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Impacts of Invasive *Alliaria Petiolata* on Two Native Pieridae Butterflies, *Anthocharis Midea* and *Pieris Virginiensis*

Danielle Marie Thiemann
Wright State University

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IMPACTS OF INVASIVE ALLIARIA PETIOLATA ON TWO NATIVE PERIDAE
BUTTERFLIES, ANTHOCHARIS MIDEA AND PIERIS VIRGINIENSIS

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

By

DANIELLE MARIE THIEMANN
B.S., University of Dayton, 2014

2017

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WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

April 20, 2017

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Danielle Marie Thiemann ENTITLED Impacts of Invasive *Alliaria petiolata* on Two Native Pieridae Butterflies, *Anthocharis midea* and *Pieris virginiensis* BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Donald F. Cipollini, Ph.D.
Thesis Director

David L. Goldstein, Ph.D., Chair
Department of Biological Sciences

Committee on Final Examination

Donald F. Cipollini, Ph.D.

Thomas Rooney, Ph.D.

John O. Stireman III, Ph.D.

Robert E.W. Fyffe, Ph.D.
Dean of the Graduate School

ABSTRACT

Thiemann, Danielle Marie. M.S. Department of Biological Sciences, Wright State University, 2017. Impacts of Invasive *Alliaria petiolata* on Two Native Pieridae Butterflies, *Anthocharis midea* and *Pieris virginiensis*

Invasion of *Alliaria petiolata* has negative direct and indirect impacts on the systems in which they invade. This study focuses on further identifying impacts which this non-native *A. petiolata* has on herbivores whose range they have invaded. Oviposition on *A. petiolata* by the specialist butterfly, *Pieris virginiensis*, is known to be a mismatch event leading to larval death from sinigrin and alliarinoside. To observe if the related specialist, *Anthocharis midea*, falls into the same oviposition sink paired plot comparisons between native *Cardamine concatenata* and non-native *A. petiolata* were conducted. Early in the season paired-plot comparisons showed a preference for native *C. concatenata* while later comparisons a preference for *A. petiolata*. A significant influence of the date of oviposition on selected host was seen. Environmental stressors such as drought and disease can lead to changes in plant development and productivity. Trade-offs exists between defenses so as one area of defense is invested in other areas of defense will not be allocated resources because of the energetic costs. Under these environmental stressors resources should be

shifted away from herbivory defense and with the reduction of secondary metabolites herbivores will be expected to perform better. Environmental stressors including drought and disease on larval performance and preference were investigated. Influences of drought stress on non-native *A. petiolata* were not sufficient enough to allow for specialist herbivores *A. midea* and *P. virginiensis* to reach pupation. Generalist herbivore *Trichoplusia ni*, the cabbage looper, was short lived and unable to reach pupation on any *A. petiolata*, normally watered or drought stressed. *Anthocharis midea* preference assays show a clear preference for native *C. concatenata* over non-native *A. petiolata*, severely drought stressed *C. concatenata* over normally watered plants and no preference between drought stressed or normally watered *A. petiolata*. Presence of white rust, *Albugo candida*, on the native host negatively influenced growth and larval weight of *P. virginiensis*. As larvae develop, they become more mobile and have been seen to move from leaves to floral parts of host. As native *C. concatenata* and invasive *A. petiolata* grow in close proximity transfer between the native and non-native *A. petiolata* is possible. Simulation of this transfer resulted in larval death for *A. midea*, while once transferred, later instar *P. virginiensis* ceased feeding and began pupation.

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INTRODUCTION

Plant Insect Interactions

When invasive plant species are introduced into a system this invasion impacts direct competitors as well as those herbivore species whose natural hosts are compromised. Systems evolve over time resulting in a 'balance'. In the case of plants and their herbivores, native plants have defenses against herbivory and herbivorous insects have evolved strategies to allow them to utilize these plants. Herbivory can damage a plant but may also be of no consequence or benefit to the plant depending on the plants ability for compensatory growth (Maschinski and Whitham 1988). Negative effects, including reduction of reproductive potential and compromising the competitive ability of the plant (Maschinski and Whitham 1988), may exert selective pressure great enough to influence a plant populations genotypic frequency to favor the spread of anti-herbivore defenses (Berenbaum 1986). Change arises over evolutionary time driving a positive feedback loop wherein an adaptation of the insect species for herbivory exerts a selective pressure on the plant defensive adaptations which in turn places pressure on the insect to adapt and so on. This evolutionary arms race results in constitutive, ever present defenses, as well as inducible defenses, which are elicited by feeding (Schardl 2002). These inducible defenses are often in the form of toxic or deterrent chemicals termed secondary metabolites that are

compounds that serve in plant defense by providing feeding deterrence, toxicity or acting as a precursor to physical defense systems (Bennett and Wallsgrove 1994).

The Specialist and Generalist Strategies of Herbivores

Herbivores have evolved mechanisms to combat the defenses of plants they feed on. Many insect species evolved methods to avoid toxic and deterrent defenses of these plants and are even able to utilize these chemicals as host recognition cues or for nutrients (Bennett and Wallsgrove 1994). For example, the myrosinase catalyzed breakdown of glucosinolates, a group of secondary metabolites, into isothiocyanates and other compounds results in toxic effects on non-adapted organisms but serve as oviposition attractants or feeding stimulants for adapted specialists (Hopkins et al. 2008; Winde and Wittstock 2011). Many specialist insects have a preference for specific metabolite profiles and have developed mechanisms to process this narrow range of plant chemical defenses (Fox and Morrow 1981). Some specialists have turned secondary plant defenses' against their hosts by using these secondary metabolites as signals of host presence (Bennett and Wallsgrove 1994). While the specialization on hosts allows for great efficiency of food processing, meaning they procure as much nutrients as possible from the food source, the range of resources they are able to utilize is limited. In contrast, generalist herbivore species are able to utilize a

wide variety of resources by being able to detoxify or metabolize secondary chemicals from a wide array of plants (Fox and Morrow 1981). The cost of this strategy is that although they are able to metabolize an array of chemicals, the efficiency with which these species can process their food is decreased (Fox and Morrow 1981).

Plant Insect Interactions with Non-Native Species

When a novel species is introduced able to colonize and then expand within the new region, the invasion can impact competitors and disrupt co-evolved plant-host systems. In North America, many endangered or threatened species are at high levels of risk due to interactions with novel or invasive species. About 42% of all species on the endangered or threatened list in the United States are listed as such mainly due to competition or predation from an invasive species (Pimentel 2005). When novel interactions between herbivorous insects and invasive plants occur they have three potential results: 1. Novel host recognition and utilization by native species to its benefit 2. Failure of novel host recognition by the native species, resulting in no interaction. 3. Novel host recognition and utilization by native species to its detriment (Davis, 2014). When the invasive species is recognized and used to the detriment of the native species these interactions are termed mismatch events (Davis 2014).

The successful adoption of sweet fennel, *Foeniculum vulgare*, by *Papilio zelicaon*, Anise swallowtail, illustrates novel host recognition to the benefit of the native herbivore. Utilization of this novel host resulted in a transition from univoltinism to multivoltinism for *Papilio zelicaon* shortening generation time and allowing for an increase in population size (Tong and Shapiro 1989).

When invasive species are not recognized by native herbivores, these herbivores could be missing a potential reservoir of resources. This is seen with the honeysuckle sawfly (*Zaraea inflata*) which rarely selects non-native *Lonicera* in nature as a host for larvae even though they can feed and reach pupation on some of these species in laboratory settings (Lieurance and Cipollini 2013). Lieurance et al. (2015) studied herbivory rates and secondary metabolite presence in native and non-native *Lonicera* species North American species. The non-native species *Lonicera maackii* is known to produce secondary metabolites that have roles in alleopathy and defense against herbivores (Cipollini et al. 2008; Dorning and Cipollini 2006; Skulman et al. 2004). Non-native *Lonicera* species also receive low levels of herbivory both in natural and experimental settings (Lieurance et al. 2015; Lieurance and Cipollini 2013). There is evidence that phenolic compounds, their glycoside derivatives and iridoid glycosides (IGs) are phytotoxic and deter generalist feeders (Boeckler et al. 2011; Cipollini et al. 2008; Hay and Fenical 1990). IGs are also seen to be oviposition and feeding cues for co-evolved specialist (Bowers 1984; Peñuelas et al. 2006; Reudler

Talsma et al. 2008). If certain IGs, or increased levels of IGs, stimulate oviposition for specialist herbivores of *Lonicera*, which has been demonstrated for other plant species containing IGs (Reudler Talsma et al. 2008), the lack of these IGs could explain why specialists rarely choose non-native *Lonicera* in the field (Lieurance et al. 2015). An example of the failure to recognize a suitable novel host by native herbivores also include the interaction of the Clouded Sulphur butterfly (*Colias philodice*) and Crown Vetch (*Securigera varia*) (Karowe 1990), and the West Virginia White butterfly (*Pieris virginiensis*) and watercress (*Nasturtium officinale*) (Bowden 1971).

Examples where native herbivores select a novel host that cannot support larval development can be seen in many Lepidoptera including in the Papilionidae (Berenbaum 1981) and Pieridae (Chew 1977). For example, the preferential use of non-native garlic mustard, *Alliaria petiolata*, by the West Virginia White butterfly is a mismatch event as use of this host plant results in larval mortality and negative consequences for the population (Davis and Cipollini 2014).

Impacts of *Alliaria petiolata* on Insects

In this study, I investigated the effects of the presence of the novel host *A. petiolata* and environmental stress on plant-insect interactions. *Alliaria petiolata*, garlic mustard (Brassicaceae) is biennial herb native to Europe that has become

a major North American invasive pest since its introduction in the 1800s. In the mid-to-late 1900s *A. petiolata* was recognized as a major invasive plant (Davis and Cipollini 2014). Since its introduction, *A. petiolata* has spread across the continent, aided through directly out competing native plants for light and nutrients as well as alleopathy which reduces native seed germination (Meekins and McCarthy 1999; Prati and Bossdorf 2004). The increased presence of *A. petiolata* with native Brassicaceae species such as *Cardamine concatenata*, *C. diphylla*, *C. douglassii*, *C. heterophylla* has increased the chance that native mustard-feeding pierid species will encounter and attempt to use *A. petiolata* (Davis and Cipollini 2014). Differences in secondary metabolites between the native Brassicaceae species and the novel *A. petiolata* may cause problems for herbivore species that are adapted to combat the chemical defenses of their native hosts but not equipped to overcome the novel defenses of *A. petiolata*. The phytochemical profile of *A. petiolata* shares almost no overlap with native Brassicaceae species of North America, illustrating that *A. petiolata* alleopathic secondary chemistry is novel in its invaded range (Barto et al. 2010). *Alliaria petiolata* produces alliarinoside, a hydroxynitrile glucoside unknown from other species, and glucosinolates, predominantly sinigrin (allylglucosinolate) (Agerbirk et al. 2010; Haribal et al. 2001; Huang et al. 1994; Vaughn and Berhow 1999). The sinigrin present in *A. petiolata* is absent in native Brassicaceae which have

been examined, while native Brassicaceae contains some flavonoids that *A. petiolata* lacks (Barto et al. 2010).

