardizing the independent variables offered the same benefits as standardizing the dependent variables, but also improved model inference. When using two or more continuous independent variables in a regression, each variable is provided with a partial regression coefficient, which determines the correlation between an independent and dependent variable when all other independent continuous variables are held constant (Scheiner et al. 2000). The key here is that computationally, the other variables are held constant at a value of zero. Without standardizing the variables, this can result in non-sensical results; for example, in a regression where chlorophyll A production and glucosinolate production predict fitness, without standardization, the model would estimate the effect of glucosinolate production on fitness when there is no
chlorophyll present in the leaf – which does not make biological sense. In contrast, standardized variables have a mean at zero, therefore, the model predicts the effect of glucosinolate production on fitness given the mean chlorophyll A production of the population. Importantly, standardizing independent variables does not affect the test statistic or p-value (Crawley 2007).

2.5.2 Principal component analyses

Glucosinolate, flavonoid and chlorophyll A compounds are produced in leaf tissue and may all be produced in higher concentrations when more nutrients are available. Multicollinearity in regression analysis can result in p-values and coefficients which are unreliable, shared among correlated variables, and susceptible to small changes in the data (Crawley 2007). To test for correlation among glucosinolate, flavonoid and chlorophyll A, I used linear regressions (see Fig. B1). I then used a PCA to obtain biologically relevant variables that reflect the chemical profile in the leaf tissue of a plant. These variables were used instead of glucosinolate, flavonoid and chlorophyll A concentrations in linear models to eliminate the issues associated with multicollinearity. The variable “leaf quality” was developed, which represents the production of glucosinolate, flavonoid and chlorophyll A, and explains most of the variation in these compounds. Two other variables were developed to represent the production of glucosinolates or flavonoids relative to chlorophyll A. These variables are relative glucosinolate investment (RGI), and relative flavonoid investment (RFI) and explain a small amount of the total variation in glucosinolate, flavonoid and chlorophyll A production.
Two other PCAs were conducted to summarize the initial transplant size measurements of *A. saccharum*, and the bolt size measurements of *A. petiolata*. For the measure of initial *A. saccharum* size, the PCA consisted of all three initial (i.e. stem height, largest leaf length and number of leaves) and final measurements of *A. saccharum* shoot mass (i.e. stem height, number of leaves and total leaf area) in the first growing season. For the measure of *A. petiolata* bolt size, the PCA consisted of all four *A. petiolata* second-year shoot measurements (i.e., bolt height, number of leaves, largest leaf length and width).

### 2.5.3 Model selection

In the fertilizer treatment, only the fertilizer and competition treatment effects were of interest. The effect of fertilizer and treatment on shoot mass and glucosinolate, flavonoid, and chlorophyll A concentration were assessed using two-way ANOVAs. In the main experiment, I needed to compare the effects of many independent variables (i.e., treatment, leaf chemistry traits, life history traits, pathogen and fern abundance, competitor traits, greenhouse and field location, and seed family) on five dependent variables (i.e., rosette size, survival, fecundity, *A. saccharum* TLA in year 1 and shoot mass in year 2). For this reason, I used backwards model selection and likelihood ratio tests to determine significance between models. Backwards model selection involves starting with a maximum model including all hypothesized effects. From the maximum model, I sequentially determined if removing an effect from the model significantly worsened the model’s predictive power, based on a likelihood ratio test with a $\chi^2$ distribution and degrees of freedom equal to the number of parameters removed from the model. If significant, the term was retained in the model, otherwise it was removed.
Stepwise deletion continued until all variables were assessed. This yielded the minimum adequate model (MAM), which contained only significant predictor variables. This method eliminates type-1 error inflation associated with independently assessing the association between many independent variables and a single dependent variable (Crawley 2007). I started by first assessing the random effects of family, greenhouse and field location, then the nuisance effects of pathogens and ferns, and then other effects of interest, such as leaf chemistry traits.

Generalized linear mixed effects models were used to fit and test all linear models, using the package glmmTMB in R (R Core Team 2014; Brooks et al. 2017). I calculated selection gradients with the three measures of success (i.e., rosette size, survival and fecundity as response variables). Selection gradients are partial regression coefficients that estimate the direct relationship between a trait and fitness (e.g., fecundity) by holding all other traits in the analysis constant (Lande and Arnold 1983). For this reason, I included other correlated traits to assess direct selection on glucosinolate, flavonoid and chlorophyll A production. Specifically, selection gradients represent direct selection on the plant traits leaf quality, RGI and RFI, growth rate, rosette size and bolt size, and account for the nuisance variables of pathogen damage, field and greenhouse location, and fern abundance.

2.5.3.1 Selection on \textit{A. petiolata} traits and the effects of treatment, pathogens and fern on \textit{A. petiolata}

I determined selection acting on leaf quality, RGI and RFI in each treatment, for each of the three measures of fitness (rosette size, survival and fecundity). In terms of rosette size, the maximum model from which backwards selection commenced was:
\[\text{Rosette Size} = (T \ast \text{Leaf quality}) + (T \ast RGI) + (T \ast RFI) + (P_1 \ast P_2 \ast P_3) + \\
Fern + \text{Leaf area} + RGR1 + 1|\text{Family} + 1|\text{GH bench}\]

where \(T\) denotes “treatment” (i.e. alone, interspecific and intraspecific), \(\text{Leaf area}\) denotes the leaf area of the sampled leaf from which plant tissue was sampled, \(RGR1\) denotes the first relative growth rate value, \(\text{GH bench}\) denotes the greenhouse bench where each plant was grown, \(\text{Family}\) denotes the seed family of the plant, \(P\) represents “pathogen” and subscripts denote the type of pathogen (i.e. black pathogen abundance, thrip damage and powdery mildew presence correspond to subscripts 1, 2 and 3). The symbol \(*\) represents an interaction as well as all lower order effects. For example, \(P_1 \ast P_2 \ast P_3\) denotes the three-way interaction between all three pathogens, all possible two-way interactions between these variables (there are three possible combinations), and the main effect of each pathogen. The last two variables with a preceding “1|” represent a random effect.

Two-way interactions between treatment and leaf quality, treatment and RFI, and treatment and RGI were included to test for treatment-dependent selection gradients. A three-way interaction between all three pathogens was included to test for synergistic and antagonistic effects of pathogen on performance and fitness. \(\text{Leaf area}\) was included as a covariate to account for the confounding autocorrelation between leaf size and rosette size: The size of the sampled leaf was part of the measurement of TLA (rosette size). This is problematic because many variables may be influenced by leaf size. For example, secondary compounds can be diluted in larger leaves (Cipollini and Gruner 2007); larger leaves can take on more pathogen damage; and larger, older leaves may provide more
opportunity for infection by powdery mildew fungus. If these variables are correlated with leaf area, they would be autocorrelated with rosette size. Including leaf area controls for the variation in rosette size explained by leaf area and thus eliminates the connection between relevant traits and rosette size.

The model predicting *A. petiolata* survival followed mostly the same format but used a binomial error distribution. In addition, *Leaf area* was removed and rosette size was included as a predictor to determine whether performance in year 1 affected survival. The field location of the plant (i.e. field column and field row) were also included as random effects because survival measurements occurred after plants had been moved to the field:

\[
Survival = (T \ast Leaf\ quality) + (T \ast RGI) + (T \ast RFI) + (P_1 \ast P_2 \ast P_3) + Fern \\
+ Rosette\ size + RGR1 + 1|Family + 1|GH\ bench + 1|Field\ row \\
+ 1|Field\ col
\]

where *Field col* denotes the field column. The model predicting fecundity incorporated the same variables as in the survival model, but also included the additional variable “bolt size”. Bolt size was not included in the survival model because it was measured after mortality took place and before the fecundity measurement:

\[
Fecundity = (T \ast Leaf\ quality) + (T \ast RGI) + (T \ast RFI) + (P_1 \ast P_2 \ast P_3) + Fern \\
+ Rosette\ size + Bolt\ size + RGR1 + 1|Family + 1|GH\ bench \\
+ 1|Field\ row + 1|Field\ col
\]
The effect of fern abundance was re-assessed separately. Fern abundance was recorded in each pot, and as there were two plants per pot in the intraspecific treatment, fern data from this treatment was pseudo-replicated. Data were averaged to the pot level in the intraspecific treatment only and then merged with the other (unchanged) data. The effect of fern abundance on performance, survival and fecundity were assessed using this data. Coefficients and p-values of fern effects come from the modified data, whereas other coefficients come from the models included above.

To understand the average effect of inter- and intraspecific competition on *A. petiolata*, the effect of treatment on rosette size, survival and fecundity were calculated by removing plant traits from the previous models. Therefore, the models only contained random effects, treatment, and the significant effects of fern and pathogen damage if present. Pathogens and fern abundance were included in models when significant as they can directly influence fitness and are thus important variables to account for when assessing treatment effects. For example, if the interspecific treatment has more thrip damage than other treatments and thrip damage reduces fitness, the predicted effect of intraspecific competition would be reduced. In contrast, including plant traits as predictors would result in multicollinearity and produce inaccurate results. This is because many plant traits will also respond to treatment just as performance and fecundity measurements do. For example, if leaf quality exhibits a response to treatment, then using leaf quality and treatment as predictors in a model of fecundity will result in some treatment variation being explained by the variable treatment, and some by the variable leaf quality. This multicollinearity would reduce the apparent effect of treatment...
by dispersing the effect of treatment across predictors, giving an inaccurate estimate of treatment effects.

2.5.3.2 Effects of treatment, fern and pathogen damage on *Acer saccharum*

It was also important to understand how pathogen damage, ferns, and treatment influenced *A. saccharum* performance. To determine this, maple performance in year 1 and 2 were the dependent variables models predicted by treatment (i.e., maple control vs. interspecific competition), initial maple size, pathogen damage, and fern abundance as fixed effects and greenhouse bench as a random effect:

\[
\text{Maple size}_1 = \text{Maple size}_0 + T_m + \text{Fern} + \text{Maple damage} + 1|\text{GH bench} \quad (2.7)
\]

\[
\text{Maple size}_2 = \text{Maple size}_0 + T_m + \text{Fern} + \text{Maple damage} + 1|\text{GH bench} \quad (2.8)
\]

where *Maple size* \(_1\) denotes *A. saccharum* TLA at the end of year 1 and *Maple size* \(_2\) denotes shoot biomass at the end of year 2. *Maple size* \(_0\) is the principal component denoting initial maple size, *T* \(_m\) denotes treatment (i.e. the maple alone or interspecific treatment) and *Maple damage* describes the proportion of damaged leaves on the plant. Initial maple size was important to control for because *A. saccharum* were of different sizes in the beginning of the experiment.

