

Biogeography and phenology of oviposition preference and larval performance of *Pieris virginiensis* butterflies on native and invasive host plants

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Received: 10 February 2017 / Accepted: 14 August 2017
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Abstract In invaded environments, formerly reliable cues might no longer be associated with adaptive outcomes and organisms can become trapped by their evolved responses. The invasion of *Alliaria petiolata* (garlic mustard) into the native habitat of *Pieris virginiensis* (West Virginia White) is one such example. Female butterflies oviposit on the invasive plant because it is related to their preferred native host plant *Cardamine diphylla* (toothwort), but larvae are unable to complete development. We have studied the impact of the *A. petiolata* invasion on *P. virginiensis* butterflies in the Southeastern USA by comparing oviposition preference and larval survival on both plants in North Carolina (NC) populations without *A. petiolata* and West Virginia (WV) populations where *A. petiolata* is present. Larval survival to the 3rd instar was equally low in both populations when raised on *A. petiolata*. Mean oviposition preference on the two plants also did not differ between populations. However, we found a seasonal effect on preference between early and late season flights within WV populations. Late season females laid 99% of total eggs on *A. petiolata* while early season females utilized both host plants. Late season females were also less likely to lay eggs than early season females. This change in preference toward *A. petiolata* could be

driven by the early senescence of *C. diphylla* and suggests a seasonal component to the impact of *A. petiolata*. Therefore, the already short flight season of *P. virginiensis* could become further constrained in invaded populations.

Keywords West Virginia White · *Alliaria petiolata* · Evolutionary trap · Novel plant–insect interactions

Introduction

Invading species can lead to novel ecological communities in which existing biotic interactions are altered and new interactions are created (van der Putten et al. 2004). Specifically, the introduction of invasive plant species often changes environments by altering biogeochemical cycles, decreasing community diversity, and increasing competition with already established plants for nutrients, light, and pollinators (Gordon 1998). Invasive plant species can also impact native herbivores by altering plant–herbivore interactions, including interactions with insect herbivores (Pimentel et al. 2005).

Many plant–insect interactions have co-evolved as insect herbivores adapt to specific secondary defensive compounds of their native host plant species (Cornell and Hawkins 2003). For example, plants are able to perceive a wide range of herbivore-associated cues to elicit the release of defensive secondary compounds

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(Ali and Agrawal 2012). Many specialized insect herbivores use these plant chemical cues to locate and identify preferred plant species for oviposition (Renwick 1989; Schoonhoven et al. 2005). Additionally, plant defensive compounds act as feeding stimulants and deterrents during larval development and the balance of these stimulatory and inhibitory compounds controls larval acceptance or rejection of the host plant initially chosen by the ovipositing female (Schoonhoven et al. 2005). Therefore, plant invasions have the potential to “trap” ovipositing females into laying their eggs onto host plants that are unsuitable to larvae depending on the chemical similarity of the invasive plant species to native host plants. Oviposition mistakes such as these have the potential to threaten and endanger the persistence of insect species. Here we explore how the ongoing range expansion of an invasive plant, garlic mustard (*Alliaria petiolata*), is altering oviposition and larval success of a native herbivore, the West Virginia White butterfly (*Pieris virginiensis*), by comparing herbivore populations inside and outside the current range of garlic mustard.

A. petiolata is an invasive biennial crucifer that was introduced to North America from Europe in the late 1800s (Cavers et al. 1979; Rodgers et al. 2008). It is highly successful as an invasive plant as it competes with native plants for nutrients and light and uses allelopathy to reduce native seed germination (Vaughn and Berhow 1999; Meekins and McCarthy 1999; Prati and Bossdorf 2004), and negatively affects beneficial soil microbes which indirectly affects native plant health (Roberts and Anderson 2001; Callaway et al. 2008; Wolfe et al. 2008; Burke 2008). As a result, *A. petiolata* is now common in many habitats across the eastern United States. It has become abundant as far south as Virginia during the past 20 years, and it is continuing to spread southward into North Carolina where it is now present in some habitats but not yet widespread or abundant (Rodgers et al. 2008).

Most *Pieris* butterflies utilize hostplants in the family Brassicaceae, and glucosinolates produced by these plants are important stimuli for female oviposition and larval feeding. *A. petiolata* produces similar glucosinolates to native crucifer species, but the specific glucosinolates produced are unique and only found in *A. petiolata* (Barto et al. 2010). Additionally, *A. petiolata* also produces the novel hydroxynitrile glucoside alliarinoside which is unknown from other

Brassica species (Frisch et al. 2014). Alliarinoside has been shown to function as a feeding deterrent to young larval instars of *P. virginiensis* (Davis et al. 2015). In invaded habitats, *A. petiolata* has been documented as an oviposition site for several Pierid species in North America despite its unique glucosinolate composition (Huang et al. 1995; Keeler and Chew 2008), including *P. virginiensis* (Davis and Cipollini 2014a). *Pieris rapae* is native to Europe, where *A. petiolata* is endemic, and is able to successfully use *A. petiolata* as a host plant (Huang et al. 1995). Evidence also shows that a native cogener, *Pieris oleracea*, has been successfully utilizing *A. petiolata* as a host as the plant has invaded and spread throughout New England (Huang et al. 1995; Keeler and Chew 2008). Keeler and Chew (2008) found that *P. oleracea* populations where *A. petiolata* is well established have improved larval performance and increased adult female oviposition preference for *A. petiolata* relative to populations that do not co-occur with *A. petiolata*, suggesting invaded populations are adapting to the invasive plant.