These different secondary metabolite profiles lead to consequences for those herbivores which encounter the novel host in the field, such as adult oviposition preference, larval performance and potentially quality of offspring. For example, studies of *Pieris oleracae*, the multivoltine mustard white, a relative of the focal study species, show differential responses to use of novel *A. petiolata* as a host based on the duration of populations exposure. In areas with long-established *A. petiolata*, *P. oleracae* may be adapting in terms of both larval performance, though survival is still generally low on *A. petiolata*, and adult preference toward the invader (Keller & Chew 2008). In other areas where *A. petiolata* is not well established, there is a large range in preference and survivorship on *A. petiolata* (Keller & Chew 2008). The divergent response of *P. oleracae* to novel *A. petiolata* indicates that native herbivores may be capable of adapting in both larval performance and adult oviposition preference to the invader (Keller & Chew, 2008).

Likewise, oviposition preference of *Anthocharis midea* will be investigated in the field using paired plot comparisons. *Anthocharis midea*, the Falcate Orangetip butterfly of the Peiridae family, is a butterfly found in southeastern North America which specializes on members of the Brassicaceae family.

Anthocharis midea has been anecdotally observed ovipositing on the novel host *A. petiolata* but larval performance is unknown. Host preference of the mother in the presence of both the novel and native hosts has not yet been identified. Through field plot comparisons oviposition preference will be examined to see if this species falls into the same mismatch pattern as its relative *P. virginiensis*.

Pieris virginiensis is a univoltine butterfly which is native to eastern North America and is a specialist, feeding on spring ephemeral crucifers as larvae (Davis et al. 2015; Bess 2005) including *Cardamine concatenata* and *C. diphylla* most frequently (Shuey and Peacock 1989; Finnell and Lehn 2007; Keeler and Chew 2008). The impact of *A. petiolata* on oviposition preference for *P. virginiensis* has been established both in the field and in the laboratory. Davis and Cipollini (2014) showed that both in field and in experimental settings that *P. virginiensis* preferentially oviposits on *A. petiolata*. Use of *A. petiolata* as a host plant by *P. virginiensis* is a sink for this butterfly as larvae die on it (Davis and Cipollini, 2014), indicating that for this species within-habitat reproduction is insufficient to balance local mortality when *P. virginiensis* utilizes *A. petiolata* (Pulliam 1988). Research has been conducted to identify secondary metabolites in this species that could be responsible for the larval mortality of *P. virginiensis*. Two chemicals were identified, sinigrin and alliarinoside, that could be the cause of larval death. When tested in a no choice feeding assay, both chemicals were seen to negatively impact larval survival, leaf consumption, and larval mass when

painted on leaves of an otherwise acceptable host, with the effects of alliarinocide being more severe (Davis et al., 2015).

Anthocharis midea, the falcate orangetip butterfly, is a spring-univoltine specialist on Crucifers. Like its' relative *A. sara*, *A. midea* is found in wooded areas (Shapiro 1980). *Anthocharis midea* has been reported to lay eggs only singly on plants (Clark 1932). *Anthocharis midea*, like many of the pierid butterflies, express the red egg syndrome meaning that once laid the eggs turn a bright orange red color (Shapiro 1981). This non-cryptic coloration aids in intra- and interspecific egg-load assessment, which is the evaluation of potential hosts for oviposition based on the presence or absence of other eggs (Shapiro 1981). For *A. midea* these bright red orange eggs should serve as a visual cue to adult *A. midea* females that the host is occupied and hence not suitable for oviposition. Little else has been published on the ecology of this species, other than records of sightings. For example, Sites and McPherson (1981) established that *A. midea* has a flight activity period from mid-April to early May and is seen in southern Illinois. This means there are a great number of questions with regards to interactions of *A. midea* with potential suitable and non-suitable host plants. Preliminary studies have shown that *A. midea* will oviposit on *A. petiolata* in the field, and that its larvae, as for the related *P. virginensis*, die upon initial feeding on this novel host (Davis et al. unpublished data).

Host Transfer

As larvae mature, gain mass and thereby become more mobile, they may exhibit either a feeding preference or a need to move to other hosts as resources from their host are exhausted. For the European orange tip butterfly, *Anthocharis cardamines* that feeds on *A. petiolata* in its native range in Europe adults have been seen to oviposit and larvae subsequently feed mainly on floral parts or siliques of their host rather than leaf parts (Wiklund and Åhrberg 1978). In experiments, these larvae will readily feed on leaves but in their final instar they will move toward and feed from floral parts (Ågerbirk et al. 2010). This establishes that at later stages of development transfer of larva between parts of the host plant, such as between floral parts or leaves, or possibly between neighboring plants can occur. In general as larvae become more mature they become more tolerant to defensive compounds produced by host plants (Schoonhoven et al. 2007). For *Ematurgo atomaria*, the common heath moth, young larvae are unable to feed on *Vaccinium myrtillus*, known as bilberry which uses tannins as chemical defenses, but can utilize this host at older instars (Vellau et al. 2013). The maturity of larvae will also influence their ability to utilize hosts that express constitutive defenses, as seen with *Iridopsis ephyraria* (pale-winged gray moth) and *Tsuga canadensis* (eastern hemlock). For older instar *I. ephyraria* feeding from older foliage of *T. canadensis* higher survival rates than those of younger instars were observed. This is most likely due to the larger

mouthparts and muscles of older instar *I. ephyraria* which are better equipped to handle the constitutive defense of the toughened needles of *T. canadensis* (Pinault et al. 2009).

As larva of *P. virginiensis* and *A. midea* are mobile at later instars once food sources are exhausted they must travel to other plant parts or possibly a neighboring plant. This mobility thereby may result in the host transfer, in either direction between native hosts and potential invasive hosts. If in a natural setting native and novel species are found in close proximity to each other, there is potential for larva to move from a suitable native to the novel species or vice versa. Given the different tolerance of larval instars, this may have implications for the potential survival of the larva. If larva move from a native species to *A. petiolata* at a later instar in development these larva may be able to complete their development on this host, while earlier instars may fail on it.

Impact of Environmental Stress on Plant Insect Interactions

Drought

Environmental stress can result in changes in plant chemistry which can influence the interactions between these host plants and herbivores that utilize them (Chaves et al. 2003). Weldegergis et al. (2014) found that drought stress leads to many changes plants ranging from morphological, physiological,

biochemical and molecular changes. These changes can lead to severe effects on plant growth, development and productivity. As a consequence of these changes, the interaction between plants and insects can be altered. For *Brassica oleracea* under drought conditions, changes in volatile production and chemical defenses have been seen (Weldegergis et al. 2014). Drought in *B. oleracea* significantly impacted salicylic acid (SA) level and had a significant interactive effect with herbivory and indole-3-acetic acid (IAA) production (Weldegergis et al. 2014). When *Mamestra brassicae* moths were presented with drought stressed and control or normally watered *B. oleracea* they preferred to lay eggs on drought stressed individuals over control plants (Weldegergis et al. 2014).

Plant responses to herbivory are plastic and are subject to change with the conditions that the plants are experiencing (Maschinski and Whitham 1988). The univariate trade-off model argues that because investment in defense comes at the expense to other areas of plant development such as growth (Bazzaz et al., 1987), trade-offs will exist among defenses such that only one defense performing a particular protective function will be invested in by a plant at any given time, and all comparable redundant defenses will not be allocated resources because of the energetic cost (Agrawal, 2007). Biochemical changes in plants in response to drought events are variable and dependent on the plant species and the drought condition (Turtola et al. 2005, Mody et al. 2009). These drought stress events have been reported to increase populations of herbivorous

insects on plant populations, thereby promoting insect outbreaks in natural ecosystems (Weldegergis et al. 2014; Mattson and Haack 1987). In Britain, areas that are experiencing both climate change and habitat loss have resulted in variable response of butterfly species to these changing environments (Warren et al. 2001). Generalists had mixed responses with half of the studied species increasing their distribution in response to climate expansion and other generalists and the majority of specialists declining in distribution, which is consistent with habitat loss (Warren et al. 2001). Decline in specialist species is likely to be seen due to the combined forces of habitat loss and climate change (Warren et al. 2001).

The glucosinolate concentration of drought-stressed *A. petiolata* was substantially lower than that of normally watered *A. petiolata* (Gutbrodt et al. 2011). When presented with drought stressed and well-watered plants, larvae of the specialist herbivore *Pieris brassicae*, preferred to feed on well-watered plants, while *Spodoptera littoralis*, a generalist herbivore, had a preference for drought stressed plants (Gutbrodt et al. 2011). Contrary to its feeding preference, specialist *P. brassicae* developed faster on drought stressed plants (Gutbrodt et al. 2011).

Disease

Disease is a biotic stressor which can lead to tradeoffs with defense. Under the univariate tradeoff model, assuming different mechanisms are utilized for defense against pathogens and herbivores, while defense is focused on combating threats from pathogens resources able to be allocated to defenses against herbivory would be lowered. These lowered defenses could then facilitate better larval performance on the host plant. For example, *Spodoptera exigua*, the beet armyworm, larvae have faster development rates when feeding on cotton plants infected with the fungus, *Chartomium globosum* (Zhou et al. 2016). Although colonization by the fungus had no significant influence on larval weight, *C. globosum* colonization negatively affect the fecundity of *Aphis gossypii* (cotton aphids) and *S. exigua* (Zhou et al. 2016). The varied responses of herbivores, both between species and between stages of development, to the presence of pathogens serves to illustrate that further research needs to be done to identify how pathogen presence will affect herbivore feeding.