2.5.3.3 Effects of neighbor *Acer saccharum* traits on focal *Alliaria petiolata*

Aside from the average effects of treatment, the traits of competitors (i.e., neighbors in the same pot) could also affect the performance and fitness of *A. petiolata* and *A. saccharum*. For example, *A. saccharum* were of different sizes and *A. petiolata* of different sizes, leaf chemistry profiles, and seed families. The effects of competitor traits are referred to within-treatment effects, because they are assessed using data from only
one treatment. The following model assessed the effect of *A. saccharum* on *A. petiolata* performance using the interspecific treatment data:

\begin{equation}
\text{Rosette size} = \text{Maple size}_{1,N} + (P_1 \ast P_2 \ast P_3) + \text{Fern} + 1|\text{Family} + 1|\text{GH bench}
\end{equation}

where the subscript “N” denotes a neighbor’s (i.e. the competitor in the same pot) trait. Only *A. saccharum* size in year 1 was included in the model, as maple shoot mass in year 2 could not affect rosette size in year 1. I assessed the influence of *A. saccharum* on *A. petiolata* survival with this model:

\begin{equation}
\text{Survival} = \text{Maple size}_{1,N} + (P_1 \ast P_2 \ast P_3) + \text{Fern} + 1|\text{Family} + 1|\text{GH bench} + 1|\text{Field col} + 1|\text{Field row}
\end{equation}

where the effect of field column and row are now added to the model because survival was recorded in the field. *Acer saccharum* shoot mass in year 2 is not included in the model because it was measured at the end of year 2 whereas *A. petiolata* mortality took place at the beginning of year 2. I assessed the

the influence of *A. saccharum* on *A. petiolata* fecundity using this model:

\begin{equation}
\text{Fecundity} = \text{Maple size}_{1,N} + \text{Maple size}_{2,N} + (P_1 \ast P_2 \ast P_3) + \text{Fern} + 1|\text{Family} + (1|\text{GH bench}) + (1|\text{Field col}) + (1|\text{Field row})
\end{equation}

where the size of *A. saccharum* in year 1 and 2 were assessed because both of these traits may have contributed to the final fecundity of *A. petiolata*. 

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2.5.3.4 Effects of neighbor *Alliaria petiolata* traits on focal *Alliaria petiolata*

In this section, I discuss the models used to assess the effect of a neighboring *A. petiolata* plant on a focal *A. petiolata* plant. The following three models were assessed using only data from the intraspecific competition treatment. I assessed the within-treatment effects of intraspecific competition on rosette size with this model:

\[ (2.12) \]

\[
Rosette \ size = Rosette \ size_N + Family_N + (P_1 * P_2 * P_3) + Fern + 1|Family \\
+ 1|GH \ bench
\]

where neighbor rosette size and family predict the focal plant’s rosette size. The variable neighbor family \((Family_N)\) reflects genetic variation for competitive ability that is not captured by rosette size. I assessed the within-treatment effects of intraspecific competition on survival using this model:

\[ (2.13) \]

\[
Survival = Rosette \ size_N + Family_N + Survival_N + (P_1 * P_2 * P_3) + Fern \\
+ 1|Family
\]

where neighbor survival \((Survival_N)\) is now added to the model. Neighbor survival indicates whether or not a plant is more or less likely to survive, depending on whether the competitor survived or not. Due to low sample size in the intraspecific treatment, field and greenhouse effects were eliminated from the model because the model would not converge with any more parameters. I assessed the influence of intraspecific competition on fecundity using this model:

\[ (2.14) \]
\[ \text{Fecundity} = \text{Rosette size}_N + \text{Family}_N + \text{Survival}_N + \text{Bolt size}_N + (P_1 \times P_2 \times P_3) + \text{Fern} + 1|\text{Family} \]

where neighbor bolt size is now added to the equation because this variable was recorded after mortality took place but before fecundity measurements and, therefore, could have influenced fecundity. Like the last model, random effects of field and greenhouse were omitted from this analysis due to low sample size.

2.5.3.5 Effects of neighbor *Alliaria petiolata* traits on focal *Acer saccharum* performance

Lastly, I discuss the effect of *A. petiolata* traits on *A. saccharum*. Selection gradients on putative allelochemicals were calculated with Equation 2-4, 2-5 and 2-6, however, a positive selection gradient for allelochemical production in the interspecific treatment is not sufficient to conclude that selection acted in response to allelopathy. In agreement with the allelopathy hypothesis, secondary compounds are only predicted to benefit *A. petiolata* through the intermediate, detrimental effect on *A. saccharum*. For this reason, allelopathy mediated selection can be confirmed by (1) a negative effect of glucosinolate or flavonoid concentration on maple performance, and (2) a negative effect of maple performance on *A. petiolata* fitness (Kingsolver and Schemske 1991; Scheiner et al. 2000). The following two models were assessed using only data from the interspecific competition treatment. I assessed the effect of *A. petiolata* on *A. saccharum* TLA in year 1 with the following model:

\[ \text{(2.15)} \]
\[
\text{Maple size}_1 = \text{Leaf quality}_N + \text{RGI}_N + \text{RFI}_N + \text{Rosette size}_N + \text{Family}_N \\
+ \text{Maple damage} + \text{Maple Size}_0 + \text{Fern} + 1|\text{GH bench}
\]

where the effect of \textit{A. petiolata} secondary compounds (i.e., leaf quality, RGI, RFI), rosette size and seed family predicted \textit{A. saccharum} TLA in year 1. I assessed the effect of \textit{A. petiolata} on \textit{A. saccharum} shoot mass in year 2 with the following model:

\[
(2.16)
\]

\[
\text{Maple size}_2 = \text{Leaf Quality}_N + \text{RGI}_N + \text{RFI}_N + \text{Rosette Size}_N + \text{Family}_N \\
+ \text{Survival}_N + \text{Bolt size}_N + \text{Maple damage} + \text{Maple size}_0 + \text{Fern} \\
+ 1|\text{GH bench} + 1|\text{Field col} + 1|\text{Field row}
\]

where bolt size and field location have now been added to the model as these measurements were taken after \textit{A. saccharum} was moved to the field.

2.5.4 Estimating genetic variation, plasticity and genetic variation for plasticity

A mixed model analysis was performed on all \textit{A. petiolata} traits in order to calculate genetic variation, plasticity, and genetic variation for plasticity. To do this, a variance component analysis was performed on each trait. Each trait (i.e., chlorophyll A, glucosinolate and flavonoid production, leaf quality, RGI, RFI, rosette size, bolt size, RGR1, survival and fecundity) was used as the dependent variable in a mixed model with family, treatment, and a family-by-treatment interaction as random effects. For each trait, the percent of variation explained by seed family (genetic variation), treatment
(environmental variation/plasticity) and family-by-treatment interactions (genotype by environment/genetic variation for plasticity) was deduced. The amount of variation explained by seed family denotes the total genetic variation of the trait, as this is the percent of variation attributable to genetic variation. Likewise, the amount of variation in a trait explained by treatment represents plasticity, because this is the amount of variation that responded to treatment. A treatment-by-family interaction creates another set of parameters to allow each family to exhibit a unique response to treatment. Therefore, a family by treatment interaction represents genetic variation for plasticity.

The significance of each variable was assessed using a likelihood ratio test. Leaf area of the sampled leaf was used as a fixed effect in models involving glucosinolate, flavonoid, and chlorophyll A expression, leaf quality, RFI and RGI, as leaf size influenced these variables. Greenhouse bench, field row and field column were used as fixed effects in models where significant and appropriate (i.e., field measurements were only used as a predictor on traits that were measured in year 2). To obtain mean values and confidence intervals around the mean, leaf quality, RFI, and RGI were also used in a model with treatment and leaf area of the sampled leaf as a fixed effect. This analysis allowed the mean expression in each treatment to be determined and parametric confidence intervals to be calculated after accounting for leaf area. In these models, treatment and leaf area were used as fixed effects and greenhouse bench was a random effect.
Chapter 3

Results

3.1 General results

Mortality in the greenhouse was 2.04% for *A. petiolata* and 1.02% for *A. saccharum* in year 1. However, this increased after plants were moved into the field, where mortality increased more for *A. petiolata* (69.66%) than for *A. saccharum* (6.70%). In general, *A. petiolata* performed better in the alone treatment than in competition, with those in intraspecific competition suffering the most. Individuals facing intraspecific competition had the lowest growth rate, performance and fecundity and had the highest mortality in the greenhouse (Table 1). For example, mean rosette size was 9562.91 cm² in the alone treatment, 8547.18 cm² in the interspecific competition treatment and only 5119.92 cm² in the intraspecific treatment; mean fecundity was 3.13 g in the alone treatment, 1.78 g in the interspecific treatment and only 1.07 g in the intraspecific treatment. Similarly, *A. saccharum* tended to perform better when grown alone in terms of performance in year 1 (i.e., TLA) and 2 (i.e., shoot mass), although *A. saccharum* was also initially larger in the control treatment (Table 2; for a visualization, see Fig. B6). For example, mean *A. saccharum* shoot mass in year two was 1.18 g in the alone treatment but only 0.72 g in the interspecific treatment.

In terms of leaf chemistry traits, chlorophyll A, glucosinolate and flavonoid production were higher in the alone treatment than in the inter- and intraspecific treatments. For example, mean glucosinolate production was 0.22 mg/mL in the alone treatment, but only 0.18 mg/mL and 0.20 mg/mL in the inter- and intraspecific treatments, respectively. Unlike the biomass and fecundity results above, chlorophyll A and
glucosinolate production were higher in the intraspecific treatment than in the interspecific treatment. For example, chlorophyll A concentration was 0.34µg/mL in the intraspecific treatment but only 0.30µg/mL in the interspecific treatment. It is important to note, however, that leaf area of the sampled leaf (i.e., the area of the leaves from which leaf chemistry was measured) was also smaller in the intraspecific treatment than the other treatments. Leaf area of the sampled leaf was only 2140.96cm² in the intraspecific treatment compared to 3235.20cm² and 3193.63cm² in the alone and interspecific treatment, respectively (Table 1).

Pathogen damage on *A. petiolata* was distributed fairly evenly across treatments. Thrip damage was slightly higher in the intraspecific treatment (4.38 spots/leaf) than in the alone and interspecific treatments (2.87 and 2.82 spots/leaf, respectively). Powdery mildew infection occurred more frequently in the alone treatment (35.10% of plants were infected) than in the inter- and intraspecific treatments (20.80% and 26.80% of plants were infected, respectively) (Table 1).