Like *P. oleracea*, *P. virginiensis* is native to eastern North America and has also been shown to lay eggs on *A. petiolata* (Courant et al. 1994; Porter 1994; Davis and Cipollini 2014a). It is a relatively rare, univoltine butterfly found where native crucifer species are abundant in beech-maple-hemlock woods (Courant et al. 1994; Porter 1994). Most studies on this species have been conducted on populations throughout New England, Pennsylvania, and Ohio, but the range of *P. virginiensis* extends throughout the Appalachians southward into Virginia and North Carolina (Mather 1964). The native ephemeral forb *Cardamine diphylla* is the most common larval host plant of *P. virginiensis*, but there are also occasional small populations that use *Cardamine concatenata*, *Cardamine dissecta*, or *Boechera laevigata* when *C. diphylla* is absent (Calhoun and Iftner 1988; Shuey and Peacock 1989). *C. diphylla* emerges in early April, completes leaf expansion by May, and senesces by early June when the tree canopy begins to shade out the lower understory. The life cycle of *P. virginiensis* is tightly coupled to the early spring phenology of *C. diphylla*. After overwintering as pupae, the adult butterflies emerge in early to mid-April, flying 4–5 weeks through May, and new larvae must complete development and pupate before senescence of the hostplant in June (Shapiro 1971; Cappuccino and Kareiva 1985). Research on *P. virginiensis* in Connecticut by

Doak et al. (2006) in the 1980 s suggests that low egg loads and time limitation in this species contribute to the “choosiness” of females’ oviposition sites.

The northern distribution of *P. virginensis* (New York, Ohio, Pennsylvania) overlaps strongly with the *A. petiolata* invasion. Adult female butterflies from these populations readily oviposit on the invasive *A. petiolata* (Davis et al. 2015), and lab and field experiments show that oviposition is significantly greater on invasive *A. petiolata* than on native *C. diphylla* (Davis and Cipollini 2014a). However, larvae are unable to complete development on these novel host plants (Bowden 1971; Chew 1980; Porter 1994; Davis and Cipollini 2014a). Davis et al. (2015) found that the hydroxynitrile glucoside alliarinoside and high concentrations of the glucosinolate sinigrin contribute to larval failure on this novel host, but sinigrin and alliarinoside did not contribute to oviposition choices in the adult females. Finnell and Lehn (2007) suggest that the perceived decline of *P. virginensis* may be due to exotic plant invasions, in addition to habitat loss and fragmentation. In contrast to *C. diphylla*, *A. petiolata* is shade tolerant and does not senesce until late summer. The biennial stalks grow over 40 cm between April and May reaching close to 70 cm in height before producing fruits and senescing from June through September (Anderson 1996), resulting in quite different seasonal phenologies between the two plants.

The invasive *A. petiolata* is now widespread throughout West Virginia and parts of Virginia, but has only recently established as far south as North Carolina, where it is not yet common in *P. virginensis* habitats (Rodgers et al. 2008). In this study, we investigated how the ongoing southern range expansion of *A. petiolata* will affect populations of *P. virginensis*, and the interactions of *P. virginensis* with its native hostplant *C. diphylla* in the southeast, by conducting oviposition preference and larval survival experiments with *P. virginensis* from *A. petiolata*-absent populations in North Carolina and comparing them to *A. petiolata*-present populations in West Virginia in 2013 and 2014. Because *A. petiolata* has been shown to be toxic to larvae, we expected the invaded butterfly population to respond in one of three ways: (1) avoidance of laying eggs on *A. petiolata* by choosy, adult butterflies, (2) increased survival of larvae feeding on *A. petiolata*, or (3) no difference in either response between populations. Differentiation

and avoidance of *A. petiolata* was quantified and compared by conducting ovipositional assays on both host plants between the North Carolina and West Virginia populations. Similarly, larval survival was quantified and compared by hatching and raising larvae on both host plants and comparing between both populations. Additionally, because relative attractiveness between host plants is unlikely to be static throughout the flight season due to differences in phenology between the two species we assayed *P. virginensis* oviposition preference in West Virginia twice within one flight season—once during the first 2 weeks of the flight and again during the last 2 weeks—during 2015.

Methods

Study populations

Adult *P. virginensis* butterflies were collected from *A. petiolata*-absent field sites located near Highlands Biological Station in North Carolina (NC) from April 15, 2013 to May 15, 2013 and April 20, 2014 to May 9th, 2014. NC populations were primarily sampled from two field sites along the Appalachian Trail including Stecoah Gap in Graham County (35.3578678, -83.7185053; elevation: 3200 ft) and the Wasilik Poplar area in Macon County near Rainbow Springs (35.0940, -83.5221; elevation: 3310 ft). Both field sites are rich cove hardwood ecosystems located in the Nantahala National Forest. *P. virginensis* butterflies were also collected from *A. petiolata*-present sites located in Monongahela National Forest in West Virginia (WV) from May 18th, 2014 to May 26th, 2014 and again during 2015 in early spring from April 28th, 2015 to May 8th, 2015 and late spring from May 20th, 2015 to May 27th, 2015. *A. petiolata* has been recorded in West Virginia since 1933 (Huebner 2003), therefore *P. virginensis* populations in these field sites have been potentially interacting with *A. petiolata* for 84 generations (1 generation per year for 84 years). Butterflies were primarily collected from two sites in Randolph County: Bickle Run (38.91209, -79.71172) and along Whites Run Road (38.85046, -79.48707). *P. virginensis* in these study locations emerge in early spring around mid-April, flying 4–5 weeks through May (Shapiro 1971; Cappuccino and Kareiva 1985).