The obligate biotrophic oomycete pathogen family Albuginaceae (white blister rust) typically infects Brassicaceae species (Ploch et al. 2010). *Albugo candida* is a generalist parasitizing a broad range of Brassiceae, including *C. diphylla* (Choi et al. 2009), while *Albugo hesleri* specializes on *C. diphylla* alone (Ploch et al. 2010). This white blister rust has been observed infecting

Cardamine species in the field. *Erysiphe cruciferarum*, a causal agent of powdery mildew disease for Brassicaceae plants, has been seen to infect *A. petiolata* in Southwest Ohio, largely centered in Montgomery and Greene Counties (Ciola and Cipollini 2011). The non-native *A. petiolata* has also been observed in the field to be infected with *Xanthomonas campestris*, black rot, a bacterium which infests crucifer crops worldwide (Cornell 1994). With novel species introduction, the interaction between non-native species, pathogens and the herbivores which utilize them is also of interest given that disease presence may allow for better use of the novel host.

Objective and Hypothesis

The objective of this study is to observe the divergence in generalist and specialist response to invasion by *A. petiolata* and how environmental stressors including disease and drought influence these interactions. To this end, we observed the oviposition preference of the specialist *Anthocharis midea*, in the field for its native host, *Cardamine concatenata*, cutleaf toothwort, in relation to *A. petiolata*. Field observations of *P. virginiensis* oviposition on *C. diphylla* and *A. petiolata* were also observed while collecting specimens for laboratory testing to confirm the continuation of previously established oviposition preference for *A. petiolata* established by Davis (2014). The mother knows best theory states that mothers will select a host that is best for the fitness of their offspring (Davis et al.

2015). Due to this, I hypothesize that *Anthocharis midea* will preferentially oviposit on native host types when present to maximize the fitness of their offspring.

To observe the impact that *A. petiolata*, drought stress and disease have on larval performance; no choice bioassays were conducted. Larvae were provided with either the native host or invasive host from one of three levels of drought stress and, when possible, diseased host plants, and performance on that host was measured. To investigate larval preference, bioassays have been conducted presenting larva with a choice between the invasive and native host and choices of drought stress levels within the one host species. I hypothesize that both 'specialist' species, *P. virginensis* and *A. midea*, will prefer and perform better on native Brassicaceae species. I hypothesize that the generalist *Trichoplusia ni*, cabbage looper, will not have a preference and will perform equally well on both the native Brassicaceae species and the invasive *A. petiolata*. I hypothesize that if *A. midea* is a sequestering herbivore, the performance will be best when the plant is at moderate stress levels, so these herbivores should also show a preference for moderately stressed plants. *Anthocharis midea* and *P. virginensis* will have better survival when feeding on moderately stressed *A. petiolata*. This also suggests that these 'specialist' herbivores perform better when feeding on hosts with disease. I hypothesize that the generalist *T. ni* will perform better on invasive *A. petiolata* than the

specialists. This generalist will be able to utilize the invasive host but will perform best when hosts are greatly stressed and producing low levels of defenses from severe drought stress or disease.

METHODS

Oviposition Preferences in the Field

Adult oviposition preference was examined at Scioto Trail State Park in Chillicothe Ohio on two dates April 14, 2016 and April 21, 2016. To examine oviposition preferences of *A. midea*, a paired plot survey was conducted. Plots were identified by driving the park trails looking for adjacent road side areas where both *C. concatenata* and *A. petiolata* grew side by side. Suitable plots were ten meter stretches containing an average of twenty plants of each host type. All *A. petiolata* and a similar number of *C. concatenata* in the patch were counted recording the total number of each host, number of plants of each host with eggs, and number of each host with multiple eggs. All eggs laid on *A. petiolata* were collected for laboratory use. Eggs were moved from *A. petiolata* to *C. concatenata* and stored in tupper ware containers and kept shaded and in a cooler. These were stored until larvae were large enough to transfer into the bioassay set up.

All statistical analyses were completed using R Studio and the package KMSurv and survival were utilized. For *P. virginensis* general trends in field oviposition were recorded. To statistically analyze oviposition of *A. midea* mean number of oviposition events were calculated for each host, *C. diphylla* and *A. petiolata*. I used a t-test to assess if the mean number of eggs per plot differed

between the two host plants. In order to analyze the interaction of survey date and host type on egg number an ANOVA was conducted. The influence of host type and date on the occurrence of multiple oviposition events on a singular host was also analyzed using an ANOVA.

An oviposition preference index (OPI) was estimated from the paired plot comparisons.

$$\frac{\sum \text{Oviposition Event on Native} - \sum \text{Oviposition Events on Invasive}}{\sum \text{Total Oviposition Events in Plot}}$$

A positive value indicates a preference for native host while a negative value indicates a preference for the novel host. OPI was analyzed by conducting a one-sample t-test; in a system without preference the expected calculated index would be zero, the t-test will establish if the observed value is significantly different from the expected zero value.

Egg Collection

Anthocharis midea

Anthocharis midea eggs were collected from Scioto Trail State Park in Chillicothe, Ohio where this species is abundant. Eggs were collected on two dates April 14, 2016 from *C. concatenata* and April 21, 2016 from *A. petiolata*. Park trails were driven while individuals watched out of the window for cut leaf

toothwort and garlic mustard patches. Once an area was spotted with suitable hosts car was parked at a suitable location and host plants in the area were searched for *A. midea* eggs. For eggs placed on *C. concatenata* only the area of egg placement was collected to prevent great impacts to the native flora. These were placed in tupperware containers with a moist paper towel and kept shaded in a grocery bag. For transport, containers were kept in a cooler.

On April 21, 2016 the same search and collection technique was used for *A. midea* eggs. As eggs appeared to be laid more prevalently on *A. petiolata* at this time, these non-native hosts were the main focus of search and subsequent collection of eggs on this date.

Pieris virginiensis

On April 30, 2016 Roaring Run Recreational Area, Apollo, PA inspected by Mr. and Mrs. Cipollini to identify if *P. virginiensis* were flying. *Pieris virginiensis* eggs were collected from this location on May 4, 2016. Eggs were collected by walking the gravel bike path through the park. *Alliaria petiolata* were destructively searched meaning they were pulled and searched for eggs. Native *C. diphylla* were searched along the riparian zone of Roaring Run, where it grew naturally. As *C. diphylla* grows predominately in areas close to water, paired comparisons of oviposition preference between the two patches was not undertaken. Davis and Cipollini (2014) previously established an oviposition preference of this

butterfly for *A. petiolata* over its native host. Adults were netted and identified in the field and released.

Plant Collection

Cardamine concatenata

Cardamine concatenata and *A. petiolata* utilized in bioassays were collected from the Wright State Woods. Potting soil was mixed with water in the green house in order to hydrate the soil. Moist soil was placed into the cells of potting trays. A bucket of moist soil was also made to take to the field. Trays, the soil bucket and trowels were taken into the field to facilitate transplanting plant specimens as soon as they were collected. Once transplanted, they were transported back to the greenhouse. Once back in the greenhouse all plants were watered with DI water using a watering can to help ensure the plants transplanted well.

Only flowering *C. concatenate* plants were collected as these are primarily utilized as the host plant. These were carefully removed as the bulbs easily separated from the stem of the plant. The trowel was inserted low into the ground and dug in a circle around the base of the plant. Then it was used as a lever to prop up a chunk of clay or dirt which contained the bulb. This was slowly removed by hand in order to prevent the detachment of the bulb from the plant

stem. These plants were very delicate so removal failed in about a third of the removal attempts. Successfully removed *C. concatenata* were placed into cells filled three fourths of the way with soil and once the bulb was in place soil was added up to the area of the stem which was purple and green. Six trays of thirty-two 300mL cells each were collected.

Alliaria petiolata

For *A. petiolata* a similar method of collection was used. Second year *A. petiolata* only were collected as these are primarily used as host plants. As these were not as delicate as *C. concatenata*, removal was done by digging a circle around the root, again using the trowel as a lever and lifting the root out of the ground. The plant was then shook to remove any soil. As these roots are larger than the bulbs of *C. concatenata*, trays with sixteen 500mL cells were used. Cells were filled about halfway with soil before adding the *A. petiolata* and once the *A. petiolata* was placed in the cell soil was filled in to cover the root of the plant. Six trays were collected. As *A. petiolata* persists through the summer months, more were harvested as needed in the same manner, but these were not used until acclimated to their drought condition.

Cardamine diphylla

Cardamine diphylla plants were collected carefully by hand from Roaring Run. These were collected by gently removing the top layer of leaf litter and soil with a trowel and then gently lifting the top inch of soil containing the rhizomes up. The rhizomes were removed and the plants were held delicately by the rhizomes in order to organize the plants. Healthy *C. diphylla* and *C. diphylla* infested with *Albugo hesleri*, white blister rust (Ploch et al. 2010), were removed for study. Two potting containers were created to transport the diseased and healthy two leaf toothwort. These containers were made from a standard non-scented garbage bag filled with a few inches of moist dirt. The top of the bag was rolled down in order to form a pot. Rhizomes were placed in the moist soil. For transportation the bags could be unrolled and carried by the draw strings of the garbage bag. Bags could be rolled back down to serve as pots once transported. Water was added to the soil in the bag once it was safely moved.

Plants were transplanted one plant per cell into four trays containing twenty four 500mL cells. Compacted potting soil was hydrated with water and then placed into the cells. The soil was filled leaving about five centimeters of space to the top of the cell. Rhizomes containing multiple plants were separated. Rhizomes were broken apart by hand in order to separate the plants and rhizomes were also broken into fragments about an inch long in order to allow for

easy planting. Excess rhizomes were kept moist as multiple plants grow from one rhizome with the hopes of growing new plant material. Rhizomes were then placed in the soil and covered by a shallow layer of top soil. These plants were watered daily. If the tray was dry they were given a liter of water and if the tray was still damp then they were given 500 mL of water.

A secondary plant collection was needed as many of the *C. diphylla* were damaged in transport. For this, *C. diphylla* were collected in the same manner in Hocking Hills, Ohio. These were collected and placed into a tray for transport. These were planted in the same manner in the greenhouse at Wright State University. All plants collected from this location were free of *A. hesleri*.