*Acer saccharum* suffered slightly more herbivore damage during interspecific competition. The mean number of leaves damaged per plant was 0.72% in the interspecific treatment compared to 0.53% in the maple alone treatment (Table 2). Interestingly, fern abundance was much higher in pots that contained *A. saccharum*, for example, the average abundance was 0.56 and 0.65 ferns per pot in the alone and intraspecific treatments, respectively, but in the interspecific treatment, fern abundance was nearly twice as high, at 1.18 ferns per pot, and in the maple alone treatment, fern abundance averaged 8.38 ferns per pot (Table 1 and 2).
Table 1. Summary statistics of *Alliaria petiolata* characteristics for each of the three competition treatments.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Alone</th>
<th>Interspecific</th>
<th>Intraspecific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse mortality</td>
<td>1.76% (3-170)</td>
<td>1.20% (2-167)</td>
<td>3.74% (7-187)</td>
</tr>
<tr>
<td>Field mortality</td>
<td>65.27% (109-167)</td>
<td>73.619% (120-163)</td>
<td>68.18% (120-176)</td>
</tr>
<tr>
<td>RGR1 (ln(pixels)/sec)*</td>
<td>1.07 ± 0.06</td>
<td>1.01 ± 0.07</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>Rosette size (cm²)</td>
<td>9562.91 ± 473.87</td>
<td>8547.18 ± 499.17</td>
<td>5119.92 ± 384.19</td>
</tr>
<tr>
<td>Fecundity (g)</td>
<td>0.22 ± 0.010</td>
<td>0.18 ± 0.013</td>
<td>0.20 ± 0.010</td>
</tr>
<tr>
<td>Glucosinolate (mg/mL)</td>
<td>0.11 ± 0.0049</td>
<td>0.10 ± 0.0053</td>
<td>0.10 ± 0.0057</td>
</tr>
<tr>
<td>Leaf area of sampled leaf (cm²)</td>
<td>3235.20 ± 167.03</td>
<td>3193.63 ± 160.18</td>
<td>2140.96 ± 123.20</td>
</tr>
<tr>
<td>Black pathogen damage (N)</td>
<td>1.60 ± 0.55</td>
<td>1.08 ± 0.41</td>
<td>1.64 ± 0.66</td>
</tr>
<tr>
<td>Thrip damage (N)</td>
<td>2.87 ± 0.61</td>
<td>2.82 ± 0.63</td>
<td>4.38 ± 1.49</td>
</tr>
<tr>
<td>Powdery mildew presence</td>
<td>35.10% (53-151)</td>
<td>20.80% (31-149)</td>
<td>26.80% (41-153)</td>
</tr>
<tr>
<td>Fern abundance (N)</td>
<td>0.56 ± 0.33</td>
<td>1.18 ± 0.51</td>
<td>0.65 ± 0.50</td>
</tr>
</tbody>
</table>

Data in brackets denote the fraction used to calculate the percent. Plus or minus (±) values denote Wald’s 95% confident interval (CI) around the mean in each treatment.

* RGR1 denotes the first relative growth rate. All data are \( \times 10^{-6} \).

Table 2. Summary statistics of *Acer saccharum* characteristics for each of the two competition treatments.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Maple alone</th>
<th>Interspecific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse mortality</td>
<td>6.45% (2-31)</td>
<td>1.79% (3-167)</td>
</tr>
<tr>
<td>Field mortality</td>
<td>6.89% (2-29)</td>
<td>6.13% (10-163)</td>
</tr>
<tr>
<td>Initial Size (PC1)</td>
<td>1.02 ± 0.57</td>
<td>-0.19 ± 0.24</td>
</tr>
<tr>
<td>TLA year 1 (cm²)</td>
<td>7785.55 ± 1307.07</td>
<td>4612.42 ± 406.88</td>
</tr>
<tr>
<td>Shoot mass year 2, (g)</td>
<td>1.18 ± 0.13</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Leaf damage*</td>
<td>0.53 ± 0.13</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Fern abundance (N)</td>
<td>8.38 ± 2.6</td>
<td>1.18 ± 0.51</td>
</tr>
</tbody>
</table>

Data in brackets denote the fraction used to calculate the percent. Plus or minus (±) values denote Wald’s 95% confident interval around the mean.

* Damage was calculated per plant. The proportion displayed therefore represents the mean proportion of leaves damaged per plant and the 95% confidence interval around this mean.
3.2 Principal component analysis

A PCA was performed on the variables glucosinolate, flavonoid and chlorophyll A for the purpose of creating uncorrelated, but biologically meaningful variables that can be used in analyses. The first principal component (PC1), representing leaf quality, accounted for the majority (73%) of the variation in glucosinolate, flavonoid, and chlorophyll A production. All three variables had positive loadings, meaning that they were all positively correlated with one another. The second principal component, representing relative flavonoid investment (RFI), accounted for 16% of the total variation, had a large positive loading for flavonoid production and a large negative loading for chlorophyll A production, but had a small loading for glucosinolate production. The third principal component, representing relative glucosinolate investment (RGI), accounted for 10.8% of the total variation, had a large positive loading for glucosinolate production and a large negative loading chlorophyll A production, but had a small loading for flavonoid production (Table 3).

<table>
<thead>
<tr>
<th>Principal component</th>
<th>Loadings</th>
<th>Proportion of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucosinolate</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>PC1 (Leaf Quality)</td>
<td>0.59</td>
<td>0.55</td>
</tr>
<tr>
<td>PC2 (RFI)</td>
<td>-0.24</td>
<td>0.82</td>
</tr>
<tr>
<td>PC3 (RGI)</td>
<td>0.77</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

Two other PCAs were conducted to summarize the initial size measurements of *A. saccharum*, and the bolt size measurements of *A. petiolata*. The first PCA was used to
summarize the initial measurements of *A. saccharum* size. It consisted of all three initial measurements (i.e., stem height, largest leaf length and number of leaves) and final measurements of *A. saccharum* shoot mass (i.e., stem height, number of leaves and TLA) in the first growing season. The first principal component, representing the initial size of *A. saccharum*, had positive loadings for all six variables and explained 41.0% of the total variation in these 6 traits (Table B2). The second PCA was used to summarize the bolt size measurements of *A. petiolata* and consisted of the 4 traits measured in year 2 (i.e., bolt height, number of leaves, largest leaf length and width). The first principal component, representing bolt size, accounted for 91.2% of the total variation in these 4 traits and also had positive loadings for each variable (Table B3).

### 3.3 Trait selection and allelopathy

#### 3.3.1 Selection gradients of leaf traits

I used Lande-Arnold (1983) selection gradients, which estimate the partial regression coefficients (PRC) of multiple traits in a single model with fitness as the dependent variable. In this context, the PRC of a trait denotes the expected change in fitness per standard deviation change in the trait. Selection gradients were obtained from models of rosette size, survival and fecundity (MAMs can be found in Table B1 corresponding to models 1, 2, and 3). In the model of rosette size (TLA), leaf area of the sampled leaf was positively (+0.51) correlated with rosette size ($\chi^2=118.4$, $p < 0.001$), and the random effect of greenhouse bench was a significant predictor of rosette size ($\chi^2=6.37$, $p=0.01$). In terms of survival, the random effect of field row ($\chi^2 = 5.1$, $p=0.02$) and family ($\chi^2 = 10.7$, $p = 0.001$) were significant. There were no significant random effects in the model that predicted fecundity.
In models of rosette size and fecundity, leaf quality (PC1; indicative of glucosinolate, flavonoid and chlorophyll A production), interacted significantly with treatment (Table 4). In both models, leaf quality exhibited a significantly positive selection gradient only in the interspecific treatment (Table 5). In the interspecific treatment, a 1 standard deviation (σ) increase in leaf quality resulted in a +0.30σ increase in rosette size (TLA) \((z=2.3, p=0.02; \text{Fig. 1a})\), and a +0.73σ increase in fecundity \((z=2.9, p=0.004; \text{Fig. 1b})\). Leaf quality (PC1) was not a significant predictor of survival \((p=0.06)\).

In contrast, RFI (PC2) was not a significant predictor of either rosette size or fecundity but interacted significantly with treatment in the model predicting *A. petiolata* survival (Table 4). In this model, RFI exhibited a negative selection gradient in the inter- and intraspecific treatments only. The model estimated that a 1σ increase in RFI would decrease the likelihood of survival by -0.30 log odds in the intraspecific treatment \((z = -2.2, p = 0.03)\) (equivalent to reducing the probability of survival by 57.4%) and reduce the likelihood of survival by -0.50 log odds in the interspecific treatment \((z = -2.7, p = 0.006)\) (equivalent to reducing the probability of survival by 62.2%) (Table 5). Unlike the other two leaf chemistry variables, RGI (PC3) was not a significant predictor of rosette size, survival or fecundity (Table 4).

### 3.3.2 Selection gradients of other traits

The influence of RGR1 (i.e., the first relative growth rate measurement) on *A. petiolata* rosette size, the effect of rosette size and RGR1 on survival, and the effect of rosette size, bolt size and RGR1 on fecundity were also assessed in the same models (i.e. models 1, 2, and 3 in Table B1) that assessed leaf chemistry traits above. I did not detect a significant effect of rosette size in models that predicted survival \((\chi^2 = 2.3, p = 0.13)\) or
fecundity ($\chi^2 = 1.2, p = 0.27$). Furthermore, relative growth rate was not a significant predictor of rosette size, survival or fecundity (Table 4). In contrast, bolt size was associated with fecundity, whereby a $1\sigma$ increase in bolt size was estimated to increase fecundity by $+0.50\sigma$ ($\chi^2 = 32.7, p < 0.001$).

**Table 4. Selection gradients of *Alliaria petiolata* traits.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Rosette Size</th>
<th>Survival</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma$ ($\chi^2$, $p$)</td>
<td>log odds ($\chi^2$, $p$)</td>
<td>$\sigma$ ($\chi^2$, $p$)</td>
</tr>
<tr>
<td>Leaf Quality (PC1)</td>
<td>$T \times E (6.6, 0.04)$*</td>
<td>0.28 (3.8, 0.06)</td>
<td>$T \times E (8.2, 0.02)$</td>
</tr>
<tr>
<td>RFI (PC2)</td>
<td>0.27 (0.5, 0.48)</td>
<td>$T \times E (6.9, 0.04)$</td>
<td>0.14 (2.4, 0.13)</td>
</tr>
<tr>
<td>RGI (PC3)</td>
<td>0.01 (0.0, 0.95)</td>
<td>0.31 (1.6, 0.21)</td>
<td>-1.11 (1.4, 0.23)</td>
</tr>
<tr>
<td>Rosette size</td>
<td>N/A</td>
<td>-0.21 (2.3, 0.13)</td>
<td>0.06 (1.2, 0.27)</td>
</tr>
<tr>
<td>Bolt size</td>
<td>N/A</td>
<td>N/A</td>
<td>0.50 (32.7, &lt; 0.001)</td>
</tr>
<tr>
<td>Relative Growth Rate</td>
<td>0.02 (0.5, 0.49)</td>
<td>-0.07 (0.01, 0.91)</td>
<td>0.05 (0.2, 0.69)</td>
</tr>
</tbody>
</table>

Significant estimates ($p < 0.05$) are in bold.
* $T \times E$ indicates a trait-by-environment interaction, with treatment-specific selection gradients shown in Table 5.