Although the species' range is expected to shift and contract with climate change, predictions of advancing emergence times due to environmental cues including temperature are difficult to make due to geographic variation in emergence times due to latitude (Davis and Cipollini 2016). However, timing of flight periods does not differ significantly between the North Carolina and West Virginia study populations despite a difference in latitude (K. Augustine, pers. obs.). Additionally, the West Virginia locations sampled as part of this study had a noticeably greater population density than the North Carolina locations (K. Augustine, pers. obs.).

Oviposition experiment

Female adult butterflies from the NC field sites were brought back to the laboratory facilities at Highlands Biological for female host plant oviposition preference assays. Each adult female collected (Spring 2013: $n = 19$, Spring 2014: $n = 9$) was placed individually inside a 1 ft \times 1 ft flight cage for 4 h, outdoors in full sunshine and given the choice between 4 cuttings of the two host plant species: the native host plant *C. diphylla* and the invasive *A. petiolata*. Two stems per species were randomly placed inside each flight cage and the cutting arrangement was randomized between cages and between trials. Each cutting was placed in a water wick to prevent desiccation during the course of the trial. At the end of each trial, cuttings were collected and the number of eggs laid per female on each host plant species was counted and recorded. These oviposition trials were repeated up to 4 times per female across multiple days, weather depending. In NC, the *C. diphylla* host plant cuttings were collected from local populations near Highlands Biological Station and from field sites with permission from the Nantahala Forest Service. The invasive *A. petiolata* specimens were collected from a wild infestation located near Asheville, NC and brought to the field station. Oviposition preference assays in WV were conducted in a similar manner as above within sunny roadside clearings along Whites Run Road in Randolph County with native and invasive host plant cuttings collected from the surrounding forest and roadsides with permission from the Monongahela Forest Service. NC populations were caught and assayed in early spring during 2013 and 2014, and WV populations were sampled in late spring of 2014

($n = 24$) and again in early spring 2015 ($n = 32$) and late spring 2015 ($n = 19$) to test for seasonal differences in preference.

Larval survival experiment

Eggs laid during the oviposition preference assays were collected for larval hatching and survival analyses during 2013 and 2014 in both field sites. During 2013 in North Carolina, a total of 320 eggs were collected from 14 females; 108 eggs laid on *C. diphylla* and 212 eggs laid on *A. petiolata*. During 2014 in North Carolina, a total of 143 eggs were collected from 7 females; 62 eggs on *A. petiolata* and 81 eggs on *C. diphylla*. During 2014 in West Virginia, a total of 135 eggs were collected from 10 females; 134 eggs were laid on *A. petiolata* and only 1 egg on *C. diphylla*.

To compare larval survival between the two plant species, eggs laid on both host plants were removed within 24 h of being laid using a paint brush and individually transferred to a vented plastic cup containing a cut leaf of either host plant placed on moist filter paper. Eggs from each female were randomly assigned a host plant species for hatching in order to assess the effect of the invasive plant on hatching rate. When a female laid multiple eggs during an oviposition trial or between trials, eggs were randomly divided between each host plant species. Eggs were hatched and larvae reared individually inside portable mini environmental chambers (TriTech Research DigiTherm DTM-MP-38) set at 25°C with 14L:10D light cycle until field collections were completed, at which point all larvae were brought back to lab facilities at the University of North Carolina, Chapel Hill and kept in lab environmental chambers (Percival 36VL; Geneva Scientific, Wisconsin) set to the same conditions for the remainder of the experiment.

Of the total 426 eggs laid in North Carolina in 2013 and 2014, 212 eggs were transferred and raised on *C. diphylla* and 214 were transferred and raised on *A. petiolata*. Of the total 135 eggs laid in West Virginia in 2014, 66 eggs were transferred and raised on *C. diphylla* and 62 were transferred and raised on *A. petiolata*. Eggs and larvae were checked daily in order to assess development time and leaves were replaced as needed. Only leaves from secondary *A. petiolata* branches were used. Larval fitness was quantified by

calculating survival rate and development time. Due to the limited availability of host plant material in the lab as host plant material was sourced from each source population and brought back to the lab (see description of the oviposition experiment above), larvae were tracked to the start of the 3rd out of 5 instars rather than to pupation.