Drought Treatment

Drought stress was imposed on all collected plant types at three different levels, normal, moderate and severe. All watering was done from the underside of the plants by lifting the cells and placing water in the trays using graduated cylinders. When the trays were dry plants were given 1 L of DI water. When trays still held residual moisture plants were given 500mL of DI water. This was done to ensure that the plants were not over watered and thus damaged in any way. For normally watered plants these were watered daily. For moderately watered plants, these were given water when half of the plants in the tray appeared to be

wilting. For severely stressed plants, these were watered only when three fourths of the trays' plants were wilting.

Larval Bioassay Conditions

All bioassays were set up in the same general format. A petri dish was used as an enclosed testing area. Each dish contained a half a piece of Kim-wipe folded into a small square and saturated with DI water. This was done in order to keep the environment inside of the petri dish humid to prolong leaf quality. Leaves were placed in the petri dish. Larva were transferred from the containers in which they were collected as eggs and allowed to feed on until they were large enough for transfer (about one week). To transfer larva from one container to another, a small paint brush was used. Larvae were allowed to crawl next to the brush and then gently lifted and moved from one container to another. With all trials, leaves were kept as close to the same volume between each container as possible. To do this, leaves of similar sizes were chosen for feeding or if flowering material was used, the same volume of buds were used. The amount of material provided to the larva was also adjusted to the instar of the larva. As the larva developed they required a greater volume of food so the volume was increased to reflect the larval needs; all increased volumes were kept as similar as possible. Larvae were weighed every Monday, Wednesday and Friday. They were transferred using a paint brush, placed in a small weigh dish and weighed

using a balance. Larva were returned using a paint brush and placed on their food source within the dish or, if in a choice trial, placed in the center of the dish. All dishes were sealed with parafilm to preserve the conditions within the dish and in order to prevent loss of larvae in case of an accident.

Larval Performance in No Choice Bioassays

Drought Stress

Anthocharis midea

To test the effects of drought stress on *A. midea* larval development larvae were enclosed with leaves of the appropriate stress treatment in petri dishes as described above. Each individual larva was subjected to one treatment type, for example one larva was placed in a petri dish with material from moderately stressed garlic mustard. The different treatments provided for *A. midea* larvae were as follows: normal *C. concatenata* (n= 8), normal *A. petiolata* (n= 7), moderate *C. concatenata* (n= 8), moderate *A. petiolata* (n=9), severe *C. concatenata* (n=8) and severe *A. petiolata* (n=9).

Pieris virginiensis

Bioassays were also conducted to examine the effect of drought stress in an invasive host on larval performance. No choice assays were conducted offering normal *C. diphylla* (n= 7), normal *A. petiolata* (n=5) and severely

stressed *A. petiolata* (n=7) to *P. virginiensis* larvae. Larval mass was recorded in the same manner as in *A. midea* bioassays, every Monday, Wednesday and Friday until all larvae pupated or died. Days to death and days to pupation were recorded for each assay as well.

Statistical Analysis

Performance of larvae of both species was analyzed and displayed using the package *survival* through Kaplan-Meier curves. Curves were constructed to display the survival probability to pupation through the span of the experiment and how weight changed through the course of the experiment. One-way anovas were used to analyze how days to pupation, days to death, and larval mass were influenced by drought stress level of hosts. Impacts of drought on survival of both *A. midea* and *P. virginiensis* are also examined using Kaplan-Meier curves.

Impacts of Disease on Larval Performance

To measure the effects of disease on *P. virginiensis* larval performance, no choice bioassays were performed. Five days after hatching, when larva were large enough to be transferred into the bioassay setup, larvae were provided leaves of healthy native *C. diphylla* (n=7) and native *C. diphylla* with disease (n=7). Larvae were allowed to feed for 15 days from May 10, 2016 to May 25, 2016. To analyze the effects of disease on *P. virginiensis* larval performance,

average growth rates on healthy and diseased hosts were calculated. This was calculated for each larva over each feeding period by taking the change in weight divided by the number of days feeding. Average growth rates were compared using a t test.

Host Transfer

To observe how host transfer impacts larval performance larvae of both *A. midea* and *P. virginiensis* were first allowed to feed from the native host and roughly halfway through development transferred to the non-native *A. petiolata*. For *A. midea*, larvae were allowed to feed from *C. concatenata* for ten days and then were transferred to *A. petiolata*. Every Monday, Wednesday and Friday larval mass, days to death and days to pupation were recorded.

To observe the effect transfer has on *P. virginiensis* performance, seven larvae were randomly selected from the normal and disease performance test. These were switched after ten days of feeding on the native *C. diphylla* leaves to non-native *A. petiolata* leaves, from the collection done in Wright State Woods. The host larvae selected for feeding was noted. Every Monday, Wednesday and Friday larval mass, days to death and days to pupation were recorded.

Kaplan Meier Curves were constructed to observe larval performance over time. An ANOVA was conducted to test the influence transfer has by comparing

species to the event observed, the pupation or death of the larva, and the time of the event.

Larval Preference Bioassays

Three different choice tests were conducted to identify feeding preference of the *A. midea* larva. Larvae were again enclosed within the petri dish and presented with two different food types. To test the preference between native and non-native hosts, the larva in the petri dish was presented with a native plant, *C. concatenata*, and a non-native plant, *A. petiolata*. Feeding materials were placed on either side of the dish and each time a larva was introduced to the dish it was placed in the center. Larvae were weighed every Monday, Wednesday and Friday and at these times the food sources were replenished. Food source preference was also noted every time the food was replaced and the larvae were measured. To test the preference between food sources of different drought stress levels, larvae were enclosed with severely stressed and normally watered material of each host type. Choice tests were run between non-native food sources with different drought stress levels, normal and severely drought stressed *A. petiolata* (n= 4), and between native food sources with different drought stress levels, normal and severely stressed *C. concatenata* (n=4). In order to identify the preference between the native and non-native

feeding, larvae were presented with normally watered *A. petiolata* and *C. concatenata* (n=6).

Trichoplusia ni

As these insects were studied in the off season for native growth, and previous work demonstrated that these species are able to feed from *A. petiolata* to some extent (unpublished data), only *A. petiolata* was grown for testing. *Alliaria petiolata* seeds were collected from plants and stratified to induce germination. Seeds were placed on coffee filters inside of petri dishes; these filters were kept wet providing moisture to the seeds. Petri dishes were sealed with parafilm to preserve moisture loss and ensure that the seeds were kept inside the dishes in case of an accident. Dishes were stored at 4°C in a refrigerator and continually given water, simulating winter, until the start of germination was seen. Once seeds began to germinate they were moved to room temperature on the laboratory bench, and provided water as needed, until the seedlings emerged. These were then transplanted into trays containing 32 300 mL cells, three seedlings to each cell. Cells were filled with soil three holes were placed into the cell so the long root could be placed into the hole and then filled with soil. These seedlings were watered daily. Once seedlings of *A. petiolata* reached the four leaf stage, trays were watered from below, being given 1L of water when soil was dry and 500mL when soil was moist. When these plants

were well established, having at least four fully expanded leaves, drought stress was applied as described in the previous experiments. All *A. petiolata* planted were fertilized at the end of every week. *Tricholpusia ni* eggs were ordered from Benzon Research, Carlisle, PA.

Bioassays were conducted in order to test if the impacts of drought stress, and preference between drought stressed and normally watered *A. petiolata*. Eggs were placed on normally watered leaves of *A. petiolata* and larvae were allowed to feed on the normal *A. petiolata* until they were past the first larval instar and were large enough to transfer. Once at this stage they were transferred to the experiments. Larvae were weighed three times weekly and days to death and days to pupation were noted. Number of deaths both on and off of the provided normally watered *A. petiolata* leaf was recorded for those larvae which were not large enough to measure. Seven were raised on normally watered, moderately drought stressed and severely drought stressed *A. petiolata* each. To analyze and display the data Kaplan Meier Survival Curves were constructed and an ANOVA was used to compare drought treatment, event (pupation or death of larva) and the time to the event.

RESULTS

Oviposition Preferences in the Field

Anthocharis midea oviposition was seen to display a clear trend. Earlier in the season *A. midea* displayed a preference for the native *C. diphylla* while later in the season the preference shifted toward the non-native *A. petiolata* (Figure 1). On the first survey date, most oviposition events occurred on the native host, *C. diphylla*, while on the second survey date most oviposition events occurred on novel, *A. petiolata* (Figure 2B). On average over the entire reproductive season the invasive *A. petiolata* received more frequent oviposition events than the native *C. concatenata* (Figure 2A). No significance differences in preference were seen when preference index was compared among hosts across the entire season ($t = -0.917$, $p = 0.369$) or between means of oviposition events on the host type ($t = 1.719$, $p = 0.336$); however, a significant interaction of both selected host ($F = 0.028$, $p = 0.003$) and date of oviposition ($F = 5.191$, $p = 0.0278$) on preference index was seen.

Anthocharis midea lays eggs singly and these eggs are adapted to develop a bright red orange coloration to indicate that the host is occupied.. Multiple eggs were seen on both native and invasive host types, though more frequently on *A. petiolata*. During paired plot comparisons multiple events were only observed on *A. petiolata*. Multiple events seen on native hosts were often in

locations on the plant far removed from each other. Multiple events seen on the invasive host were observed at distal locations, as well as located on the same floral structure, or most often, the leaf of the plant. Multiple oviposition events were seen to be significantly influenced by host type ($F= 4.442$, $p=0.041$) but not by date ($F= 2.555$, $p=0.117$).

At Roaring Run, only one *P. virginiensis* egg was found on a native *C. diphylla* ($n=40$) while roughly 35 were found on *A. petiolata* ($n=70$).

Larval Performance in No Choice Bioassays

Drought Stress

Anthocharis midea

For those larva fed native hosts under the three levels of drought stress there seemed to be little impact on the development of the larvae. Moderately stressed native hosts seem to have the largest negative impact on larval development as these were seen to have the lowest larval masses at pupation. However probability of survival remained high in all levels of drought stress within the native host type (Figure 5A).