**Table 5. Selection gradients of *Alliaria petiolata* traits in a significant interaction with treatment.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th>Rosette Size</th>
<th>Survival</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma$ ($z$, $p$)</td>
<td>log odds ($z$, $p$)</td>
<td>$\sigma$ ($z$, $p$)</td>
<td></td>
</tr>
<tr>
<td>Leaf Quality (PC1)</td>
<td>Alone</td>
<td>0.07 (0.9, 0.36)</td>
<td>N/A</td>
<td>-0.39 (-1.96, 0.05)</td>
</tr>
<tr>
<td></td>
<td>Intraspecific</td>
<td>0.10 (0.4, 0.71)</td>
<td>N/A</td>
<td>-0.01 (1.5, 0.13)</td>
</tr>
<tr>
<td></td>
<td>Interspecific</td>
<td><strong>0.30 (2.3, 0.02)</strong></td>
<td>N/A</td>
<td><strong>0.73 (2.9, 0.004)</strong></td>
</tr>
<tr>
<td>RFI (PC2)</td>
<td>Alone</td>
<td>N/A</td>
<td>0.41 (1.6, 0.10)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Intraspecific</td>
<td>N/A</td>
<td><strong>-0.30 (-2.2, 0.03)</strong></td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Interspecific</td>
<td>N/A</td>
<td><strong>-0.50 (-2.7, 0.006)</strong></td>
<td>N/A</td>
</tr>
</tbody>
</table>

Significant estimates ($p < 0.05$) are in bold.
Figure 2. Association of leaf quality (PC1) with *Alliaria petiolata* rosette size and fecundity.

Green circles denote *A. petiolata* plants grown in the alone treatment, orange squares denote *A. petiolata* plants grown in the interspecific treatment and blue triangles denote *A. petiolata* plants grown in the intraspecific treatment. The colored lines show the estimated effect of leaf quality on rosette size and fecundity (i.e., selection gradients), with color corresponding to treatment. In the case where the effect was not significant, a slope of zero is shown to reflect the null hypothesis that the slope is equal to zero.

3.4 Effect of pathogens and competition

3.4.1 Effect of competition on *Alliaria petiolata*

Both inter- and intraspecific competition reduced *A. petiolata* performance and fecundity, but *A. petiolata* was more affected by intra- than interspecific competition (Table 6). On average, intraspecific competition reduced rosette size by -1.12σ ($\chi^2 = 137.0$, $p < 0.001$) and fecundity by -0.81σ ($\chi^2 = 18.3$, $p < 0.001$), whereas interspecific competition reduced rosette size by -0.36σ and fecundity by -0.51σ ($\chi^2 = 18.3$, $p < 0.001$). Treatment was not a significant predictor of survival (Table 6).
There were also significant effects of competitor traits on *A. petiolata* rosette size, survival and fecundity. The influence of competitor traits was assessed using data from only the interspecific treatment (i.e., models 4, 5 and 6 in Table B1) or intraspecific treatment (i.e. models 7, 8 and 9 in Table B1) and controlled for the significant effects of fern and pathogen abundance when present. Intraspecific competitor traits had a larger influence on *A. petiolata* than did interspecific competitor traits. In intraspecific competition, a 1σ increase in the rosette size of the competitor was estimated to reduce focal plant rosette size by -0.55σ ($\chi^2 = 56.6, p < 0.001$) and fecundity by -0.29σ ($\chi^2 = 6.5, p = 0.01$). In the interspecific treatment, a 1σ increase in *A. saccharum* TLA (year 1) was estimated to reduce *A. petiolata* rosette size by -0.24σ ($\chi^2 = 13.74, p < 0.001$) and fecundity by -0.09σ ($\chi^2 = 5.4, p < 0.05$).

*Acer saccharum* size also reduced the likelihood of *A. petiolata* survival on average, whereby a 1σ increase in *A. saccharum* TLA (year 1) was estimated to reduce survival by -0.82 log odds (equivalent to reducing the probability of survival by 69%) ($\chi^2 = 11.5, p < 0.001$). Oddly, neighbor *A. petiolata* size was associated with an increased likelihood of survival, whereby a 1σ increase in rosette size was estimated to increase survival by 1.1 log odds (equivalent to increasing the probability of survival by 75%) ($\chi^2 = 5.7, p = 0.02$). Competitor size in the second year (i.e. bolt size and *A. saccharum* shoot mass in year 2) did not influence *A. petiolata* fecundity (Table 6).

Other unmeasured competitor traits also affected *A. petiolata* survival and fecundity, resulting in genetic variation for intraspecific competitive ability. After accounting for neighbor size, the seed family of the neighbor *A. petiolata* plant was a significant predictor of both the focal *A. petiolata* plant’s survival ($\chi^2 = 37.7, p < 0.05$)
and fecundity ($\chi^2 = 48.5$, $p < 0.001$). Furthermore, neighbor survival was estimated to increase the likelihood of focal plant survival by 8.54 log odds ($\chi^2 = 81.1$, $p < 0.001$). This is equivalent to increasing the likelihood of survival by 99.98%. In other words, if the neighbor survived, there was a good chance the focal plant also survived, and vice versa. This effect can be immediately observed in the raw data; for example, 90.3% of dead individuals in the intraspecific competition treatment also had dead neighbors, and 79.2% of individuals that survived also had neighbors that survived. On the contrary, neighbor survival also reduced fecundity by a very large degree. Neighbor survival reduced fecundity by -1.64σ ($\chi^2 = 13.9$, $p < 0.001$). Therefore, while neighbor survival was associated with an increased probability of survival, it also greatly reduced fecundity in those that survived (Table 6).

The effect of fern abundance on rosette size, survival and fecundity was assessed with models 1, 2, and 3 (Table B1). Fern abundance was associated with reduced rosette size. For example, a 1σ increase in fern abundance was estimated to reduce rosette size by -0.18σ ($\chi^2 = 12.5$, $p < 0.001$). In contrast, fern abundance increased the likelihood of *A. petiolata* survival. A 1σ increase in fern abundance was estimated to increase the likelihood of survival by 0.36 log odds (equivalent to increasing the probability of survival by 58.9% ($\chi^2 = 14.1$, $p < 0.001$). Fern abundance did not, however, affect fecundity.
Table 6. Effects of competition and pathogen infection on *Alliaria petiolata* performance and fitness.

<table>
<thead>
<tr>
<th>Competitor/Pathogen trait</th>
<th>Rosette Size</th>
<th>Survival</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma$ ($\chi^2$, p)</td>
<td>log odds ($\chi^2$, p)</td>
<td>$\sigma$ ($\chi^2$, p)</td>
</tr>
<tr>
<td><em>A. saccharum</em> (mean effect)</td>
<td>-0.36 (137.0, &lt; 0.001)</td>
<td>-0.56 (5.00, 0.08)</td>
<td>-0.51 (18.3, &lt; 0.001)</td>
</tr>
<tr>
<td><em>A. saccharum</em> TLA (Year 1)</td>
<td>-0.24 (13.74, &lt; 0.001)</td>
<td>-0.82 (11.5, &lt; 0.001)</td>
<td>-0.09 (5.4, 0.02)</td>
</tr>
<tr>
<td><em>A. saccharum</em> shoot mass (Year 2)</td>
<td>N/A</td>
<td>N/A</td>
<td>-0.11 (0.88, 0.35)</td>
</tr>
<tr>
<td><em>A. petiolata</em> (mean effect)</td>
<td>-1.12 (137.0, &lt; 0.001)</td>
<td>-0.11 (5.00, 0.08)</td>
<td>-0.81 (18.3, &lt; 0.001)</td>
</tr>
<tr>
<td>Rosette size</td>
<td>-0.55 (56.6, &lt; 0.001)</td>
<td>1.11 (5.7, 0.02)</td>
<td>-0.29 (6.5, 0.01)</td>
</tr>
<tr>
<td>Bolt size</td>
<td>N/A</td>
<td>N/A</td>
<td>-0.06 (0.66, 0.42)</td>
</tr>
<tr>
<td><em>A. petiolata</em> survival</td>
<td>N/A</td>
<td>8.54 (81.1, &lt; 0.001)</td>
<td>-1.64 (13.9, &lt; 0.001)</td>
</tr>
<tr>
<td><em>A. petiolata</em> family*</td>
<td>(23.2, 0.39)</td>
<td>(37.7, 0.02)</td>
<td>(48.5, &lt; 0.001)</td>
</tr>
<tr>
<td>Fern (Year 1 abundance)</td>
<td>-0.18 (12.5, &lt; 0.001)</td>
<td>0.36 (14.1, &lt; 0.001)</td>
<td>-0.04 (0.33, 0.56)</td>
</tr>
<tr>
<td>Thrip damage</td>
<td>-0.07 (2.96, 0.08)</td>
<td>0.05 (0.0, 0.91)</td>
<td>-0.04 (0.2, 0.65)</td>
</tr>
<tr>
<td>Black pathogen damage</td>
<td>-0.19 (24.5, &lt; 0.001)</td>
<td>0.03 (0.0, 0.87)</td>
<td>-0.11 (1.4, 0.23)</td>
</tr>
<tr>
<td>Powdery mildew presence</td>
<td>-0.19 (5.3, 0.02)</td>
<td>-0.09 (0.2, 0.65)</td>
<td>0.08 (0.6, 0.42)</td>
</tr>
</tbody>
</table>

Significant estimates (p < 0.05) are in bold.

*This variable is categorical, and each genetic family may have different effects on the variable if significant.

### 3.4.2 Effect of competition on *Acer saccharum*

The average effect of treatment and fern abundance on *A. saccharum* TLA in year 1 was obtained from a model that also included the initial transplant size of *A. saccharum*
and pathogen damage (model 10 from Table B1). The effects of treatment and fern abundance on *A. saccharum* shoot mass in year 2 were assessed in a model that included only initial size (model 11 from Table B1). Initial size was a significant predictor in both models. A 1σ increase in initial size was associated with a +0.65σ increase in *A. saccharum* TLA in year 1, and a +0.42σ increase in *A. saccharum* shoot mass in year 2. After controlling for the effect of initial size, *A. saccharum* was strongly affected by interspecific competition. On average, interspecific competition reduced *A. saccharum* TLA in year 1 by -0.62σ (χ² = 19.01, p < 0.001) and shoot mass in year 2 by -0.87σ (χ² = 22.7, p < 0.001) (Table 7). Fern abundance significantly affected *A. saccharum* TLA in year 1 whereby a 1σ increase in fern abundance was predicted to reduce *A. saccharum* TLA by -0.13σ (χ² = 6.8, p = 0.009). The effect of ferns, however, did not persist to influence *A. saccharum* shoot mass in year 2.

To test for allelopathy and other competitive effects on *A. saccharum*, I analyzed whether leaf chemistry traits and life history traits of *A. petiolata* were associated with reduced *A. saccharum* performance in year 1 and 2. These effects were estimated from modelling *A. saccharum* TLA in year 1 with the initial size, fern abundance and leaf damage as covariates; and shoot mass in year 2 with initial size as a covariate (models 12 and 13 in Table B1). After controlling for these effects and significant life history traits (outlined below), leaf quality (PC1) was negatively correlated with *A. saccharum* TLA in year 1 (χ² = 7.4, p = 0.006), whereby a 1σ increase in leaf quality was estimated to decrease *A. saccharum* TLA by -0.20σ, consistent with allelopathy (Table 7; Fig. 2a). Contrary to predictions, however, RGI (PC3) was positively correlated with *A. saccharum* TLA in year 1, whereby a 1σ increase in RGI was estimated to increase *A. saccharum* TLA in year 1, whereby a 1σ increase in RGI was estimated to increase *A. saccharum* TLA in year 1.
saccharum TLA by +0.16σ (χ² = 11.4, p < 0.001; Fig. 2b). RFI (PC2) was not associated with A. saccharum TLA in year 1 and no leaf chemistry traits were associated A. saccharum shoot mass in year 2 (Table 3).