Statistical analyses

Because both populations were sampled between multiple years, we first compared data from oviposition assays between years within each population to test for variation and determine if data could be aggregated to compare preferences between *A. petiolata*-absent populations in NC and *A. petiolata*-present populations in WV. The effect of year was analyzed by fitting mixed effects models using the glmer function from the lme4 package (Bates et al. 2015) that included the number of eggs laid on each host plant as a binomial response variable, female as a random effect, and comparing models with and without year included as a fixed effect using the likelihood ratio to test for differences between years within each population. Any females collected and assayed but that did not lay eggs during the oviposition trials were excluded from oviposition preference analyses. Year did not have a significant effect in NC ($\chi^2 = 1.4181$, $p = 0.2337$, $df = 1$), or WV ($\chi^2 = 1.2951$, $p = 0.2251$, $df = 1$) and therefore data was aggregated between years within each population for all remaining preference analyses.

The categorical variable season had 2 levels defined as “early” season for females sampled on or before May 15th, and “late” season for females sampled after May 15th, as females generally fly from mid-April to late May. May 15th was used as a cutoff date to denote the last 2 weeks of their flight period as the “late” season. Female oviposition preference was compared between populations by including only early season females, and fitting mixed effects models using the glmer function from the lme4 package (Bates et al. 2015) that included the number of eggs laid on each host plant as a binomial response variable, population and the total number of eggs laid per female as fixed effects, and individual female and trial number as random effects. Models were run with and without population as a fixed effect and compared using the likelihood ratio to test for

population differences in oviposition preference. Early season versus late season effects on oviposition preference were compared for WV in 2015 only and between all data collected in WV during 2014 and 2015 by fitting similar mixed effect models including the categorical variable season as a fixed effect instead of population. Preliminary analyses of the WV data collected in 2015 only showed qualitatively similar results to that of the combined WV 2014 and 2015 dataset and therefore only the results from the combined WV dataset are reported for the seasonal effect analysis.

The effect of season on overall egg laying success was also analyzed by designating egg laying success for all females caught and assayed as a binomial string where 0 represented failure to lay any eggs across all trials and 1 represented any egg laying success across all trials. This was included as a binomial response variable, analyzed using the glm function (R Core Team 2016) with and without season as a fixed effect to test for seasonal differences in laying success within WV during 2015, and models were compared using a Chi square analysis. Population differences in laying success in the early season only were tested for in a similar manner by comparing models with and without population as a fixed effect.

Hatching success and larval survival were also analyzed as binomial response variables using the glm function (R Core Team 2016) with population and the plant species on which each individual was raised as fixed effects, and mother as a random effect. All statistical analyses were done in R version 3.1.1 and p values are reported based on analysis of deviance in the text.

Results

Oviposition preference

Mean oviposition preference did not differ between populations during the early season ($\chi^2 = 0.0587$, $p = 0.8085$, $df = 1$). Females in NC laid 71% of total eggs on *A. petiolata* and females in WV laid 68% of total eggs on *A. petiolata* (Fig. 1). However, oviposition preference shifted between early and late season flights within WV populations ($\chi^2 = 13.258$, $p = <0.001$, $df = 1$), such that late season females laid 97% of total eggs on *A. petiolata* (Fig. 1).

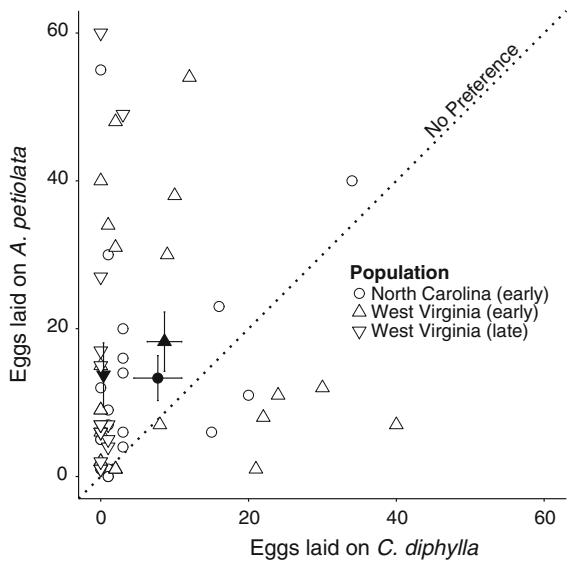


Fig. 1 The proportion of total eggs laid per female on both *Alliaria petiolata* and *Cardamine diphylla* for all females collected in 2013, 2014, and 2015 between both populations. Circles represent North Carolina and triangles represent West Virginia. Filled symbols indicate the mean number of eggs laid on each plant for each population during each season sampled with standard errors

Oviposition success in WV declined significantly from 75% in the early season to 37% in the late season ($\chi^2 = -10.884$, $p = 0.001$, $df = 1$, Fig. 2a). Oviposition success did not differ between populations in the early season at 79% in NC and 75% in WV ($\chi^2 = -0.10681$, $p = 0.7438$, $df = 1$, Fig. 2a). Of the successful egg-laying females, 12 out of 22 (54%) laid eggs on multiple days in NC, 16 out of 24 (66%) laid eggs on multiple days in early season WV, and 4 out of 16 (25%) laid eggs on multiple days in late season WV (Fig. 2b).

Larval survival

Hatching success of eggs was not significantly influenced by host plant species ($\chi^2 = 2.4517$, $p = 0.1174$, $df = 1$, Fig. 3a) or population ($\chi^2 = 0.0302$, $p = 0.862$, $df = 1$, Fig. 3a). The eggs collected from *A. petiolata*-absent populations in North Carolina had an 83% hatching rate on *C. diphylla* and an 86% hatching rate on *A. petiolata* (Fig. 3a). The eggs collected from *A. petiolata*-present populations in West Virginia had an 82% hatching rate

on *C. diphylla*, and an 89% hatching rate on *A. petiolata* (Fig. 3a).