For larvae being fed on varying levels of drought stressed *A. petiolata* feeding was much lower than that observed on *C. concatenata* at any drought stress level (Figure 4A&B). Drought level of the *A. petiolata* produced a shift in

the duration of survival of the larvae but mortality was still high across all treatments (Figure 4B). Larvae were unable to survive feeding on any level of drought stressed *A. petiolata*, and no significant impact of drought stress level on survival time was seen ($F= 0.414$, $p= 0.663$). Larvae feeding on the normally watered *A. petiolata*, were seen to steadily decline in probability of survival, and did not survive past five days of feeding. Drought stress was seen to increase the probability of survival over time but not enough to allow survival to pupation on this host plant. Moderately stressed *A. petiolata* extended the survival of larvae until ten days in development but larvae did not survive past this to pupation. Larvae feeding from severely stressed *A. petiolata* did not feed as long as those feeding from moderately stressed *A. petiolata*. These larvae feeding from severely stressed *A. petiolata* were seen to survive to seven days until death. A significant influence of host species was seen on the duration of time spent feeding ($F= 65.941$, $p=3.2e-10$), while the drought treatment had no significant effect ($F= 0.414$, $p=0.663$). There was also a significant impact of duration of feeding and the event, pupation or death ($F= 9.713$, $p=0.003$).

As expected with the trend seen in survival, larval mass was greater on the native host type than on the novel host type (Figure 5). Host type had a significant impact on larval mass ($F= 236.829$, $p=2e-16$). However, drought stress did not significantly impact larval mass ($F= 0.816$, $p=0.449$).

Pieris virginiensis

After three days of feeding, all *P. virginiensis* placed on hosts of both *C. diphylla* and *A. petiolata* were seen to have a negative trend in probability of survival over time (Figure 6), and the effect of drought treatment was not significant ($F= 0.253$, $p=0.623$). Normally watered native host allowed for the survival of larvae to pupation, but again with a declining probability of survival as time went on. Most larvae on normally and severely drought stressed *A. petiolata* did not survive past 6 days. One larva survived until 13 days on severely stressed *A. petiolata*. A significant influence of host type, native *C. diphylla* or non-native *A. petiolata*, was seen on the duration of time feeding ($F= 20.179$, $p=0.0005$). There was no significant influence of treatment or event, pupation or death, on the duration of feeding ($F=0.253$ $p=0.623$, $F= 0.117$ $p=0.738$).

As *P. virginiensis* larvae were unable to survive to pupation feeding from any level of drought stressed *A. petiolata* it is not surprising that larva feeding from *C. diphylla* reached much greater larval weights before pupation (Figure 7). However larvae feeding on the severely drought stressed *A. petiolata* on average had a larger larval weight than those feeding from the other levels of drought stressed *A. petiolata* (Figure 7). A significant influence of host was seen on the

mass of the larvae ($F= 182.203$ $p<0.0001$) but no significant influence was seen of the drought treatment on the mass of the larva ($F= 1.496$ $p=0.24$).

Impacts of Disease on Larval Performance

When *P. virginiensis* fed on healthy *C. diphylla* they experienced an average growth rate of 0.027g/day ($n=7$, $df=0.013$). When *P. virginiensis* fed on diseased *C. diphylla* they experienced an average growth rate of 0.0097g/day ($n=7$, $df=0.004$). For *P. virginiensis* fed on normal *C. diphylla* average mass achieved was 0.137829g ($n= 7$, $df= 0.045$) while those larvae fed on diseased *C. diphylla* had an average mass of 0.0508g ($n=7$, $df= 0.021$). Disease presence on the host plant had a significant effect on the growth and mass of larva ($t= -3.33$ $p= 0.013$, $t= -4.66$ $p= 0.0014$).

Host Transfer

For larvae of *A. midea* transferred from their native *C. concatenata* to the non-native *A. petiolata* 10 days into development, survival quickly declined (Figure 3). All but one of these larvae died quickly and were unable to complete development to pupation once transferred. The one which survived to pupation fed minimally and began pupation shortly after transfer. For larvae of *P. virginiensis* transferred from native *C. diphylla* to non-native *A. petiolata*, probability of survival only slightly decreased once transferred from the native

host type to the invasive *A. petiolata*. Once presented with the novel host type six out of nine of the larvae minimally fed, under 5% of leaf area was consumed, and then they began pupating. Only one larva transferred to *A. petiolata* fed and had died upon the next observation. These interactions displayed a strong impact of the species on the survival to pupation of the larva when transferred from the native host to invasive host (n=9 (*A. midea*), 7 (*P. virginiensis*)). The species undergoing transfer did not have an influence of the event observed, the event being the pupation or death of the larva (F= 1.066 p=0.321). The transfer did have a significant influence on the event, death or pupation of the larvae (F= 5.128 p=0.04128).

Larval Preference Bioassays

Anthocharis midea larvae clearly showed a preference for the native host *C. concatenata* over the non-native *A. petiolata* (Table 1). *Anthocharis midea* larvae offered a choice of severely stressed or normally stressed *C. concatenata* showed a preference for normally watered material (Table 1). When provided severely drought stressed and normally watered *A. petiolata*, larvae displayed no feeding preference. All feeding from *A. petiolata* was at a lower volume than that on the native *C. concatenata*.

Trichoplusia ni

No *T. ni* feeding from any *A. petiolata* was able to survive to pupation. For larva fed on normally watered *A. petiolata* the average days to death was 6.33 days (n=3, df =3.786). There were 24 deaths of larva too small for measurements on *A. petiolata*. These either fed or died or did not feed and travelled off the leaf and thereby died. For larva fed on moderately drought stressed *A. petiolata* the average days to death was 12 days (n=4, df= 2.0). For severely stressed *A. petiolata* fed larvae the average days to death was 7.4 days (n=5, df= 4.775). Feeding from moderately drought stressed *A. petiolata* tended to be more beneficial than feeding from severely stressed *A. petiolata* (Figure 8). However, no significant impact of drought treatment was seen on the duration of feeding (F= 2.348 p=0.151).

Drought stress had a significant impact on the mass of larvae (F= 8.573 p= 0.0084). For larvae feeding on the normally watered *A. petiolata* the average mass was 0.000267g (n=3, df= 0.0002) which was much less than the mass which was able to be obtained by feeding on drought stressed *A. petiolata*. For larva feeding from moderately stressed *A. petiolata* they were able to obtain a larval weight of 0.010625g (n=4, df=0.0065) while those feeding from severe obtained a mass of 0.00142g (n=5, df= 0.0012)(Figure 9).

DISCUSSION

Novel species have been an increasing cause for concern in ecology as with increased continental travel invasion has become more frequent. In systems where the co-evolution of plants and herbivores has resulted in a natural 'balance' when the novel species is introduced there can be negative consequences (Pimentel 2005; Davis 2014). This is the case with non-native *A. petiolata* that has been seen to have negative direct and indirect impacts on areas which it has invaded from alleopathic suppression of native seed germination to mismatch oviposition events of *P. virginensis*. The objective of this study was to further identify effects of this non-native on specialist and generalist herbivores as well as the impacts that environmental stress may have on these interactions. This study has (1) assessed the oviposition preference of *A. midea* between native and non-native hosts, (2) measured larval performance of *A. midea*, *P. virginensis* and *T. ni* under on native and non-native hosts under various drought levels, (3) assessed *A. midea* and *P. virginensis* larval preference between native and non-native hosts and for hosts under different levels of drought stress (4) tested the effect host transfer from native to non-native hosts, and (5) tested the impacts disease presence on host has on larval performance of *P. virginensis*.

Oviposition Preferences in the Field

Observations made in the field of *P. virginiensis* oviposition support previous findings that *P. virginiensis* has a preference for the novel *A. petiolata* over its native hosts (Davies and Cipollini 2014). For *A. midea* no significant preference between hosts was seen when looking at the preference index over the entire season. However, when taking into account date of the observation, significant influences of host identify on oviposition event were seen. Oviposition events on the novel *A. petiolata* should be considered mismatch events as the chosen host does not benefit the larva and in fact leads to the death of any offspring placed on it.

When butterflies are identifying a potential host they undergo a number of behaviors to identify the suitability of the host. The sequence consists of searching, orientation, encounter, landing, surface evaluation and then finally acceptance (Renwick and Chew 1994). During the searching phase, the cues for suitable host are visual, extending from the shape and color of the plant to the apparency of the host (Renwick and Chew 1994). On the dates that the areas were surveyed there were distinct differences in appearance of the host plants. On the first survey date, the native host, *C. concatenata* was in bloom and receiving the majority of oviposition events while the novel host was not yet blooming and received few oviposition events. On the second survey date, *A.*

petiolata was in bloom and received the majority of oviposition events while the majority of *C. concatenata* were past the blooming stage and received few oviposition events on this date. As *A. petiolata* and *C. concatenata* are in the same family, floral structures of these two plants are similar and both species produce glucosinolates; these floral structures could serve as a visual cue for suitable host and similar chemosensory profiles could lead to the mismatch oviposition events. For *P. virginiensis*, the novel host *C. diphylla* has floral parts similar to *A. petiolata* and the leaflet structure is also similar. *Cardamine diphylla* has two trifoliate leaves emerging from the stem while *A. petiolata* has multiple larger heart shaped leaves emerging from the stem. Visually the appearance of second year *A. petiolata* may serve as a supernormal stimulus resulting in the preferential selection of the host.

As these hosts flower at different times, *A. midea* could be cueing in on this unsuitable host through these visual cues to the demise of the offspring. Influences of the date of these events could have repercussions for populations of *A. midea* in Southern Ohio. Host plant associations play a profound role in the evolution of butterflies, shifts between chemically distinct plant group usage shift populations and drive evolution of different butterfly species (Fordyce 2010). Velzen et al. (2013) demonstrated that historically climate change resulting in temporal shifts promoted the diversification within the *Cymothoe* and *Harma* genera. Resulting host plant shifts from these temporal changes for *Cymothoe*

may have triggered differential diversification rates from the related *Harma* lineage (Velzen et al. 2013). For *A. midea*'s relative *P. oleracea*, where the population was exposed to *A. petiolata* over a longer duration of time, a preference for the novel *A. petiolata* as a host was seen with slight increase in the performance of the offspring. This response supports the idea that adaptation of both host preference and larval performance may be possible with regard to *A. petiolata* use (Keeler and Chew 2008).

For *A. midea* over time as mothers select different hosts preferentially at different periods during the mating season two outcomes may occur, the early emerging genotypes will be selected for as the offspring of later emerging and ovipositing adults will perish. If the population shifts to an earlier spring phenology to avoid *A. petiolata*, this could be detrimental to the species as this could result in consequences from environmental factors. As these are early spring emerging butterflies this may jeopardize the population if early emergence would result in the exposure of adults to late winter weather. If the temporal separation continues the population could over time diverge into two distinct races. This would be possible only if the larvae were to experience some adaptation that allowed them to utilize the *A. petiolata* as a host.