Using the models just mentioned, neighbor rosette size did not affect A. saccharum TLA in year 1 but a 1σ increase in rosette size was estimated to reduce A. saccharum shoot mass in year 2 by -0.25σ (χ² = 12.2, p < 0.001). Neither A. petiolata survival nor bolt size affected A. saccharum shoot mass in year 2. The within-treatment effect of A. petiolata seed family was also assessed as a predictor of A. saccharum TLA in year 1 and shoot mass in year 2 but was not significant; neither was the effect of A. petiolata survival on A. saccharum shoot mass in year 2 (Table 7).

Table 7. Effects of competition, pathogen infection and transplant size on Acer saccharum performance in year 1 and 2.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Total leaf area (Year 1)</th>
<th>Shoot mass (Year 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>σ (χ², p)</td>
<td>σ (χ², p)</td>
</tr>
<tr>
<td>A. petiolata (mean effect)</td>
<td>-0.62 (19.01, &lt; 0.001)</td>
<td>-0.87 (22.7, &lt; 0.001)</td>
</tr>
<tr>
<td>Rosette size (TLA)</td>
<td>0.04 (0.4, 0.51)</td>
<td>-0.25 (12.2, &lt; 0.001)</td>
</tr>
<tr>
<td>Bolt size</td>
<td>N/A</td>
<td>-0.06 (0.76, 0.38)</td>
</tr>
<tr>
<td>A. petiolata survival</td>
<td>N/A</td>
<td>0.17 (0.02, 0.86)</td>
</tr>
<tr>
<td>A. petiolata family*</td>
<td>(24.5, 0.32)</td>
<td>(30.6, 0.10)</td>
</tr>
<tr>
<td>Fern abundance</td>
<td>-0.13, (6.8, 0.009)</td>
<td>0.05 (0.8, 0.36)</td>
</tr>
<tr>
<td>Leaf Quality (PC1)</td>
<td>-0.20, (7.4, 0.006)</td>
<td>0.02 (0.31, 0.58)</td>
</tr>
<tr>
<td>RFI (PC2)</td>
<td>0.06 (1, 0.32)</td>
<td>-0.14 (2.2, 0.14)</td>
</tr>
<tr>
<td>RGI (PC3)</td>
<td>0.16 (11.4, &lt; 0.001)</td>
<td>0.17 (1.9, 0.17)</td>
</tr>
<tr>
<td>Leaf damage</td>
<td>-0.22 (9.5, 0.002)</td>
<td>0.05 (0.1, 0.76)</td>
</tr>
<tr>
<td>Initial size of A. saccharum</td>
<td>0.65 (80.6, &lt; 0.001)</td>
<td>0.42 (36.7, &lt;0.001)</td>
</tr>
</tbody>
</table>

Significant estimates (p < 0.05) are in bold.
*This variable is categorical, and each genetic family may have different effects on the variable if significant.
Figure 3. The association of competitor leaf quality (PC1) and RGI (PC2) with *Acer saccharum* total leaf area in year 1.

The y-axis denotes *A. saccharum* total leaf area in year 1, while the x-axis denotes leaf quality (PC1) (a) and relative glucosinolate investment (PC3) (b) of the competitor *A. petiolata* grown in the same pot. Data are displayed in units of standard deviation and lines show the partial regression coefficients.

### 3.4.3 Pathogen effect on *Alliaria petiolata* and *Acer saccharum*

The effects of pathogens on *A. petiolata* were assessed using models 1, 2 and 3 in Table B1. A 1σ increase in black pathogen damage was estimated to reduce rosette size (TLA) by -0.19σ ($\chi^2 = 24.5$, $p < 0.001$). The presence of powdery mildew infection elicited a similar negative effect, whereby presence of the fungus was estimated to reduce rosette size (TLA) by -0.19σ ($\chi^2 = 5.3$, $p < 0.05$). Neither of these pathogens affected survival or fecundity and thrip damage was not associated with rosette size, survival or fecundity (Table 6).
The effect of pathogen damage on *Acer saccharum* TLA in year 1 and shoot mass in year 2 using models 10 and 11 in Table B1. A 1σ increase in leaf damage was estimated to reduce *A. saccharum* TLA in year 1 by -0.22σ ($\chi^2 = 9.5, p = 0.002$). Like *A. petiolata*, however, the effect of pathogens did not carry through to year 2 as leaf damage was not a significant predictor of *A. saccharum* shoot mass (Table 7).

### 3.5 Genetic variation and plasticity

To determine the amount of variation in each trait attributable to plasticity, genotype and genotype-by-environment effects was assessed using a variance component analysis. Each *A. petiolata* trait was used as a dependent variable in a linear mixed-effects model with the random effects of treatment, seed family and a treatment-by-family interaction. Glucosinolate, flavonoid, and chlorophyll A were all affected by treatment: 8% of each trait was explain by treatment (all $p < 0.001$; Table 8). Of these three variables, only glucosinolate production had detectable genetic variation, with 7.47% of glucosinolate variation being explained by seed family ($\chi^2 = 14.0, p < 0.001$). Leaf quality (PC1) exhibited no genetic variation while treatment accounted for 10.36% of variation ($\chi^2 = 27.1 p < 0.001$). A small proportion (~3%) of the variation in RFI (PC2) was explained by treatment and had no detectable genetic variation (Table 8). In contrast, RGI (PC3) was the trait with the most genetic variation in this experiment: 19.03% of the variation was explained by family ($\chi^2 = 44.0, p < 0.001$) and none of the variation in RGI was attributable to treatment. Leaf quality was highest in the alone treatment whereas RFI was highest in interspecific treatment (Fig. 3).

Rosette size and relative growth rate did not exhibit any significant genetic variation, but a large amount of the variation was explained by treatment (26.72% and
10.36%, respectively; both p < 0.001). While there was no genetic variation for fecundity, which was explained only by treatment, there was genetic variation for both survival and bolt size (Table 5). In fact, 15.72% of the variation in survival ($\chi^2 = 22.0$, p < 0.001) and 7.57% of the variation in bolt size could be attributed to seed family ($\chi^2 = 16.3$, p < 0.001). While a small proportion of the variation in bolt size could be attributable to treatment (2.18%), none of the variation in survival was influenced by treatment (Table 5). I did not detect any genetic variation for plasticity (i.e. a treatment-by-family interaction) for any trait.

### Table 8. Percent of total trait variation predicted by family, treatment and family-by-treatment interactions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Family % ($\chi^2$, p)</th>
<th>Treatment % ($\chi^2$, p)</th>
<th>Family × Treatment % ($\chi^2$, p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll A</td>
<td>2.21 (1.7, 0.19)</td>
<td>8.54 (27.1, &lt; 0.001)</td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>Glucosinolate</td>
<td><strong>7.47 (14.0, &lt; 0.001)</strong></td>
<td>8.78 (26.3, &lt; 0.001)</td>
<td>3.02 (0.6, 0.43)</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>&lt; 0.1 (0, 1)</td>
<td><strong>8.00 (16.2, &lt; 0.001)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>Leaf quality (PC1)</td>
<td>2.31 (1.2, 0.27)</td>
<td><strong>10.36 (27.1, &lt; 0.001)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>RFI (PC2)</td>
<td>0.53 (0.3, 0.59)</td>
<td><strong>3.1 (5.3, 0.02)</strong></td>
<td>2.57 (0.3, 0.56)</td>
</tr>
<tr>
<td>RGI (PC3)</td>
<td><strong>19.03 (44.0, &lt; 0.001)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
<td>0.94 (0.1, 0.78)</td>
</tr>
<tr>
<td>Relative growth rate</td>
<td>0.43 (0.1, 0.70)</td>
<td><strong>15.10 (65.9, &lt; 0.001)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>Rosette size (TLA)</td>
<td>0.24 (1.0, 0.32)</td>
<td><strong>27.46 (141.4, &lt; 0.001)</strong></td>
<td>2.78 (1.7, 0.19)</td>
</tr>
<tr>
<td>Survival</td>
<td><strong>15.72 (22.0, &lt; 0.001)</strong></td>
<td>0.24 (0.9, 0.77)</td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>Bolt size</td>
<td><strong>7.57 (16.3, &lt; 0.001)</strong></td>
<td><strong>2.18 (6.5, 0.01)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
<tr>
<td>Fecundity</td>
<td>&lt; 0.1 (0, 1)</td>
<td><strong>10.0 (10.0, 0.002)</strong></td>
<td>&lt; 0.1 (0, 1)</td>
</tr>
</tbody>
</table>

Significant estimates (p < 0.05) are in bold.
3.6 Fertilizer experiment

The fertilizer experiment was designed to determine the effect of fertilizer application on *Alliaria petiolata* in each treatment using two-way ANOVAs. In the fertilizer experiment, unlike the main experiment, the effect of treatment (i.e. the alone, inter- and intraspecific treatments) was not a significant predictor of glucosinolate, flavonoid, or chlorophyll A production. Although, the production of all three traits significantly increased with fertilizer application (glucosinolate: $f = 76.35; p < 0.001$; flavonoid: $f = 26.78; p < 0.001$; and chlorophyll A: $f = 245.75; p < 0.001$) (See figures B3-B5 for a visualization). In the two-way ANOVA predicting *A. petiolata* shoot mass (year 1), competition and fertilizer were in a significant interaction ($f = 21.88; p < 0.001$). When
grown without fertilizer, intraspecific competition greatly reduced shoot mass, while interspecific competition did not have an effect. Interestingly, the effect of intraspecific competition on shoot mass was not reduced when fertilizer was added, in fact, the influence of intraspecific competition may have been exacerbated (Fig. 4).

Figure 5. The effects of fertilizer application and competition on *Alliaria petiolata* shoot mass.
Boxplots denote shoot mass in each of three competition treatments, from left to right: alone (green), intraspecific competition (blue), and interspecific competition with *Acer saccharum* (yellow).
Chapter 4

Discussion

4.1 Overview

I measured selection on glucosinolate and flavonoid expression in three different competitive environments to determine whether or not selection is acting directly on these compounds in response to allelopathy. If selection is acting on allelochemicals, this would suggest that allelopathy is an important mechanism of competition and may facilitate *A. petiolata* invasion. I also measured the effects of *A. petiolata* and *A. saccharum* on each other to shed light on an important species interaction during *A. petiolata* invasion. In general, I found that selection acted on many correlated traits and there was very little total genetic variation for many of these traits, suggesting that although there may have been selective pressure, a response to selection is unlikely for many traits. While *A. petiolata* was the superior competitor in competition with *A. saccharum*, there is not strong evidence to suggest that allelopathy contributed to this.