Survival to 3rd instar differed significantly between host plant species ($\chi^2 = 262.42$, $p < 0.0001$, $df = 1$, Fig. 3b), and between populations ($\chi^2 = 27.26$, $p < 0.0001$, $df = 1$, Fig. 3b) but survival on the invasive plant did not differ between populations ($\chi^2 = 0.0182$, $p = 0.8926$, $df = 1$). Instead the population difference in survival was driven by differences in survival on the native host plant between populations ($\chi^2 = 27.934$, $p < 0.0001$, $df = 1$). For the *A. petiolata*-absent site in North Carolina 105 larvae (50%) fed *C. diphylla* survived to the 3rd instar, while only 4 larvae (0.01%) fed *A. petiolata* survived to the 3rd instar (Fig. 3b). For the *A. petiolata*-present site in West Virginia, 51 larvae (77%) fed *C. diphylla* survived to the 3rd development instar, while only 1 larva (0.01%) fed *A. petiolata* survived to the 3rd instar (Fig. 3b). Notably, all larvae from both source populations that reached the 3rd instar when fed *A. petiolata* were very small and, at the time the experiment was terminated, looked unlikely to survive to the 4th instar.

Discussion

The variation in female oviposition preference in the North Carolina populations of *P. virginiensis* from *A. petiolata*-absent sites initially observed in 2013 combined with the low larval survival rate when larvae are raised on *A. petiolata* in this population suggested that this host plant would be a strong force driving selection for oviposition preference for the native *C. diphylla* host plant in adult *P. virginiensis* females. However, we did not observe a difference in mean oviposition preference between populations which suggests that females have not yet adapted to avoid laying on *A. petiolata* within the *A. petiolata*-present West Virginia population. Additionally, there was no difference in larval survival to 3rd instar between populations when larvae were raised on *A. petiolata* leaves, suggesting that local adaptation of larvae has also not yet occurred. This is in contrast to the congeneric native *P. oleracea* which has improved larval performance and increased adult female oviposition preference for *A. petiolata* in *A. petiolata*-present populations in New England (Keeler and Chew 2008).

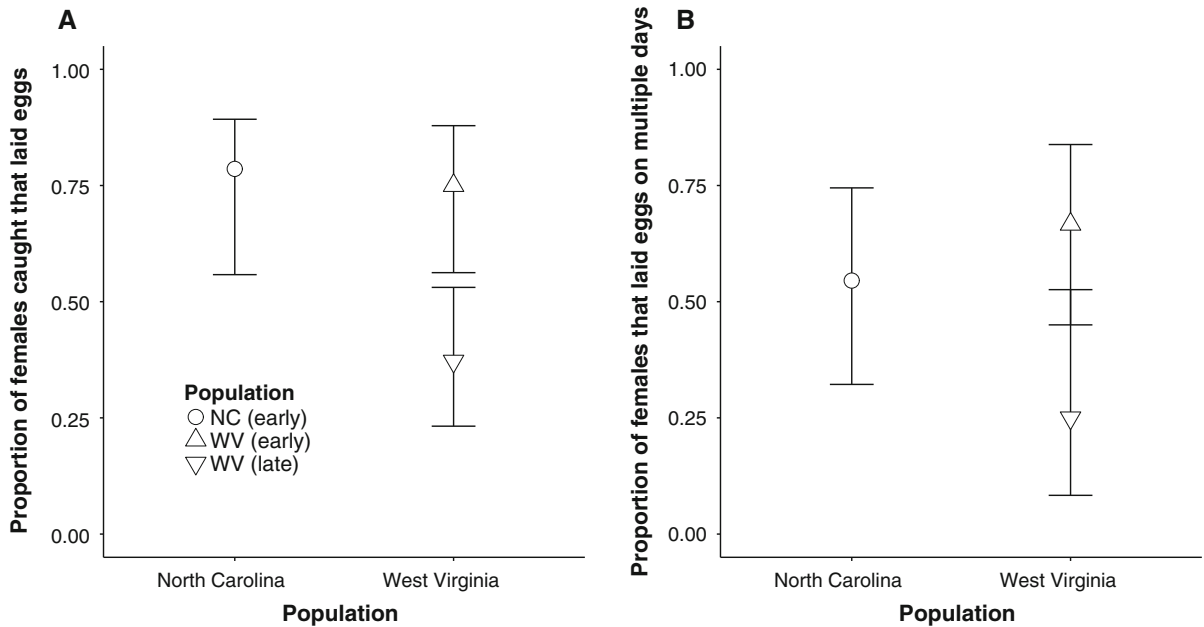


Fig. 2 Oviposition trial success. **a** The proportion of females caught that successfully laid eggs calculated as the number of females that laid eggs over the total number of females placed in oviposition trials between populations and between seasons in WV. **b** The proportion of females that successfully laid eggs on

multiple trial days calculated as the number of females that laid on more than 1 day over the total number placed in oviposition trials between populations and between seasons in WV. Circles represent North Carolina and triangles represent West Virginia with 95% confidence intervals

