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**Context-dependent patterns, determinants and demographic consequences of herbivory in
an invasive species**

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Abstract

Non-native invasive plants often lose many of their specialist herbivores in their invaded ranges, but new enemies may be recruited from the local generalist fauna, or from enemies themselves introduced to the same region. As a result, few invaders are free of herbivore damage; however, the incidence of this herbivory and its consequences for plant demography may depend on an array of biotic and abiotic factors. In *Alliaria petiolata*, a non-native invasive forb in Canada, we quantified the incidence of leaf herbivory, rosette survival and seed production along with conspecific plant density and environmental site conditions in wild populations in mesic forest understory and moist grasslands. We complemented the field survey by a laboratory experiment with *Trichoplusia* to test whether the palatability of leaf tissue of *A. petiolata* varies with plant life stage and habitat type. Incidence of herbivory was significantly higher in adults than in rosettes, and significantly higher in grasslands than in forest understory. The palatability experiment confirmed that leaves of adults were more palatable to a generalist herbivore than leaves of rosettes. Path analysis revealed negative plant density-dependent herbivory of adults in forest understory but positive density-dependent herbivory in grasslands. Incidence of herbivory in rosettes was positively associated with soil moisture in grasslands, but not in forest understory. Seed production was significantly positively associated with herbivory in adults and soil moisture, whereas rosette survival was significantly negatively associated with conspecific plant density. We conclude that the patterns, determinants and demographic consequences of herbivory in invasive *Alliaria* may strongly depend on a plant's life stage and plant density and may further be confounded by landscape heterogeneity.

Key Words: *Alliaria petiolata*; density-dependent herbivory; natural enemies; path analysis

Introduction

Plant-herbivore interactions play a crucial role in structuring ecological populations and communities (Harper 1977; Crawley 1989; Moran and Crone 2006). However, non-native plants often lose many of their specialized enemies in invaded regions, potentially resulting in reduced damage in their new environment (Elton 1958). While many empirical studies support this view (Keane and Crawley 2002; Torchin and Mitchell 2004; Mitchell et al. 2006; Liu and Stiling 2006), numerous exceptions exist (see reviews by Colautti et al. 2004; Heger and Jeschke 2014). One reason for these exceptions is that, even if invasive plants lose old enemies in new areas, they may quickly acquire new ones from the local fauna (Levine et al. 2004; Hawkes 2007; Hatcher and Dunn 2011, Dostal et al. 2013). Damage by these new herbivores may depend on an array of biotic and abiotic factors, but these sources of variation are still poorly understood (Dostal et al. 2013).

Environmental site conditions often govern the presence and abundance of plants and herbivores (Scholwaller and Lowman 1999). High herbivory is expected in habitats where environmental conditions are favourable for herbivores (Hawkes and Sullivan 2001; Dostal et al. 2013). As well, factors beyond environmental conditions may moderate herbivory, such as density of the host plant (Parmesan 2000; Giffard et al. 2012). Host plants offer food or shelter for herbivores, thus a reduced density of host plants may be less attractive to herbivores (Torres 1992) due to increased foraging costs or the deterring effects of co-occurring non-host plants or bare ground (Risch 1980; Visser 1986; Scholwaller and Lowman 1999; Giffard et al. 2012). Per-capita herbivory may thus increase with increasing plant density. Alternatively, increasing plant density may reduce per-capita herbivory: if a fixed number of herbivores feeds on a large number of plants, per-capita herbivory would be lower than if they feed on fewer plants.

However, while plant density may affect herbivory directly, environmental conditions may modulate herbivory directly as well as indirectly via plant density (Fig. 1).

Herbivory may further vary between life stages of a plant (Louda and Potvin 1995; Boege and Marquis 2005; Boege et al. 2007; Barton and Koricheva 2010; Massad 2013). A plant species may encounter higher herbivory in its juvenile stage than in its adult stage because the tissue of juvenile plants contains less lignin (Lowman and Box 1983; Loney et al. 2006, Barton and Koricheva 2010, Massad 2013) or because the acquisition or allocation of resources limit juvenile investment in defense (Herms and Mattson 1992). On the other hand, selection pressure by herbivores in the long run may lead to high levels of defense investment in juveniles (McKey 1974; Barton and Koricheva 2010), since a plant's investment in chemical defense depends on the functional value of a living tissue to the plant's life cycle (McCall and Fordyce 2010). The dependence of herbivory on plant life stage may further vary with habitat type because a plant's defense expression against herbivory may depend on environmental site conditions such as soil moisture, light or site productivity (Lewis et al. 2006; Cipollini and Lieurance 2012). As well, environmental cues may provide an indication about the risk of herbivory, allowing plants to initiate appropriate defenses (see review by Frost et al. 2008).