4.2 Evidence for allelopathy and selection for secondary compounds

I found evidence to suggest that *A. petiolata* reduced interspecific competition through the allelopathic effects of glucosinolates and/or flavonoids. Leaf quality (PC1) represents the production of glucosinolates, flavonoids and chlorophyll A, as all loadings in the PCA were positive, and PC1 explained 73% of the total variation in these compounds. I found that leaf quality (PC1) of the *A. petiolata* competitor was correlated with reduced *A. saccharum* TLA in year 1 (Fig. 2). This may suggest that glucosinolates and/or flavonoids acted allelopathically to reduce *A. saccharum* performance. Supporting
this hypothesis, leaf quality was positively associated with both rosette size and fecundity in the interspecific treatment only (Fig. 1a, b). Taken together, these findings may suggest that secondary compounds reduced maple performance and that selection acted to favor allelochemical production because allelopathy reduced interspecific competition. If secondary compound investment was simply correlated with fitness, whereby stronger competitors obtained more nutrients and increased production, the positive selection gradients should also have been present in the alone and intraspecific treatments; however, this was not the case. This provides evidence for allelopathy and supports the novel weapons hypothesis (NWH).

Alternatively, if interpreted differently, these results may not support allelopathy-mediated selection. Rather than direct selection acting on glucosinolates and/or flavonoids, chlorophyll A may have been the primary trait under selection. If light was a driving factor in competition with *A. saccharum* (which stood taller than *A. petiolata* in this experiment, e.g., Fig. A4), the results surrounding leaf quality (PC1) may represent the benefit of chlorophyll A. That is, if chlorophyll A production increased competitive ability against *A. saccharum*, this would also explain why leaf quality was associated with reduced *A. saccharum* performance, and why leaf quality was positively correlated with rosette size and fecundity in the interspecific treatment. Competition for light in *A. petiolata* can be very strong and drive selection in natural populations (Davis et al. 2014). In this experiment, plants were shaded and only received natural light to simulate the forest understory. Therefore, light competition may have been fierce, especially with further shading from competitors.
Supporting the light competition hypothesis, selection gradients for RFI (PC2) were negative with respect to survival during intraspecific (p < 0.5) and interspecific competition (p < 0.01; Table 5). This means that selection favored a reduction in flavonoid investment and an increase in chlorophyll A investment in treatments with potential for light competition. These results highlight the importance of chlorophyll A investment on competitive interactions with *A. saccharum*, rather than the allelopathic effects of flavonoids.

Also supporting the light competition hypothesis, selection may have acted against RGI (PC3) to increase the competitive ability of *A. petiolata* against *A. saccharum*. RGI was positively correlated with *A. saccharum* TLA in year 1 (p < 0.001; Fig. 3b) and *A. saccharum* TLA in year 1 was associated with reduced *A. petiolata* performance (p < 0.001), survival (p <0.001) and fecundity (p < 0.05) (Table 7). In a path analytical framework, this would be regarded as direct selection acting on RGI. Path analysis has been proposed to improve upon Lande-Arnold selection gradients by allowing variables to have direct effects on fitness through intermediate mechanisms. This framework is applicable to my experiment because allelochemicals and chlorophyll A are expected to increase fitness through the intermediate mechanism of improving competitive ability against *A. saccharum*. In a path analysis context, the selection gradient of RGI is equal to the product of the partial regression coefficient (PRC) of RGI on *A. saccharum* performance and the PRC of *A. saccharum* performance on *A. petiolata* fitness (Kingsolver and Schemske 1991; Scheiner et al. 2000). This approach was not used because the analysis is not easily applied to survival data, where PRCs are expressed in log-odds.
In any case, the significant positive correlation between RGI and *A. saccharum* TLA is consistent with glucosinolate investment decreasing, and chlorophyll A investment increasing, the competitive ability of *A. petiolata* against *A. saccharum*. There is strong evidence to suggest that glucosinolates are responsible for the allelopathic effects of *A. petiolata* (Stinson et al. 2006; Barto et al. 2011; Lankau 2011); therefore, the positive correlation between RGI and *A. saccharum* performance provides key evidence against the allelopathy hypothesis and for the light competition hypothesis.

Selection gradients can only distinguish between direct and indirect selection for traits that have been measured. Therefore, measuring important traits that are not necessarily the focus of the experiment can help improve inference and prevent erroneous conclusions (Lande and Arnold 1983). Chlorophyll A is an important trait because it is vital to plant growth and survival. I found that selection acted on all leaf chemistry traits, although there is more evidence to suggest that there was indirect selection on allelochemicals due to direct selection acting on chlorophyll A. While there is evidence that glucosinolates are under selection in natural populations (Lankau et al. 2009; Evans et al. 2016), my experiment shows how variation in chlorophyll A can confound estimates of selection.

**4.2.1 Limits on evolution**

Natural selection is necessary but not sufficient for adaptive evolution to occur. In order for there to be a response to selection, traits must be heritable. Specifically, the response to selection is equal to the product of narrow sense heritability and the selection differential (Arnold and Wade 1984; Matsumura et al. 2012; Pujol et al. 2018). The response to selection was not calculated in this experiment because additive genetic
variation could not be disentangled from dominant and epistatic genetic variation. However, I was able to assess total genetic variation, and I can conclude that traits without detectible genetic variation also exhibit no additive genetic variation. The lack of significant genetic variation in leaf quality and RFI suggests that evolution on either leaf quality or RFI through either of the previous mechanisms is unlikely (Table 8). RGI on the other hand, likely could respond to natural selection as it exhibited significant genetic variation (19.0%, \( p < 0.001 \)).

Past research demonstrating evolution on glucosinolate production in *A. petiolata* used population means to estimate glucosinolate production and first year biomass as a proxy for fitness. Evans et al., (2016) demonstrated positive selection for sinigrin production (a glucosinolate compound) during interspecific competition with the mycorrhizal herbaceous plant, *Arisaema triphyllum* and negative selection during intraspecific competition using 12 populations and a single sinigrin value for each population. While population means will likely vary for any trait, it is important to assess whether this variation is heritable to ensure it can respond to selection. We employed a similar experimental design but used 23 populations and estimated heritability and plasticity of total glucosinolate production. I found that glucosinolate production by itself exhibits little genetic variation (7.47%, \( p < 0.001 \)) and likely could not respond to selection.

Importantly, a lack of evidence for adaptive evolution on a trait does not mean that the trait is not adaptive. For example, in this experiment, I did not detect direct selection on glucosinolate production, however, I cannot conclude that glucosinolate production is not adaptive due to allelopathy. It may be that the variation in glucosinolate production
production was insufficient to be selected on because all levels of production produced a relatively equal allelopathic effect (which may have been strong or weak). In confirming the role of allelopathy in *A. petiolata*, a valuable experiment would be one where glucosinolate or flavonoid expression is knocked-out. Plants with and without the knock-out mutation could then be grown with a susceptible competitor in both laboratory and natural populations. This would allow allelochemicals to be applied to soil at realistic concentrations to determine whether or not allelopathy occurs in *A. petiolata*.

### 4.3 Plasticity and genetic variation

I performed a PCA analysis of glucosinolate, flavonoid and chlorophyll A production to create three biologically relevant principal components. Leaf quality (PC1) represented the shared variation between glucosinolate, flavonoid and chlorophyll A investment. Leaf chemistry traits were highly correlated, because leaf quality accounted for 73% of the total variation in these traits. One explanation for the high trait correlation is genetic quality. Due to high inbreeding in *A. petiolata*, certain families may have accumulated deleterious recessive mutations, resulting in poor genetic quality and impaired production of primary and secondary compounds. Contrary to this prediction, the variance component analysis demonstrated that 10.3% of the variation in leaf quality was explained by treatment (p < 0.001), and there was no significant genetic variation (H^2 = 2.3%). Therefore, the correlation between leaf chemistry traits was likely not due to genetic quality. Instead, the correlation was likely driven by nutrient availability because much of the variation in leaf quality was explained by treatment. Supporting this, all three compounds were reduced when nutrients were limited. Specifically, leaf quality was lowest in the intraspecific treatment, where competition was strongest, and production of
all three compounds increased significantly in response to fertilizer application. Interestingly, this has the implication that most of the variation in secondary compound expression was attributable to plastic responses to nutrient availability.

High phenotypic plasticity can limit adaptive evolution by reducing variation in fitness and thus the efficiency of selection across environments; although, plastic responses themselves can be adaptive and be selected for (Snell-Rood et al. 2010). Plasticity in *A. petiolata* may be adaptive as the production of secondary compounds are decreased when nutrients are limited in order to reduce the relative cost of producing them. Optimum defense theory states that the cost of any trait is not constant across environments, because the cost of investment is relative to the amount of resources available (Fagerstrom et al. 1987). For example, if producing 1 glucosinolate molecule takes 2 glucose molecules, the cost of glucosinolate production is higher when the plant only has 4 glucose molecules compared to when it has 40. Cipollini & Lieurance, (2012) induced glucosinolate production in *A. petiolata* using jasmonic acid in high and low nutrient environments. They found that the cost of inducing glucosinolate production was greater in the low nutrient environment, consistent with optimal defense theory. Interestingly, they also found that jasmonic acid induced glucosinolate production to a lesser degree in the low nutrient environment than in the high nutrient environment. By reducing the production of glucosinolates when facing nutrient limitation, *A. petiolata* may reduce both allocation and opportunity costs, allowing a greater proportion of resources to be allocated to reproduction and competition, respectively (Koricheva 2002).

There may be a genetic constraint on the production of glucosinolate and chlorophyll A production such that increasing the phenotypic value of one compound is
not possible without increasing the other. There is evidence these traits are linked through pleiotropy and linkage: Chen et al., (2012) down-regulated two genes involved in glucosinolate synthesis and found that 11 light-harvesting chlorophyll protein complexes were also down-regulated in Arabidopsis thaliana; and Qian et al., (2016) found strong linkage disequilibrium between genes involved in chlorophyll synthesis, chloroplast membrane synthesis and glucosinolate synthesis in Brassica Napus. In contrast, flavonoids appear to be able to be synthesized independently of chlorophyll (Kubasek et al. 1998; Misyura et al. 2013).

The second principal component, RFI represented the flavonoid: chlorophyll A ratio. I did not detect significant genetic variation for flavonoid production or RFI. Instead, 8% of flavonoid production and 3% of RFI were significantly explained by treatment (p < 0.001 & p < 0.05, respectively). The environmental influence on flavonoids may have masked genetic variation for flavonoid production, although this trait may also be conserved across populations. There is as of yet little literature on genetic variation of flavonoid expression in A. petiolata. However, consistent with my study, Haribal & Renwick, (2001) did not detect genetic variation for flavonoid concentration. While they did find genetic variation for the type of flavonoids produced, my measurement of total flavonoid production did not capture this variation.