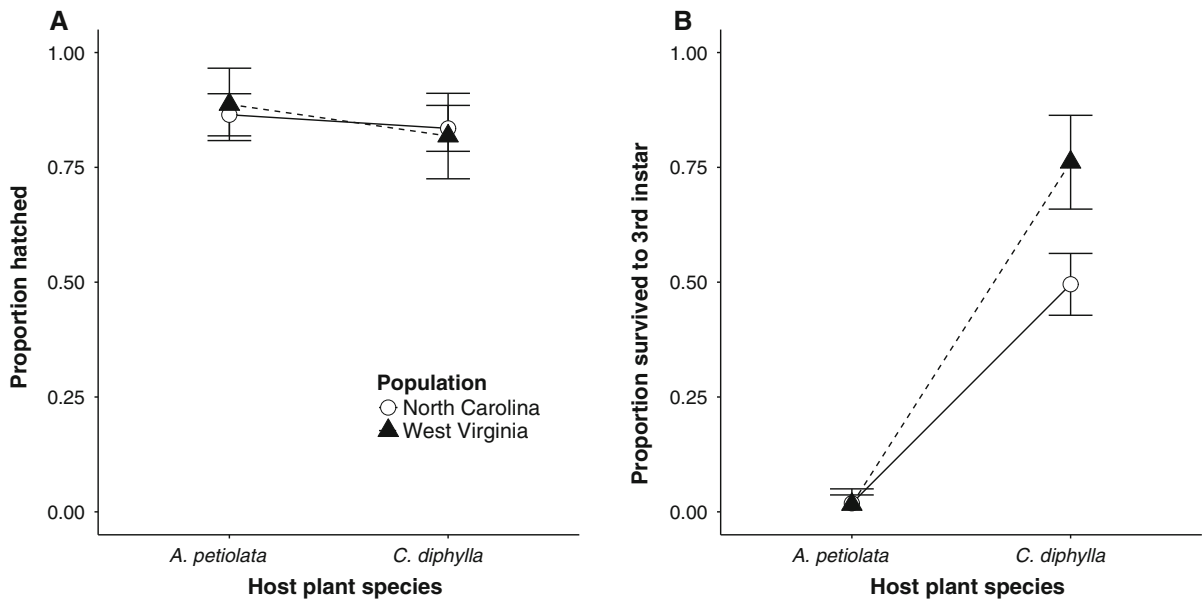


Fig. 3 Hatching success and survival to 3rd instar by host plant. **a** Larval hatching success represented as the proportion of successfully hatching eggs over the total number of eggs placed on *Alliaria petiolata* and *Cardamine diphylla* respectively. **b** Survival to 3rd instar represented as the proportion of larvae

surviving to 3rd instar over the total number of larvae placed on either *Alliaria petiolata* or *Cardamine diphylla* after hatching. Circles represent North Carolina and triangles represent West Virginia with 95% confidence intervals

However, female *P. virginiensis* preference appears to change as the season progresses. This was confirmed by resampling in both early and late season in West Virginia during April and May 2015. Mean oviposition preference does not differ between populations in North Carolina and early season West Virginia, but mean oviposition preference between early and late season in the same year in the same population (West Virginia) did differ, such that the number of eggs laid on *A. petiolata* increased in the late season. We also examined variation in preference of the subset of females that laid on multiple days within the early season in both North Carolina and West Virginia by using the estimate of the intercept of a random effect model (including mother) and standard deviation from the random effect of mother. Represented as the proportion of total eggs laid on *A. petiolata*, we found that the mean preference in early spring is slightly biased toward laying on *A. petiolata* at 0.67, but varies from 0.37 to 0.87. In addition, when analyzing the proportion of total females caught that successfully laid eggs during oviposition trials, rates were similarly high between populations in the early season but significantly declined between early and late season in West Virginia. This further suggests a seasonal component of the impact of *A. petiolata* on *P. virginiensis* populations that was previously unidentified prior to this study.

Doak et al. (2006) observed on average that *P. virginiensis* females choose to oviposit on only half of the plants that the females closely inspect, and that they fly over most *C. diphylla* ramets without any kind of inspection. However, the selected *C. diphylla* plants did not senesce later than unchosen plants. They also compared larval survival between plants picked for oviposition and plants not picked for oviposition using field collected eggs that they hatched and raised to the 1st instar in the laboratory. They found that survival was almost twice as high (45 vs. 24%) on the chosen host ramets. This female behavior and larval performance data was then used to parametrize a simulation model that suggests that female choosiness of oviposition sites increases individual larval survival as well as total female fitness and may contribute to strong host plant selection in this species (Doak et al. 2006).

We hypothesize that the change in female preference and egg laying due to season in our study is likely driven by strong host plant selection in this species and the relative changes in apparent quality of host plants

used within the oviposition assays due to the early senescence of *C. diphylla* relative to *A. petiolata*. All host plants used were field collected due to the difficulty of maintaining *C. diphylla* cultures in the greenhouse, and although only cuttings of the greenest *C. diphylla* plants were used in trials it is likely that *P. virginiensis* butterflies were picking up on early senescence cues when choosing between *A. petiolata* and *C. diphylla* cuttings during the late season trials, causing the significant decrease in the proportion of females that laid eggs during the oviposition trials between the early and late season in West Virginia. This decrease demonstrated that a higher proportion of late season females were refusing to lay eggs at all.