Herbivory often affects plant demographic traits such as reproduction of adults or survivorship of juveniles (Crawley 1989, Massad 2013), although herbivore tolerance can greatly reduce these impacts (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Bossdorf et al. 2004a; MacDonald and Kotanen 2010). In natural landscapes, plant density and environmental conditions also modulate these demographic traits (Huntly 1991). Thus, observable plant demographic traits are the combined outcome of direct and indirect effects of environmental conditions, plant density and herbivory (Moran and Crone 2006; Fig. 1). Experimental

investigation of these direct and indirect effects is challenging, as patterns observed in experimental herbivory enclosures or simulated herbivory (e.g., clipping) may differ from actual herbivory in wild populations (Baldwin 1990; Giffard et al. 2012). Hence, a survey of herbivory in wild populations may reflect natural patterns, where the relative importance of direct and indirect effects of different biotic and abiotic factors may be assessed through statistical modeling such as path analysis (Shipley 2000).

Alliaria petiolata (M. Bieb.) Cavara and Grande (Brassicaceae) is a Eurasian native and an invasive species in North America. This plant is a biennial forb with two distinct life stages: first year rosette and second year adult (Cavers et al. 1979). It occurs in diverse habitats such as forest understory, forest edges, grasslands, or roadsides verges; soil moisture and light are the important variables affecting population growth and demographic parameters of this species (Cavers et al. 1979; Nuzzo 2000; Meekins and McCarthy 2001). While *A. petiolata* encounters damage from at least 69 different species of insects and seven species of fungi in Europe (Hinz and Gerber 1998), it escapes from most in North America (Renwick et al. 2001; Lewis et al. 2006), and thus is often suggested as an example of enemy release. Still, previous studies suggest *A. petiolata* in North America encounters herbivory from generalists in both adult and rosette life stages (Lewis et al. 2006; Yates and Murphy 2008). Defense by *A. petiolata* against herbivory may depend on local factors including environmental site conditions, types and density of local populations of herbivores, connectivity of habitat and population age (Haribala and Renwick 2001; Lankau et al. 2009; Lewis et al. 2006). However, there is a lack of detailed research on causes of variation in herbivory incidence among wild populations of invasive species like *A. petiolata*.

Here, we quantified the incidence of leaf herbivory, conspecific host plant density, environmental site conditions (soil moisture and light) and two demographic traits (rosette survival and adult fitness) in established wild populations of *A. petiolata* in mesic forest understory and moist grasslands. We addressed the following questions: (i) Does the incidence of herbivory vary with plant life stage and habitat type? (ii) Does the palatability of leaf tissue to a generalist herbivore vary with plant life stage and habitat type? (iii) What are the direct and indirect effects of environmental conditions and conspecific plant density on herbivory? (iv) What are the direct and indirect effects of herbivory, environmental conditions and conspecific plant density on juvenile survival and adult fitness? This knowledge would clarify the potential dependency of herbivory and plant demography on biotic and abiotic factors.

Methods

Field sampling

We conducted this study in the Koffler Scientific Reserve (KSR) at Jokers Hill, King City, Ontario, Canada, about 40 km north of Toronto (44.0° N, 79.5° W, ~300m ASL). In the study area, *A. petiolata* occurs in several large established populations in forest understory, grasslands and along nature trails; smaller patches and scattered individuals also occur throughout this area. Naturalists working in the area suggest that these populations are at least 20-25 years old, while *A. petiolata* has occurred in the Toronto area since at least 1879 (Cavers et al. 1979). Detailed maps of topography, vegetation and soil conditions in the study area are available at <http://ksr.utoronto.ca/>.