Another possibility for the frequent selection of *A. petiolata* is the greater apparency of this plant relative to the native *C. concatenata*. Second year *A.*

petiolata has a stalk which under good growing conditions can reach a height of 1m (Sabin et al. 2017). This is far taller than the 20 cm that the native *C. concatenata* averages. These height differences may allow for the *A. petiolata* to be more apparent. However, if this was the case I would expect that the preference for *A. petiolata* would be seen throughout the entire oviposition season of *A. midea*, which was not the case.

Host species also affected the frequency of multiple oviposition events for *A. midea*. Multiple events are not typically seen for this species (Clark 1932). The red egg syndrome that *A. midea* has developed, as many other Pierid species, is a mechanism that causes their eggs to change color from light yellow to a vibrant red-orange to signal to other searching adults that the host is already in use (Shapiro 1981). Few multiple events with lower numbers of eggs were observed on *C. concatenata* while many multiple events, with the eggs laid in higher numbers, were observed on *A. petiolata*. If the novel *A. petiolata* serves as a supernormal visual or chemical stimulus this could explain the deviation of the typical behavior, and indicates that many offspring can be negatively affected at once. The more frequent occurrence of multiple oviposition events at the later survey date could also be explained as a function of time during the reproductive season. At earlier dates females may be more 'choosy' about host selection as selecting the best host would be of the greatest benefit to larval survival. Towards the end of the reproductive season this 'choosy' behavior may not be as

advantageous as the benefit of ovipositing as many eggs as possible may outweigh that of selecting the most suitable host. At this later time it may confer greater fitness to oviposit the entirety of the fertilized eggs than to reserve them for the best hosts, this may result in these multiple oviposition events on a single host as the cost of the presence of competitors is outweighed by the benefit of ovipositing all fertilized eggs.

Larval Performance in No Choice Bioassays

Drought Stress

Under normal water conditions, specialist herbivores typically feed from a narrow range of plants (Fox and Morrow 1994). These specialists are well equipped to metabolize the secondary defenses of the plant or plants they eat from but are unable to utilize a wide array of plants (Fox and Morrow 1994). When the host is drought stressed however, plants should not be able to allocate as much resources to developing secondary defenses, lowering defensive chemical concentrations (Bazzaz et al. 1987; Agrawal 2007). For sequestering specialist herbivores, these benefit most, or have the highest performance, when plant defenses are intermediately induced; non-sequestering specialist are indifferent to all but high levels of induction of defenses which result in higher levels of larval mortality (Ali and Agrawal 2012). All larvae were able to reach pupation on their native hosts. For *A. midea*, severely drought stressed native *C.*

concatenata extended the duration of feeding before pupation, while moderately stressed *C. concatenata* lowered the survival probability. This interaction demonstrates that *A. midea* is a non-sequestering specialist as the larvae benefit from what should be the lowest levels of induced defenses. With this information it would be expected that *A. midea* would perform best on *A. petiolata* when under severe drought stress. For *A. midea*, feeding from novel *A. petiolata* under drought conditions extended the duration of feeding, with moderately stressed *A. petiolata* facilitate the greatest increase in feeding duration. This does not follow the same trend seen in the native no choice feeding assays. However, even though the drought conditions improved the length of feeding possible on the novel host the shifts in secondary metabolites due to the drought stress, if they occurred, were not great enough to allow for the novel *A. petiolata* to be a suitable host to these larvae. For the specialist *P. virginiensis*, feeding from the severely drought stressed *A. petiolata* extended the duration of feeding, but again, did not allow them to reach pupation. This demonstrates that under drought conditions, though probability of survival is increased over a longer duration of time it is still not enough to allow for the release from the ecological sink of the novel *A. petiolata*. Drought conditions could potentially facilitate increased survival if drought occurs and increasing the length of feeding long enough to allow for the larva to reach a later stage of development at which they are mobile and could be able to reach a more suitable native host.

Generalist insects benefit most from suppressing induction of host defenses (Ali and Agrawal 2012). Thus, generalists should exhibit highest performance on severely stressed plant material which would have the lowest level of secondary metabolites. For *T. ni* in this study, however, larvae fed severely stressed and normally watered *A. petiolata* exhibited similar probabilities of survival over time, and moderately stressed *A. petiolata* rapidly decreased the probability of survival. The changes that drought stress created in the novel host however are not apparently enough to effectively lower the defenses and allow for the novel *A. petiolata* to be a suitable host for *T. ni* larvae. Previous research has demonstrated that *T. ni* are able to feed from *A. petiolata* at later stages in development after being reared on a suitable diet (unpublished data) to complete pupation. However, when larva feed on this novel plant early in development they are unable to process the material and perish. This illustrates that the novelty of *A. petiolata* leads to negative consequences for generalist herbivores as well as specialist herbivores.

Impacts of Disease on Larval Performance

Considering the univariate tradeoff model a plant should only be able to focus defensive resources on one area of defense at a time (Bazzaz et al., 1987; Agrawal, 2007). If the host is defending against disease, the defensive response against herbivory should be reduced. So potentially under disease conditions

secondary metabolite defenses effective against insects may not be as strong allowing better herbivore utilization of the host. Plant-pathogen-herbivore interactions are complicated, the presence of *Albugo* and *Phyllotreta nemorum*, flea beetles, on *Barbarea vulgaris*, a wild crucifer, effected each other's performance (van Molken et al. 2014). Van Molken et al. (2014) show that when infested with *Albugo* and *P. nemorum*, glucosinolate concentrations of the host plant were increased. *Phyllotreta nemorum* enhanced the spread of the sporangia but not the success of infection and the herbivore had a higher consumption rate of the host caused either by lower food quality or palatability that forces greater larval feeding to gain the required nutrients. In the current study presence of disease on the native *C. diphylla* actually negatively impacted the growth of *P. virginensis*. This indicates that either the disease itself or defenses which are elicited by the plant to defend itself from the disease are also detrimental to *P. virginensis* larva feeding from it. Further study should be conducted in order to analyze secondary chemistry of both native and non-native hosts under stress from disease and the combination of pathogen-herbivore attack. *Alliaria petiolata* is susceptible to black rot, caused by the bacterium *Xanthomonas campestris*. The non-native *A. petiolata* has been observed exhibiting this fungal disease in the Ohio area, indicating that there is a high likelihood that *A. midea* will come into contact with disease affected hosts. Effects of disease on insect resistance of the novel host, *A. petiolata*, are yet to

be investigated. *Pieris virginiensis* response to feeding from native diseased host it appears that disease on this host could lead to even greater negative consequences for the larva. Potentially this could accelerate the death of *P. virginiensis* and the related *A. midea* placed on this novel host.

Host Transfer

For *P. virginiensis* transferred from the native to novel host there was a decline in survival probability. Most of the *P. virginiensis* larva selected to forego feeding and begin pupation. For *A. midea* after transfer there was a steady decline in survival probability resulting in the death of all but one of the nine larvae. The later instar does not seem to allow for greater tolerance of the novel *A. petiolata* by either specialist species. For *A. midea* where populations of the *C. concatenata* grow in close proximity to the novel *A. petiolata* this will be detrimental to the species as mobile larvae could potentially move to this unsuitable host and perish, which could reduce future populations of the species. For *P. virginiensis*, where the native *C. diphylla* grow in close proximity to the novel *A. petiolata*, transfer from the native to the novel host would occur and result in detriment to the population. Transfer at the fourth instar did not result in the death of the larvae, but larvae forewent feeding to begin pupation. If feeding at this final stage of development is forgone there could be energetic costs to the offspring that undergo this transfer. This lowered duration of feeding could

deprive the larvae of nutrients needed to reach higher masses as adults which could impact reproductive potential by making them poorer competitors or impacting the number of eggs an adult female could produce.

CONCLUSION

These findings illustrate that generalist and specialist herbivores feeding on novel *A. petiolata* are both unable to utilize this host. For specialist herbivores, *P. virginiensis* and *A. midea*, preferential oviposition on novel *A. petiolata* over native host plants is seen, at least during certain times of year for *A. midea*. These events have been established as mismatch events as the larva are unable to feed and reach pupation on this host. Laboratory experiments examining the oviposition preference of *A. midea* still need to be conducted. Further experimentation should be completed to identify if the compounds sinigrin and alliarinose are responsible for larval death of *A. midea* as in Davis et al. (2015). Environmental stressors including drought resulted in insufficient shifts in the defensive chemical profile to allow for the utilization of the novel host for either the specialist or generalists in the study. Native host utilization under disease conditions revealed that disease on the host has negative impacts on the herbivore which feed on it. Further investigation into disease presence on host, such as *X. campestris* on *A. petiolata*, and the effect which this has on the herbivores feeding from the host is needed. Transfer from the native host to the novel resulted in differential response between the two specialists tested. When transferred from the *C. diphylla* to the novel *A. petiolata*, *Pieris virginiensis* larva did not feed from the novel host and began pupation. This could have detrimental consequences to pupation and adults as the larva are not receiving the same

nutrition which they would if they completed their development on the native *C. diphylla*. Repercussions for adult success such as reduced size and fecundity could result and future studies should rear pupae through to adulthood to observe any possible consequences. For *A. midea* when transferred from native host to feeding on novel *A. petiolata* larva subsequently perished. Many questions remain regarding the effects of *A. petiolata* on *A. midea* and members of the Peiridae family.