The third principal component, RGI represents the glucosinolate to chlorophyll ratio. I detected moderate genetic variation for glucosinolate production (H² = 7.4%, p < 0.001), however, the glucosinolate to chlorophyll ratio, RGI, exhibited the highest genetic variation in the experiment (H² = 19%, p < 0.001) despite only accounting for ~11% of the total variation in glucosinolate, flavonoid and chlorophyll A production. Furthermore,
treatment predicted 8.8% of variation in glucosinolate production but was not a significant predictor of RGI. For this reason, RGI may capture the genetic variation in glucosinolate expression and represent a genetic trade-off between investing more in glucosinolates or more in chlorophyll A. Because there is evidence of a genetic constraint on glucosinolate and chlorophyll production through pleiotropy and linkage disequilibrium, variation in the relative production of these two compounds may provide the basis of genetic variation for leaf glucosinolate production.

If genetic variation for glucosinolate production is mainly expressed through the ratio of glucosinolate to chlorophyll A, genetic variation for glucosinolate production may be higher in nutrient rich environments where chlorophyll A production is saturated, providing more opportunity for evolution. Glucosinolate heritability was found to be as high as 70% when fertilizer application was present (Hillstrom and Cipollini 2011). While low nutrient availability may have limited genetic variation in my experiment, low nutrients may be more reflective of natural populations. For example, Cipollini (2002) found that glucosinolate production was significantly lower in the field than in the greenhouse, where plants received fertilizer. Furthermore, glucosinolate production varied significantly with nutrient availability in the field, suggesting that glucosinolate plasticity occurs in natural populations in response to nutrient availability. Therefore, the low estimate of glucosinolate heritability may be more reflective of heritability in natural populations and more accurately represent how glucosinolate production could respond to selection in natural populations.

Genetic constraints may have limited the adaptive potential of glucosinolate, flavonoid and chlorophyll A plasticity. Alliaria petiolata was likely striving to increase
chlorophyll A production but not glucosinolate and flavonoid expression during interspecific competition. Supporting this, I observed selection acting to increase the ratio of chlorophyll A to secondary compounds (i.e., selection against RFI and RGI) and chlorophyll A production was 3.7-fold higher when fertilizer was applied. For this reason, down regulating glucosinolate and flavonoid expression in order to increase chlorophyll A investment in response to nutrient limitation would be beneficial. However, nutrient limitation impeding shared metabolic pathways would simultaneously result in limiting the production of all three compounds. For this reason, these traits may lack the independence necessary to elicit a unique response to nutrient availability. Because we observed selection acting to increase the ratio of chlorophyll A relative to glucosinolates and flavonoids, the ability to downregulate secondary compound production in order to free more resources for chlorophyll A production would be beneficial under limiting nutrients. Therefore, the selective pressure we observed could be considered as selection for modularity (or independence) of glucosinolate, flavonoid and chlorophyll A production (Pavličev and Cheverud 2015). In other words, selection may act to facilitate the independent expression of these compounds in order to freely down-regulate glucosinolate and flavonoid investment and increase chlorophyll A investment in response to nutrient limitation.

4.4 Ecological relevance of allelopathy

I was unable to sample root tissue in this project as destructive sampling would have prevented *A. petiolata* from completing its full two-year life cycle. By sampling leaf tissue, I assumed that there is a correlation between leaf and root glucosinolate and flavonoid production and/or that allelopathic compounds enter the soil through leaf litter.
Rosettes lost many of their leaves toward the end of the first growing season and leaves were left in the soil. Leaf litter has been suggested as the potential cause allelopathy in *A. petiolata* (Cipollini and Cipollini 2016) and many of the influential studies demonstrating allelopathy in *A. petiolata* used either leaf or whole plant extracts (Roberts and Anderson 2001; Stinson et al. 2006; Callaway et al. 2008). Rodgers, Wolfe, et al. (2008) demonstrated that leaf tissue may have a larger influence on soil processes than do root exudates and Cipollini and Flint (2013) demonstrated that leaf extracts of *A. petiolata* exhibited much stronger (> 4-fold) allelopathic effects on three native species than did root extracts, and root extracts only exhibited allelopathic effects on 1 of 3 target species.

While few studies of *Alliaria petiolata* include its complete life cycle, rosette size is often used as a proxy for fitness instead of survival and reproduction. Surprisingly, I found no correlation between rosette size and survival or fecundity, suggesting that performance in the first year is not always a good predictor of fitness in the second. This may be because much of the rosette dies back through the winter, and I observed that some plants even grew entirely new stems from a healthy root system. While our study did result in high mortality (69.66% in the field), survival is a very important aspect of selection on *A. petiolata* as mortality rates are very high in nature, with estimates ranging from 99% to a more conservative estimate of 94% (Nuzzo 1993).

My study is the first to my knowledge that measures selection gradients of allelochemicals in *A. petiolata*. This is essential to test whether allelopathy is under selection in natural populations and is a potentially ecological driver affecting invasion. I found that variation in allelochemical production likely does not contribute to *A. petiolata* fitness. These results join a growing body of literature that emphasize other ecological
effects over allelopathy in driving *A. petiolata* invasion (see Rodgers, Stinson, et al., 2008 for a review). For example, Davis et al. (2014) and Davis et al. (2012) found no evidence that allelopathy was a significant driver of competition between *A. petiolata* and tree seedlings in the field and found evidence that *A. petiolata* responds to ecological change rather than causes it. Supporting this, both Anderson et al., (2019) and Kalisz et al. (2014) found that white-tailed deer played a key role in regulating *A. petiolata* growth by selectively eating native flora but not invasive *A. petiolata*. None of these studies found evidence that *A. petiolata* competitive ability was strong enough to drive population growth. Furthermore, Huang et al. (2018) demonstrated that while sinigrin production was lower in old populations, interspecific competitive actually was higher, casting doubt that sinigrin production increases interspecific competitive ability at all.

The discrepancy between the positive evidence for allelopathy in some studies and the lack of evidence for allelopathy in my study may be due to differences in growing environments. In the field, *A. petiolata* would not only need to inhibit mycorrhizal spores, but also extraradical hyphae growing on colonized roots to prevent colonization of neighboring plants. Previous laboratory allelopathy studies of *A. petiolata* demonstrated the allelopathic effect of *A. petiolata* on AMF spores or through soil training experiments, which largely rely on spores for inoculation of mycorrhizal seedlings (Roberts and Anderson 2001; Stinson et al. 2006; Callaway et al. 2008). Barto et al. (2010) demonstrated that when root segments are used as an inoculum (propagation through extraradical mycelium), garlic mustard extracts cannot inhibit the association between AMF and *Impatiens pallida*, suggesting that *A. petiolata* can only inhibit mycorrhizal spores. The majority of fungi propagate mostly through extraradical hyphae, not spores.
(Sun et al. 2016), which may explain why allelopathy is not observed in the field where there are hyphal reservoirs that may resist *A. petiolata* allelochemicals. For example, Burke (2008) found no evidence that mycorrhizal colonization was reduced inside of *A. petiolata* invaded sites vs. outside of *A. petiolata* invaded sites in any of the three species involved in the study. In this experiment, I transplanted *A. saccharum* from the field after it would have already associated with AMF, which may explain why I did not find an effect of allelochemical production on *A. saccharum* performance.

My experiment was confounded with pathogens present in the unsterilized field soil, which may have played a role in altering the expression of glucosinolate and flavonoid compounds. But regardless of whether allelochemicals are at baseline level of production, or plastically induced, the level of allelochemical production should still have an impact on a susceptible competitor. Plasticity adds phenotypic variation but is not expected to affect selection gradients. In contrast, heritability estimates are likely lower than they would have been in the absence of pathogens. However, variation in predation and pathogen infection is expected in natural populations, therefore, using unsterilized field soil may provide estimates of heritability more similar to what would be observed in nature than using sterilized field soil.

**4.5 Outcomes of competition and selection for competitive ability**

In general, the average effect of *A. petiolata* on *A. saccharum* was almost twice as strong as that of *A. saccharum* on *A. petiolata* in terms of performance and fecundity. However, I found that the size of *A. saccharum* was a key component to the effect of *A. saccharum* on *A. petiolata* performance and survival (Table 6). Because *A. saccharum* size is correlated with its age, it is likely that older *A. saccharum* are stronger competitors.
against *A. petiolata* than are young *A. saccharum*. This suggests that an *A. petiolata* dominated forest could limit the recruitment of *A. saccharum*. Furthermore, competitive ability depends on the competitive environment (Aarssen 1984), and the conditions in which I grew these plants consisted of a small pot with very little nutrients. Because *A. petiolata* may prefer high nutrient environments (Rodgers, Wolfe, et al. 2008; Anthony et al. 2017; van Kleunen et al. 2018) and mycorrhizal fungi are increasingly worst symbionts at higher nutrient levels (Grove et al. 2017), competition in natural conditions may result in even worst outcomes for *A. saccharum*.

*Alliaria petiolata* may also benefit from rapid evolution to relative to *A. saccharum*. Due to the high natural mortality of *A. petiolata* and the high fecundity of each individual plant, evolution may quickly act to create highly competitive individuals. While I did not detect genetic variation for interspecific competitive ability, *A. saccharum* TLA in year 1 did exhibit a large influence on *A. petiolata* survival (-0.82 log odds, p < 0.001), and survival itself was a heritable trait (H$^2$ =15%; p < 0.001). Therefore, selection may have acted on *A. petiolata* in favor of interspecific competitors that could survive in competition against *A. saccharum*.

I detected genetic variation for intraspecific competitive ability. The seed family of the competitor was a significant predictor of the focal plant’s survival and fecundity in the intraspecific competition treatment. Specifically, the effect of seed family was a significant predictor in a model that assessed the effects of leaf quality, RGI, RFI, *A. petiolata* size and growth rate. Therefore, I detected genetic variation for intraspecific competitive ability that was not explained by other measured traits. Instead, the source of
variation in competitive ability likely stems from an unmeasured trait, perhaps one associated with below-ground competition.

I observed the counter-intuitive result that neighbor survival was associated with an increased chance of focal plant survival in the intraspecific treatment (+8.54 log odds, P < 0.001). This is odd because one might think that eliminating competition would increase the likelihood of survival. Furthermore, neighbor rosette size was positively associated with focal *A. petiolata* survival (+1.11 log odds, p < 0.05). This is also odd because larger neighbors should exert stronger competitive effects and reduce survival of the focal individual. These results lend support to “evolution of reduced competitive ability” (ERCA) observed in *A. petiolata* in the invasive range (Bossdorf et al. 2004).

*Alliaria petiolata* undergoes asexual reproduction and populations can be very dense due to high fecundity and limited seed dispersal; 95% of *A. petiolata* seeds disperse within 1.14m of the parent plant (Anderson et al. 1996; Loebach and Anderson 2018). For this reason, *A. petiolata* offspring are very genetically similar and may be more likely to compete against each other (kin) than non-kin. In such an environment, plants that are less competitive may have an advantage over those that are more competitive by increasing the survival of the cluster of genetically related plants, a phenomenon known as “indiscriminate altruism” (Mitteldorf and Wilson 2000; File et al. 2012). In this experiment, individuals that exerted stronger competitive effects may have reduced the likelihood that both plants would survive. In contrast, individuals with reduced competitive ability but more tolerance to competitive effects, may have allowed the neighbor to grow larger and increased the chance that both plants survive. In this way, indiscriminate altruism could evolve, however, if certain pots harbored soil pathogens,
this may explain why survival was more likely if the neighbor survived, therefore, a comprehensive test is required to test the ERCA hypothesis.