In August 2009, we marked six *A. petiolata* patches—three in forest understory and three in grasslands—and established transects of permanent sampling quadrats in each patch. Though

the number of patches sampled was small, these sites are representative of the (as yet) limited number of severely invaded sites at this location; in particular, grasslands represent open, moist habitat (soil moisture range: 22.33 – 47.40%), while the forest understory represents closed, mesic habitat (8.30 – 29.70%). Patches were separated from each other by a minimum of 75 m. *A. petiolata* patches within each of the two habitat types were comparable with respect to topography and above-ground vegetation. For each patch ($N = 6$), we laid out four perpendicular radial transects of contiguous 1 m² quadrats extending from the center to the edge of an *A. petiolata* patch. The number of 1m² quadrats per transect varied from 12 - 25 in forest understory and 7 - 14 in grasslands. In total we marked 196 permanent quadrats from mesic forest understory and 109 quadrats from moist grasslands.

In June 2010, we counted the number of adults A_{June} and rosettes R_{June} in each marked quadrat ($N = 305$), and quantified the incidence of leaf herbivory (binary; per leaf), separately for adults and rosettes. We did not identify herbivores; however, snails and slugs (class Gastropoda), a stem and leaf mining weevil (*Ceutorhynchus erysimi* Fabr.), diamondback moth (*Plutella xylostella*), meadow spittlebug (*Philaenus spumarius*), and caterpillars (Lepidoptera: Pieridae) are known herbivores of *A. petiolata* in North America (Evans and Landis 2007; Yates and Murphy 2008). Interestingly, most of these herbivores (including the gastropods, *Plutella*, *Ceutorhynchus*, *Philaenus*, and some *Pieridae*) are European in origin. We suspect gastropods were the most important herbivores at our site.

In each quadrat, we randomly selected three rosettes and three adults and counted the total number of leaves and the number of leaves with visible signs of herbivory, e.g., leaf holes, scars, chewing etc. Presence of at least three holes/scars/chewing signs on a leaf (~3% of total leaf area) was the minimum level of damage we considered to count a leaf damaged by

herbivores. Individual rosettes or adults of *A. petiolata* comprise relatively few leaves (typically, 2 - 4 leaves in a rosette and 7 - 12 leaves in a single-stemmed adult); thus, we estimated the proportion of leaves with herbivory at the quadrat level H_i by applying a correction for small samples (De Veaux et al. 2012), separately for adults and rosettes.

$$H_i = \frac{n_1 + 2}{n_2 + 4}$$

where n_1 = number of damaged leaves per quadrat, and n_2 = number of total leaves per quadrat.

Soil moisture content may affect both plant growth and moisture-sensitive herbivores such as gastropods. We measured soil moisture content and light for each quadrat ($N = 305$). Using a TDR probe, we took three measurements of soil moisture (percentage of soil volume) within each quadrat and averaged them to represent a quadrat. All soil moisture measurements were taken within a period of three days without any rainfall event for seven days prior to the sampling days. For light, we took a canopy image at breast height (using a Digital Plant Canopy Imager CI-110) in each quadrat and analyzed the image for leaf area index (Jonckheere et al. 2004). This approach uses canopy openness as a proxy for light availability, but should be less sensitive to sun angle, time of day, or cloudiness than direct measurements of light intensity.

In October 2010, we re-counted the number of rosettes in each quadrat (R_{Oct}). Then, we computed per-capita rosette survival as, R_{Oct} / R_{June} . For adult, we quantified the number of seeds per adult plant, which we measured at the same time as the herbivory measurement (June). We counted the number of pods (n_{pods}) for each of the three randomly selected adults in each quadrat and opened three randomly selected pods (if a plant had only two pods, we opened two) from each plant to count the number of seeds per pod (n_{seed}). By multiplying the average number of

seeds per pod and the average number of pods per plant, we quantified quadrat-level seed production per adult plant ($n_{pods} \times n_{seed}$).

Leaf palatability experiment

To complement the survey of natural populations, we tested in a laboratory setting whether the palatability of leaf tissue of *A. petiolata* varies with plant life stage and habitat type. We conducted a factorial experiment using *Trichoplusia ni* Hübner (Noctuidae). *Trichoplusia* is found throughout North America and considered as a major pest of crucifer crops; it is commonly used as a generalist herbivore in plant-herbivory studies (e.g., Cipollini 2002; Scott et al. 2010). We obtained *Trichoplusia* eggs from the Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Canada (<http://cfs.nrcan.gc.ca/pages/320>).