FIGURES

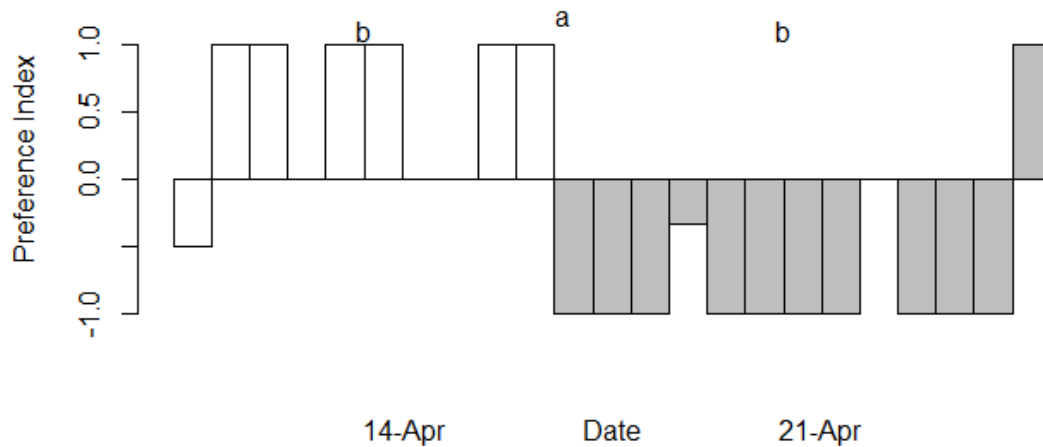


Figure 1: *Anthocharis midea* oviposition preference indexes of each plot for native and non-native hosts separated by date (white=14-Apr, grey= 21-Apr). A positive index indicates greater selection toward the native host, *C. diphylla*. A negative index indicates selection toward the non-native host, *A. petiolata*. If the proportion is zero no preference is expressed. Preference across the entire season was not significant (a) ($t = -0.917$ $p = 0.37$) but the preference seen on individual dates is significantly different than zero (b) ($t = 2.906$ $p = 0.017$, $t = -4.27$ $p = 0.001$).

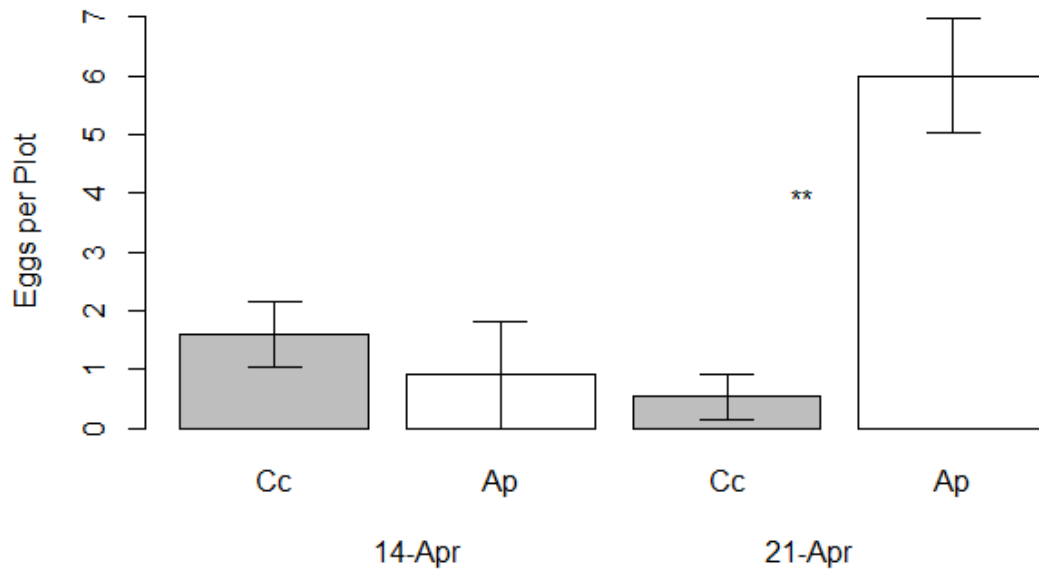


Figure 2: Average number of oviposition events by *A. midea* per plot on native *C. concatenata* (Cc, white) and non-native *A. petiolata* (Ap, grey) on two different dates. There was a significant difference on the 21-Apr between hosts selected ($F=10.028$, $p=0.003$).

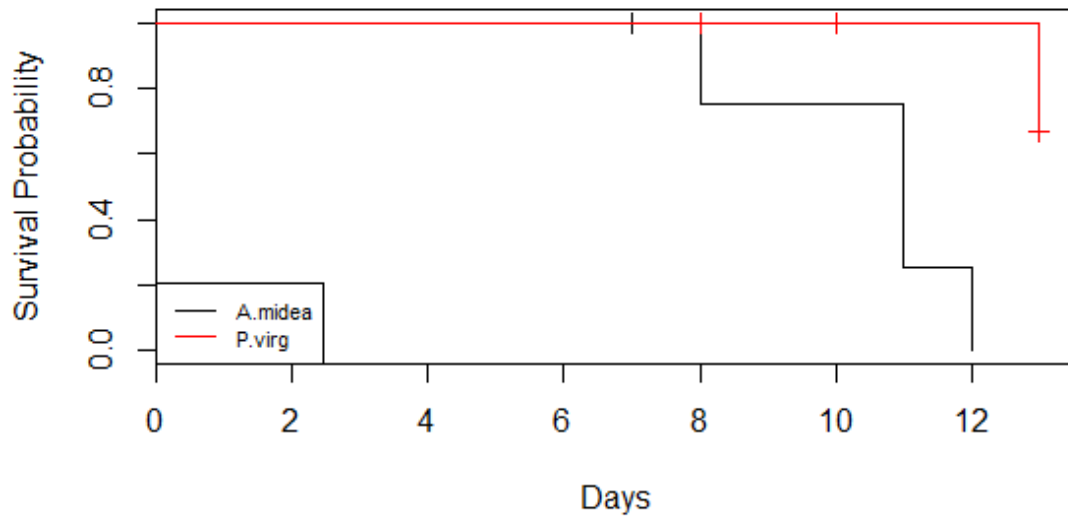
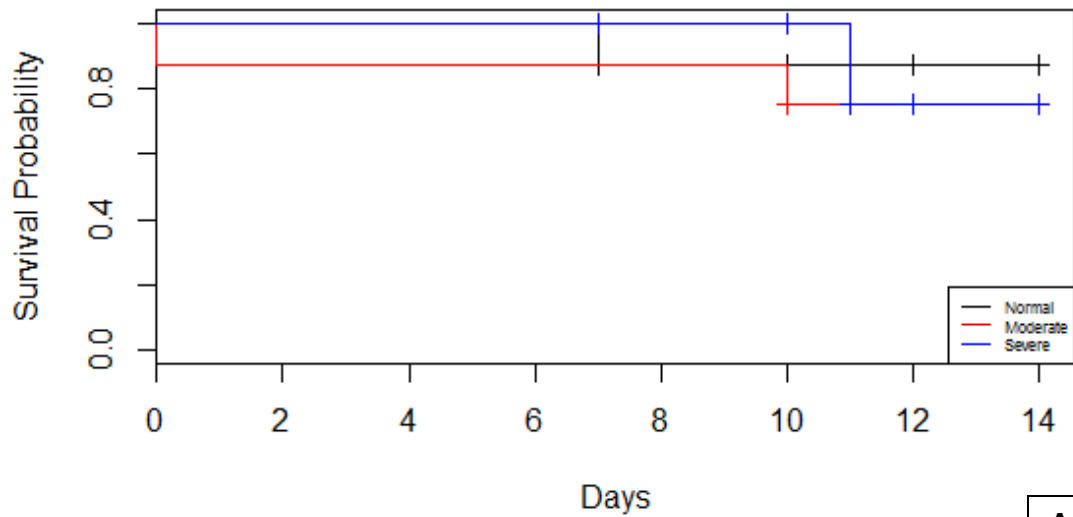
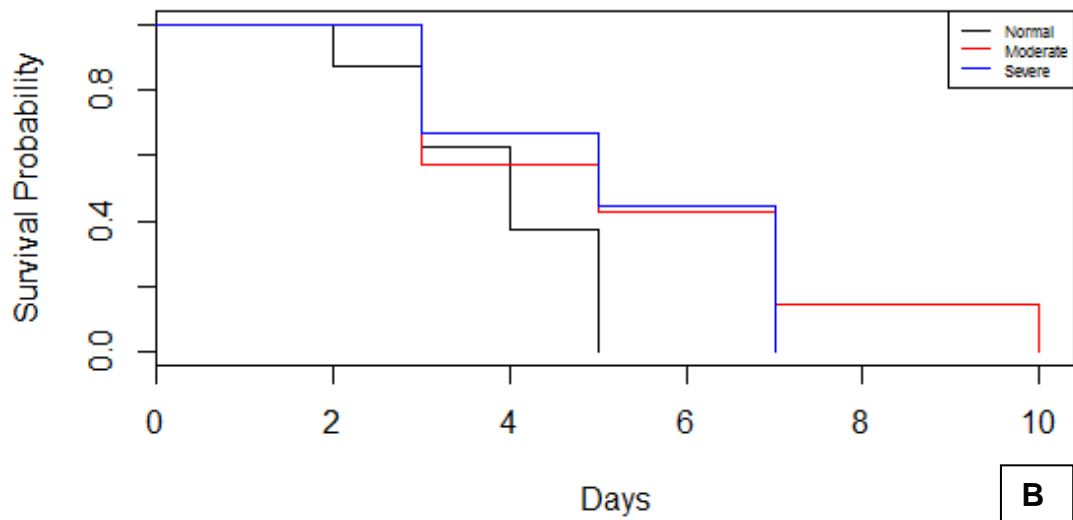


Figure 3: Survival curves of *A. midea* (n= 9) and *P. virginiensis* (n= 7) before and after transfer halfway through development from the native host to the invasive *A. petiolata*.



A



B

Figure 4: A. Survival curves of *A. midea* on a native host under three levels of drought stress, normal (black), moderate (red) and severe (blue) stress. B. Illustrates the probability of survival through development of *A. midea* on invasive host under three levels of drought stress, normal (black), moderate (red) and severe (blue) stress.

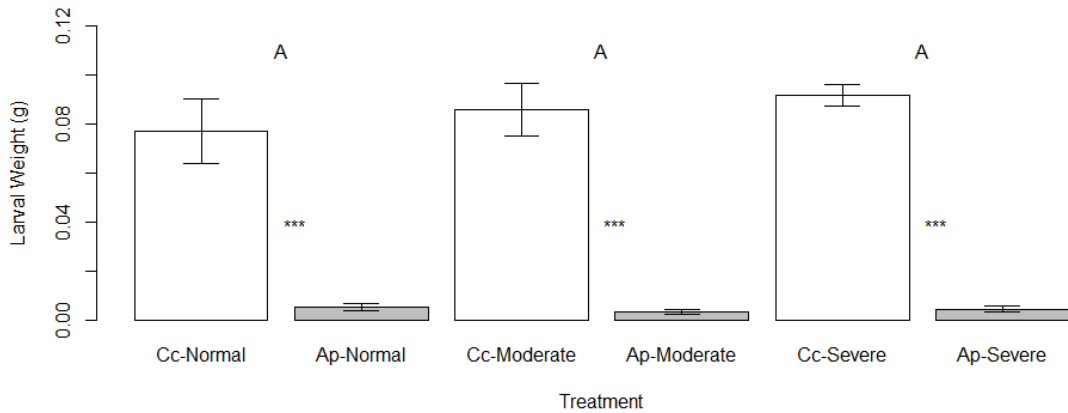


Figure 5: Average larval weight and standard error of *A. midea* after feeding on non-native *A. petiolata* (black) at three different levels of drought stress and native *C. concatenata* (white) across different levels of drought stress. A significant difference between host selected was seen ($F=236.829$ $p=2e-16$) but no significant difference between drought level was seen (A; $F=0.816$ $p= 0.449$).