Although Doak et al. (2006) suggests that females were not choosing *C. diphylla* plants that senesced later in their study, our study suggests that females in our populations are potentially using early senescence cues of *C. diphylla* in *A. petiolata*-present populations. We hypothesize that the senescence cues of *C. diphylla* make *A. petiolata* seem more preferable to the *P. virginiensis* butterflies that laid eggs during oviposition trials in the latter half of their flight season during which we sampled in West Virginia. Additionally, as *C. diphylla* senesces, *P. virginiensis* females are more likely to come into contact with *A. petiolata* as the biennial stalks increase over 40 cm in height and reach maximum leaf area towards the end of the *P. virginiensis* flight season in mid-May (Anderson et al. 2006). We suggest that strong host plant selection in invaded populations is likely driving the observed change of preference to lay on the toxic *A. petiolata* plant and decrease in preference variation during the late season. The sampling and then resampling of the same field site (West Virginia) in 2015 during the early and late part of the flight period confirmed this seasonal change in preference. Additional oviposition preference experiments should be done during the late flight season comparing preference between *A. petiolata*, late season *C. diphylla* field cuttings, and greenhouse grown *C. diphylla* cuttings in order to be certain that it is senescence cues rather than other environmental cues that the adult females are using to determine plant quality as the season progresses. Due to logistic limitations of sampling both study populations within such a short flight period, we were only able to confirm this seasonal change in oviposition preference in the West Virginia population. This shift in preference due to season should also be investigated

in the North Carolina population to understand the generality of this result.

Interestingly, we did find a significant difference in larval survival to 3rd instar when larvae were raised on the native *C. diphylla* leaves between populations such that the NC population had increased larval survival relative to the WV population. However, because the populations are feeding on different, wildy collected *C. diphylla* sources and quality may be affected by season, this result is difficult to interpret as a difference between the *P. virginiensis* populations sampled. We recommend further investigation using greenhouse grown *C. diphylla* to further investigate this difference in survival on the native host plant between populations.

Our results have important conservation implications in regards to the potential spread of *A. petiolata* in North Carolina into habitats of *P. virginiensis* as well as the spread of *A. petiolata* into other Southeastern *P. virginiensis* populations that were not sampled as part of this study. Findings from the already invaded West Virginia population suggest that *A. petiolata* has a strong, late season impact on *P. virginiensis* populations with the potential to shift phenologies earlier in the spring as later emerging butterflies are selected against due to the impact of *A. petiolata* on larval survival. This selection would further constrain the already short flight window of this butterfly, making them even more vulnerable to early spring climate variability. *P. virginiensis*' early spring emergence time is already limited due to increasing severity of weather events related to climate change (Davis and Cipollini 2016), as *P. virginiensis* currently already flies in marginally unsuitable temperatures and wind conditions (Davis and Cipollini 2014b). Furthermore, small populations like the one sampled in North Carolina may not possess the genetic variation needed for selection to occur. We recommend aggressive control and monitoring of *A. petiolata* infestations already present in North Carolina such as the infestation along the Swannanoa River in Asheville, NC before it reaches vulnerable *P. virginiensis* populations further west, as *A. petiolata* is notoriously hard to remove once present in a field site. We also recommend control of *A. petiolata* populations already present in Kentucky and Tennessee (Welk et al. 2002) as our findings suggest that the spread of *A. petiolata* into *P. virginiensis* habitats in these areas would also negatively impact

these Southeastern populations of *P. virginiensis*. Incomplete removal of *A. petiolata* is not enough to reduce the harm inflicted on *P. virginiensis* populations because females oviposit more frequently on *A. petiolata* albeit later in the season. We should therefore prevent the spread of *A. petiolata* into Southeastern habitats rather than waiting to control it once it arrives into these vulnerable, Southeastern *P. virginiensis* populations. Additionally, we recommend conserving vulnerable *C. diphylla* populations in North Carolina and elsewhere throughout the Appalachians and its habitat, as the distribution and abundance of this species may influence *P. virginiensis* choice of host plant across a landscape but this has yet to be studied in Southeastern populations.

Acknowledgements We would like to thank Nantahala National Forest and Monongahela National Forest for access to collection sites. Highlands Biological Station for access to lab space and 2 years of support from their Grant-In-Aid program. Harry LeGrand, Henry Wilbur, and Catherine Johnson for help identifying field sites. Kati Moore and Elizabeth Anglin for help with field experiments, and Jessica Higgins and Heidi MacLean for help with lab experiments. Rachel Steward and two anonymous reviewers provided valuable comments on previous versions of the manuscript. This research was funded by the National Science Foundation Grant IOS-1120500 to JGK.