In July 2012, we collected leaves that were comparable in age and size from all three forest understory and three grassland sites. Leaves of adults and rosettes were collected from separate individuals. We cut each leaf to a disk shape, so that all experimental leaves had the same leaf area (10.2 cm²), and recorded the weight of each leaf (W_i). For each of the four treatments (combination of life stage: adult vs. rosette and habitat: forest understory vs. grasslands), ten replicate leaves were treated with single *Trichoplusia* larvae (mean $\pm$ sd weight: 0.0146 $\pm$ 0.0044 g) and ten used as control (without herbivore), for a total of 40 treated leaves and 40 control leaves. We placed each leaf in a petri dish, covered them with Parafilm (Pechiney Plastics Packaging) to prevent desiccation and placed them on a laboratory table where regular room temperature (20°C) and normal day-night cycles (approximately 14 hours day length in July) were maintained.

After a week, we terminated the experiment and re-weighted each leaf (W_f) using a digital scale. For each leaf, weight loss per unit biomass (W_l) was defined as, $(W_f - W_i)/W_i$. In herbivore-treated leaves, tissue loss through herbivory and loss of leaf moisture through evaporation were the two possible reasons for weight loss, while in control leaves, weight loss was only due to loss of leaf moisture. Quantifying weight loss is a common practice in herbivory studies; although we cannot rule out the confounding effect of differential evaporation rates between intact control and damaged leaves, differences in area removed were visually obvious and agreed qualitatively with weight estimates (see below).

Statistical analyses

Survey data: To test whether the incidence of herbivory varies with plant life stage and habitat type, we used the function ‘errorsarm’ in R library “spdep” (Bivand et al. 20012) to conduct a mixed-model analysis of variance with a spatially correlated error term to account for spatial structure (coefficient of the spatial error term, λ : 0.41; $P < 0.01$). We defined the model as: incidence of leaf herbivory $\sim \mu + \text{life-stage (fixed)} + \text{habitat-type (fixed)} + \text{patch (random)} + \text{habitat-type} \times \text{life-stage} + \lambda \mathbf{W} + \text{independent error } (\varepsilon)$, where μ is the intercept, and λ is the coefficient of the spatial error term, which is defined by the spatial weight matrix $\mathbf{W}$ (adjacent quadrats were considered as neighbors following rooks connection; binary weight). To improve residual distributions, herbivory incidence data was transformed to log odds (logit), plant density to square roots, and soil moisture and light to natural logarithm. The proportion of variance explained by the model (*pseudo-R*²) was calculated as the squared correlation of the fitted values predicted from all non-spatial predictors in the model (i.e., without spatial error term) and the original transformed variable.

To evaluate the hypothesized causal relationships among factors outlined in Fig. 1, we fitted a series of path models (Shipley 2000), separately for each of the two habitat types and for adult and rosette life stages. Path analysis is similar to the linear regression model (Mysterud et al. 2008), so that the assumption of independent and normal distribution of residuals applies to path analysis. In our data from transect sampling, there was significant residual autocorrelation (see Online Resource 1 for the spatial structure and distribution of variables). We modified path analysis to include spatial error terms in each of the regression models required to fit path coefficients (Olbatus 2006). The standardized path coefficients were estimated from a series of multiple spatial regressions with correlated error terms, using type III sum of square. The regression models were defined as follows (equations 1-6):