4.6 Conclusion

By continuing the growth of *A. petiolata* until seed set, we were able to measure fitness, which is rarely performed in the biennial species *A. petiolata*. Despite the surge in literature surrounding allelopathy in *A. petiolata* and how it may influence ecology and evolution, we did not find evidence that allelopathy is a driver of *A. petiolata* fitness when competing against a naïve mycorrhizal host. Our research highlights the importance of measuring glucosinolate production in each individual in order to calculate heritability and plasticity, as well as using other important traits to strengthen selection gradients. We used 23 families from different populations to assess genetic variation but found little genetic variation for most traits, which may have been masked by strong plastic responses to nutrient availability. Given the lack of genetic variation within natural populations (Durka et al. 2005), it may be even more difficult for evolution to act in nature. Therefore, in nature, it may be unlikely that evolution would act on the traits under selection in the present study.

In competition against *A. saccharum*, *A. petiolata* was dominant and could quickly prevent the establishment of *A. saccharum*. When it comes to conservation, we echo the results of previous studies which highlight the importance of other ecological phenomena over allelopathy. While allelopathy is often reported in laboratory settings, there is limited evidence to suggest that allelopathy is relevant in regulating *A. petiolata* population dynamics.


Cipollini K, Flint W. 2013. Comparing allelopathic effects of root and leaf extracts of


Evans JA, Lankau RA, Davis AS, Raghu S, Landis DA. 2016. Soil-mediated eco-


Appendix A

Supplemental methods

Table A1. Replication in the main experiment.

<table>
<thead>
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<th>Family</th>
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<th>Intraspecific</th>
<th>Interspecific</th>
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Table A2. Replication in the fertilizer experiment.

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<td>Alone</td>
<td>Intraspecific</td>
<td>Interspecific</td>
</tr>
<tr>
<td>KVEDG1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MSMID1</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>SMITH1</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
Figure A1. Examples of leaf images used to measure pathogen data.
Figure A2. Standard curves of rutin and sinigrin.
Figure A3. Example of plant identification with machine learning used to determine growth rate.
Figure A4. *Alliaria petiolata* grown in the (A) alone treatment (B) intraspecific competition treatment and (C) interspecific competition treatment.
Figure A5. Example of greenhouse and photo apparatus used to capture images for growth rate analysis.
Appendix B

Supplemental results

B.1 Minimum Adequate Models

What predicts *A. petiolata* rosette size, survival and fecundity?

The following models represent the minimum adequate models predicting *A. petiolata* rosette size, survival and fecundity. It is from these models that coefficients were obtained. The MAM predicting rosette size was:

\[
Rosette \ Size = (T \ast Leaf \ quality) + P_1 + P_3 + Leaf \ area + (1|GH \ bench)
\]

where \(P_1\) denotes black pathogen damage, \(P_3\) denotes powdery mildew presence, leaf area denotes the area of the sampled leaf, and \(T\) denotes treatment (i.e. alone, intraspecific, interspecific). The symbol “*” denotes an interaction and all lower order effects between the variables involved. In this model, fern abundance was not significant, and the coefficients and p-values of all covariates except for fern abundance come from this model. When fern abundance was reassessed with data that did not contain pseudo-replication, the effect was significant and the coefficient and p-value for fern abundance comes from this model:

\[
Rosette \ Size = (T \ast Leaf \ quality) + P_1 + P_3 + Fern + Leaf \ area + (1|GH \ bench)
\]

The final models for survival and fecundity are:

\[
Survival = (T \ast RFI) + Fern + (1|Family) + (1|Field \ row)
\]

\[
Fecundity = (T \ast Leaf_{Quality}) + Bolt \ Size
\]

The MAM’s for the within treatment effect of *A. saccharum* on *A. petiolata* were as follows (these models only used data from the interspecific treatment):

\[
Rosette \ Size = Maple \ Size_{1,N} + P_1 + Fern + (1|Family) + (1|GH \ Bench)
\]

\[
Survival = Maple \ Size_{1,N} + (P_1 \ast P_2) \ast Fern
\]

\[
Fecundity = Maple \ size_{1,N} + (P_1 \ast P_2 \ast P_3) + Fern
\]

Where \(Maple \ Size_{1}\) denotes the total leaf area of *A. saccharum* after year 1, and the subscript “N” denotes that this trait belongs to the neighbour plant in the pot (i.e. the competitor).
The MAM’s for the within treatment effect of *A. petiolata* on *A. petiolata* were as follows (these models only used data from the intraspecific treatment):

\[
\text{Rosette Size} = \text{Rosette Size}_N + \text{Family}_N + (P_1 * P_2 * P_3) + \text{Fern} + (1|\text{Family}) + (1|\text{GH Bench})
\]

\[
\text{Survival} = \text{Rosette Size}_N + \text{Family}_N + \text{Survival}_N + (1|\text{Family})
\]

\[
\text{Fecundity} = \text{Rosette Size}_N + \text{Family}_N + \text{Survival}_N + \text{Fern} + (1|\text{Family})
\]

where family is the genetic family of the individual and survival is a categorical variable of whether the plant survived or not.

**What predicts *A. saccharum* performance in year 1 and 2?**

The following models represent the MAM’s predicting *A. saccharum* performance in year 1 (i.e. total leaf area, *Maple Size*$_1$) and year 2 (i.e. total biomass, *Maple Size*$_2$):

\[
\text{Maple Size}_1 = \text{Maple Size}_0 + T_m + \text{Fern} + \text{Maple damage}
\]

\[
\text{Maple Size}_2 = \text{Maple Size}_0 + T_m
\]

where *Maple Size*$_0$ denotes the principal component reflecting the initial size of *A. saccharum*.

The MAM’s for the within treatment effect of *A. petiolata on A. saccharum* were as follows (these models only used data from the interspecific treatment):

\[
\text{Maple Size}_1 = \text{Leaf Quality}_N + RGI_N + \text{Maple Damage} + \text{Maple Size}_0
\]

\[
\text{Maple Size}_2 = \text{Rosette Size}_N + \text{Maple Size}_0
\]
Table B1. Summary table of minimum adequate models used to calculate coefficients.

<table>
<thead>
<tr>
<th>Model</th>
<th>Dependent</th>
<th>Fixed Effects</th>
<th>Random Effects</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rosette Size</td>
<td>$(T \ast \text{Leaf quality}), P_1, P_3, \text{Leaf area}$</td>
<td>$\text{GH bench}$</td>
<td>Alone, Interspecific, Intraspecific</td>
</tr>
<tr>
<td>2</td>
<td>Survival</td>
<td>$(T \ast \text{RFI}), \text{Fern}$</td>
<td>$\text{Family, Field row}$</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>3</td>
<td>Fecundity</td>
<td>$(T \ast \text{Leaf Quality}), \text{Bolt Size}$</td>
<td>none</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>4</td>
<td>Rosette Size</td>
<td>$\text{Maple Size}_{1,N}, P_1, \text{Fern}$</td>
<td>$\text{Family, GH bench}$</td>
<td>Interspecific</td>
</tr>
<tr>
<td>5</td>
<td>Survival</td>
<td>$\text{Maple Size}_{1,N}, (P_1 \ast P_2), \text{Fern}$</td>
<td>none</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>6</td>
<td>Fecundity</td>
<td>$\text{Maple size}_{1,N}, (P_1 \ast P_2 \ast P_3), \text{Fern}$</td>
<td>none</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>7</td>
<td>Rosette Size</td>
<td>$\text{Rosette Size}_N, \text{Family}_N, (P_1 \ast P_2 \ast P_3), \text{Fern}$</td>
<td>$\text{Family, GH bench}$</td>
<td>Intraspecific</td>
</tr>
<tr>
<td>8</td>
<td>Survival</td>
<td>$\text{Rosette Size}_N, \text{Family}_N, \text{Survival}_N$</td>
<td>$\text{Family}$</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>9</td>
<td>Fecundity</td>
<td>$\text{Rosette Size}_N, \text{Family}_N, \text{Survival}_N, \text{Fern}$</td>
<td>$\text{Family}$</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>10</td>
<td>Maple Size$_1$</td>
<td>$\text{Maple Size}_0, T_m, \text{Fern, Maple damage}$</td>
<td>none</td>
<td>Interspecific, maple alone</td>
</tr>
<tr>
<td>11</td>
<td>Maple Size$_2$</td>
<td>$\text{Maple Size}_0 + T_m$</td>
<td>none</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>12</td>
<td>Maple Size$_1$</td>
<td>$\text{Leaf Quality}_N, \text{RGI}_N, \text{Maple Damage, Maple Size}_0$</td>
<td>none</td>
<td>Interspecific</td>
</tr>
<tr>
<td>13</td>
<td>Maple Size$_2$</td>
<td>$\text{Rosette Size}_N, \text{Maple Size}_0$</td>
<td>none</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

$P_1$ denotes black pathogen damage, $P_2$ denotes thrip damage and $P_3$ denotes powdery mildew presence. The subscript “N” indicates a neighbor’s trait.
Table B2. Principal component analysis of first year size measurements in *Acer saccharum*.

<table>
<thead>
<tr>
<th>Principal Component</th>
<th>Loadings</th>
<th>Proportion of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem Height (initial)</td>
<td>Largest leaf length (initial)</td>
</tr>
<tr>
<td>PC1 (Initial maple size)</td>
<td>0.45</td>
<td>0.4</td>
</tr>
<tr>
<td>PC2</td>
<td>0.42</td>
<td>0.09</td>
</tr>
<tr>
<td>PC3</td>
<td>-0.18</td>
<td>0.62</td>
</tr>
<tr>
<td>PC4</td>
<td>0.37</td>
<td>-0.49</td>
</tr>
<tr>
<td>PC5</td>
<td>0.65</td>
<td>0.18</td>
</tr>
<tr>
<td>PC6</td>
<td>-0.11</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table B3. Principal component analysis of second year shoot measurements in *Alliaria petiolata*.

<table>
<thead>
<tr>
<th>Principal Component</th>
<th>Loadings</th>
<th>Proportion of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bolt Height</td>
<td>Number of Leaves</td>
</tr>
<tr>
<td>PC1 (Bolt size)</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>PC2</td>
<td>0.83</td>
<td>-0.5</td>
</tr>
<tr>
<td>PC3</td>
<td>0.26</td>
<td>0.71</td>
</tr>
<tr>
<td>PC4</td>
<td>0.03</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Figure B1. Correlation between glucosinolate (a), flavonoid (b) and chlorophyll A concentration.
Figure B2. Average growth rate of families within treatment.
Figure B3. Effect of soil nutrients on chlorophyll A concentration.
Figure B4. Effect of soil nutrients on glucosinolate concentration.
Figure B5. Effect of soil nutrients on flavonoid concentration.
Figure B6. *Acer saccharum* performance in the interspecific and maple alone treatment.

Regression lines demonstrate the significant effect of treatment and initial maple size on performance.