References

- Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci* 17:293–302
- Anderson R (1996) Aspects of the ecology of an invasive plant, garlic mustard (*Alliaria petiolata*), in central Illinois. *Restor Ecol* 4:181–191
- Barto EK, Powell JR, Cipollini D (2010) How novel are the chemical weapons of garlic mustard in North American forest understories? *Biol Invasions* 12:3465–3471
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw.* doi:10.18637/jss.v067.i01
- Bowden SR (1971) American white butterflies (Pieridae) and English foodplants. *J Lepidopterists Soc* 25:6–12
- Burke DJ (2008) Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. *Am J Bot* 95:1416–1425
- Calhoun JV, Iftner DC (1988) An additional natural hostplant of *Pieris virginiensis* (W.H. Edwards) (Pieridae) in Ohio. *J Res Lepidoptera* 27:141–142
- Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Klironomos J (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology* 89:1043–1055

- Cappuccino N, Kareiva P (1985) Coping with a capricious environment: a population study of a rare Pierid butterfly. *Ecology* 66:152
- Cavers PB, Heagy MI, Kokron RF (1979) The biology of Canadian weeds.: 35. *Alliaria petiolata* (M. Bieb.) Cavara and Grande. *Can J Plant Sci* 59:217–229
- Chew FS (1980) Foodplant preferences of *Pieris* caterpillars (Lepidoptera). *Oecologia* 46:347–353
- Cornell HV, Hawkins BA (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *Am Nat* 161:507–522
- Courant AV, Holbrook AE, Van der Reijden ED, Chew FS (1994) Native Pierine butterfly (Pieridae) adapting to naturalized crucifer? *J Lepidopterists Soc* 48:168–170
- Davis SL, Cipollini D (2014a) Do mothers always know best? Oviposition mistakes and resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic host. *Biol Invasions* 16:1941–1950
- Davis SL, Cipollini D (2014b) How environmental conditions and changing landscapes influence the survival and reproduction of a rare butterfly, *Pieris virginiensis* (Pieridae). *J Lepidopterists Soc* 68:61–65
- Davis SL, Cipollini D (2016) Range, genetic diversity and future of the threatened butterfly, *Pieris virginiensis*. *Insect Conserv Divers* 9:506–516
- Davis SL, Frisch T, Bjarnholt N, Cipollini D (2015) How does garlic mustard lure and kill the West Virginia white butterfly? *J Chem Ecol* 41:948–955
- Doak P, Kareiva P, Kingsolver J (2006) Fitness consequences of choosy oviposition for a time-limited butterfly. *Ecology* 87:395–408
- Finnell AS, Lehn CA (2007) West Virginia White butterfly (*Pieris virginiensis*) conservation plan. Version 1.0, pp 1–14. http://leapbio.org/content/4-resources/0-west-virginia-white-butterfly/www_conservation_plan_20070326.pdf. Accessed 1 May 2016
- Frisch T, Agerbirk N, Davis S et al (2014) Glucosinolate-related glucosides in *Alliaria petiolata*: sources of variation in the plant and different metabolism in an adapted specialist herbivore, *Pieris rapae*. *J Chem Ecol* 40:1063–1079
- Gordon DR (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Ecol Appl* 8:975–989
- Huang XP, Renwick JAA, Chew FS (1995) Oviposition stimulants and deterrents control acceptance of *Alliaria petiolata* by *Pieris rapae* and *P. napi oleracea*. *Chemoeology* 5(6):79–87
- Huebner CD (2003) Vulnerability of oak-dominated forests in West Virginia to invasive exotic plants: temporal and spatial patterns of nine exotic species using herbarium records and land classification data. *Castanea* 68:1–14
- Keeler MS, Chew FS (2008) Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia* 156:559–568
- Mather B (1964) The southern limits of the range of *Pieris napi* and *Pieris virginiensis*. *J Res Lepid* 3:45–48
- Meekins JF, McCarthy BC (1999) Competitive ability of *Alliaria petiolata* (Garlic Mustard, Brassicaceae), an invasive, nonindigenous forest herb. *Int J Plant Sci* 160:743–752
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol Econ* 52:273–288
- Porter A (1994) Implications of introduced garlic mustard (*Alliaria petiolata*) in the habitat of *Pieris virginiensis* (Pieridae). *J Lepidopterists Soc* 48:171–172
- Prati D, Bossdorf O (2004) Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *Am J Bot* 91:285–288
- R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org>
- Renwick JAA (1989) Chemical ecology of oviposition in phytophagous insects. *Experientia* 45:223–228
- Roberts KJ, Anderson RC (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *Am Midl Nat* 146:146–152
- Rodgers VL, Stinson KA, Finzi AC (2008) Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. *Bioscience* 58:426–436
- Schoonhoven LM, van Loon JJA, Dicke M (2005) *Insect-plant biology*, 2nd edn. Oxford University Press, Oxford
- Shapiro AM (1971) Occurrence of a latent polyphenism in *Pieris virginiensis* (Lepidoptera: Pieridae). *Entomol News* 82:13–16
- Shuey JA, Peacock JW (1989) Host plant exploitation by an oligophagous population of *Pieris virginiensis* (Lepidoptera: Pieridae). *Am Midl Nat* 122:255
- van der Putten WH, de Ruiter PC, Martijn Bezemer T et al (2004) Trophic interactions in a changing world. *Basic Appl Ecol* 5:487–494
- Vaughn SF, Berhow MA (1999) Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *J Chem Ecol* 25:2495–2504
- Welk E, Schubert K, Hoffman MH (2002) Present and potential distribution of invasive garlic mustard (*Alliaria petiolata*) in North America. *Divers Distrib* 8:219–233
- Wolfe BE, Rodgers VL, Stinson KA, Pringle A (2008) The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *J Ecol* 96:777–783