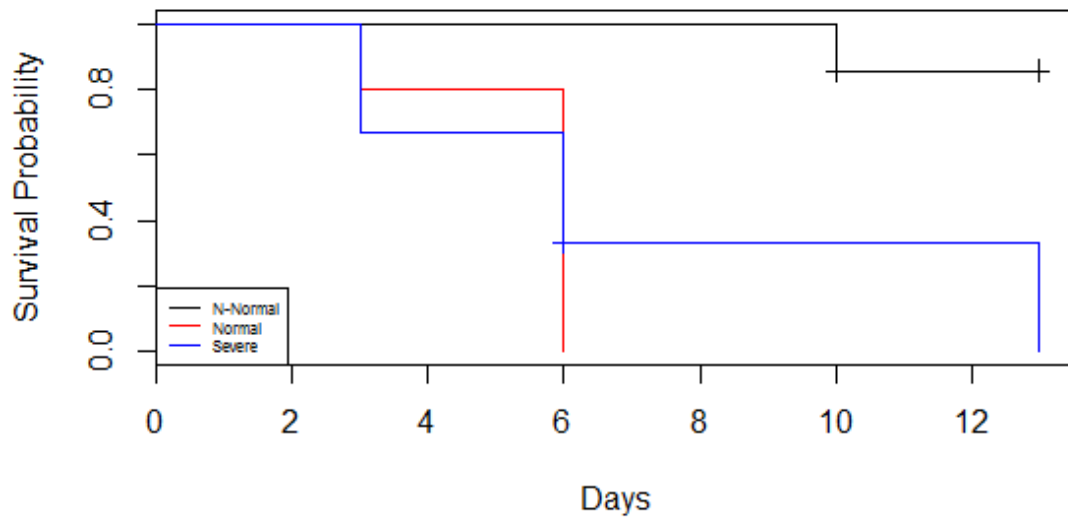


Figure 6: Survival curve of *P. virginiensis* larva feeding from normally watered native host (black), normally watered *A. petiolata* (red) and severely stressed *A. petiolata*.

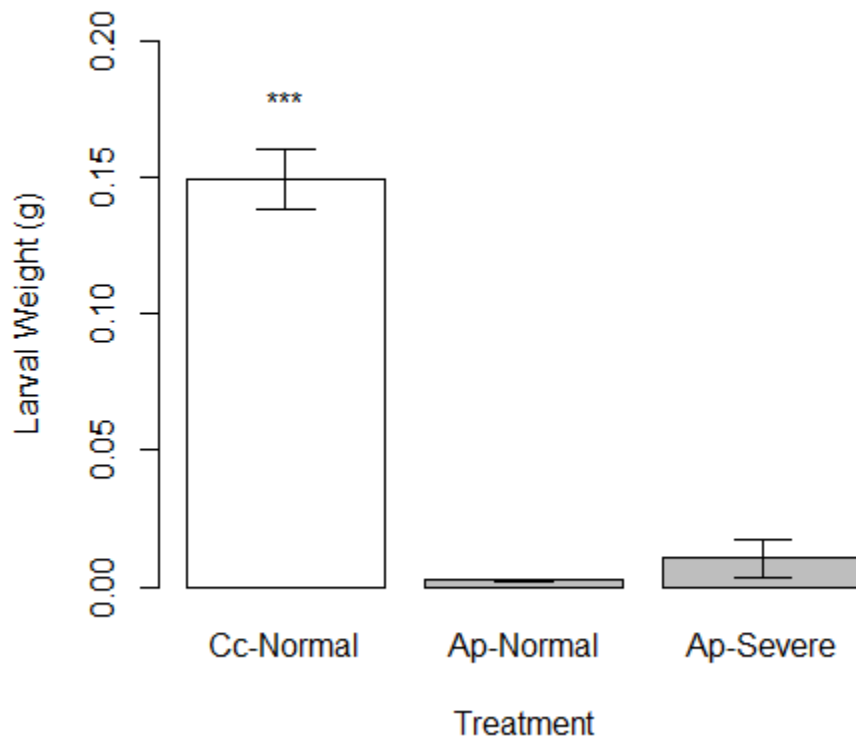


Figure 7: Average larval weight for *P. virginensis* feeding from native normally watered *C. diphylla* (white) and normal and severely drought stressed *A. petiolata* (black). There was a significant influence of the host type on larval weight ($F= 182.203$, $p=8.52e-10$).

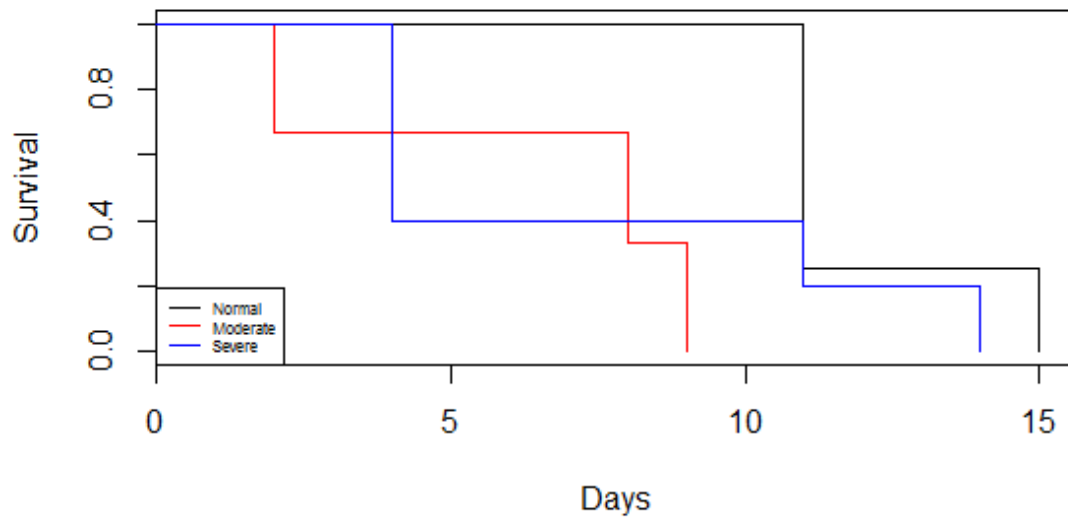


Figure 8: Probability of survival over time for generalist *T. ni* feeding on novel *A. petiolata* under different levels of drought stress, normal (black), moderate (red) and severe (blue).

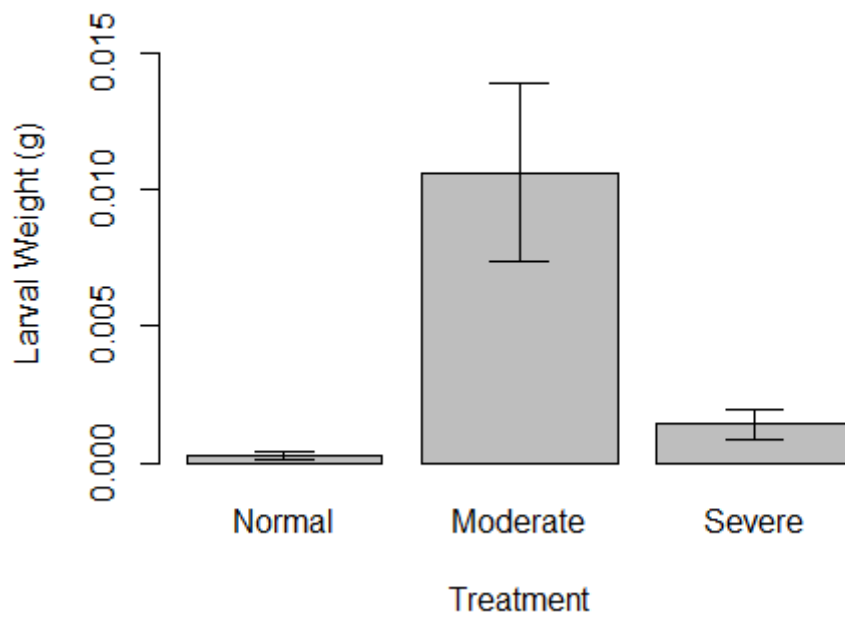


Figure 9: Average larval weights of *T. ni* seen from feeding on *A. petiolata* over varying drought stress levels, normally watered, moderately and severely drought stressed.

TABLES

Table 1: Results of larvae feeding preferences of *A. midea* and *T. ni*. The annotation 0 indicates no preference, a minus indicates a preference toward severely drought stressed host material over normally watered, a star indicates a preference toward the native or the normally watered feeding material. The number of times the symbol is repeated indicates the intensity of the response.

Species	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
<i>T. ni</i>							
	Severe vs. Normal <i>A. petiolata</i>	***	***	***	**	*	***
<i>A. midea</i>							
	Native vs Invasive	***	***	***	***	**	
	Native vs Invasive	***					
	Native vs Invasive	***	***	**			
	Native vs Invasive	***					
	Native vs Invasive	-					
	Severe vs. Normal <i>C. concatenata</i>	---	---	---	-	---	---
	Severe vs. Normal <i>C. concatenata</i>	---	---	---			
	Severe vs. Normal <i>C. concatenata</i>	---	---	---			
	Severe vs. Normal <i>C. concatenata</i>	---	---	---			
	Severe vs. Normal <i>A. petiolata</i>	0					
	Severe vs. Normal <i>A. petiolata</i>	-					
	Severe vs. Normal <i>A. petiolata</i>	0					
	Severe vs. Normal <i>A. petiolata</i>	-					

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