$$D_r \sim p_1 L + p_2 M + \lambda W + \varepsilon \quad (1)$$

$$H_r \sim p_3 D + p_4 L + p_5 M + \lambda W + \varepsilon \quad (2)$$

$$S_r \sim p_6 D + p_7 H_r + p_8 L + p_9 M + \lambda W + \varepsilon \quad (3)$$

$$D_a \sim p_1 L + p_2 M + \lambda W + \varepsilon \quad (4)$$

$$H_a \sim p_3 D + p_4 L + p_5 M + \lambda W + \varepsilon \quad (5)$$

$$F_a \sim p_6 D + p_7 H_a + p_8 L + p_9 M + \lambda W + \varepsilon \quad (6)$$

where D_r = rosette density, D_a = adult density, D = conspecific density (adult + rosette), L = light, M = soil moisture, H_a = incidence of adult herbivory, H_r = incidence of rosette herbivory, S_r = per-capita rosette survival, F_a = per-capita adult seed production, p = standardized path coefficients, λ = is the coefficient of the spatial error term, which is defined by the spatial weight matrix W where adjacent quadrats were considered as neighbours (binary weights), and ε = independent error term for corresponding regression. To improve residual distribution and model fit, conspecific plant density data was transformed to square roots, light and soil moisture to

natural logarithm, incidence of herbivory and per-capita rosette survival to logit, and per-capita adult seed production to natural logarithm. To avoid computational problems with survival data, proportions of 1 and 0 were replaced by 0.99 and 0.01, respectively. Transformed variables were standardized to mean of zero and standard deviation of one; therefore, we did not fit any intercept and the slope coefficients (p_{1-9}) represent standardized path coefficients. Residual analyses revealed that transformations were successful in normalizing the non-spatial residuals (Online Resource 2).

The standardized path coefficients (p_{1-9}) represent the direct effects. Indirect effects were quantified as follows: (i) effect of soil moisture on herbivory incidence = $p_2 p_3$, (ii) effect of soil moisture on rosette survival/adult seed production = $p_2 p_6 + p_5 p_7$, (iii) effect of light on herbivory incidence = $p_1 p_3$, (iv) effect of light on rosette survival/adult seed production = $p_1 p_6 + p_4 p_7$, and (v) effect of conspecific plant density on rosette survival/adult seed production = $p_3 p_7$. All statistical analyses were conducted in R 2.14.2 (R Development Core Team 2011).

Experimental data: To test whether the palatability of leaf tissues varies with plant life stage and habitat type, we ran a three-way fixed-effects factorial ANOVA (type III sum of squares) where W_i was the response variable, and plant life stage (Pls), habitat type (Habt) and herbivory treatment (Herb) were the predictors (equation 7). The residuals were slightly skewed (kurtosis: 1.97) but transformation did not improve residual distribution; we proceeded with untransformed data.

$$W_i \sim \mu + \text{Herb} + \text{Pls} + \text{Habt} + \text{Herb} * \text{Pls} + \text{Herb} * \text{Habt} + \text{Pls} * \text{Habt} + \text{Pls} * \text{Herb} * \text{Habt} + \text{error (iid)} \quad (7)$$

Results

Incidence of herbivory (survey data)

Incidence of herbivory in natural populations varied strongly with plant life stage ($P < 0.01$; see [Online Resource 3](#)) and habitat type ($P < 0.01$), but the interaction between plant life stage and habitat type was not significant ($P = 0.68$). Overall, incidence of herbivory was significantly higher in adults than in rosettes and higher in grasslands than in forest understory ([Fig. 2](#)). In rosettes, the mean $\pm$ sd proportion of per-capita herbivory incidence was 0.26 ± 0.11 (range: 0.12 - 0.88) in forest understory and 0.59 ± 0.20 (0.12 - 0.87) in grasslands. In adults, the mean $\pm$ sd proportion of per-capita herbivory incidence was 0.43 ± 0.17 (0.04 - 0.88) in forest understory and 0.68 ± 0.22 (0.50 - 0.97) in grasslands.

Effects of environmental factors and host plant density on herbivory

For rosettes, there was a significant positive association between soil moisture and herbivory incidence in moist grasslands ($P < 0.01$, [Fig. 3](#)); the effect was not significant in mesic forest understory ([Table 1](#)). In adults, incidence of herbivory was significantly negatively associated with conspecific plant density in forest understory ($P = 0.04$) but positively associated in grasslands ([Fig. 3](#)). We failed to detect any significant effect of light on herbivory. The effects of environmental conditions (moisture and light) on incidence of herbivory were mostly direct ([Table 1](#)).

Effects of herbivory, environmental factors and plant density on plant demography

The mean $\pm$ sd seeds per adult was 265.2 ± 276.45 (range: 6.9 - 2224) in forest understory and 855.1 ± 328.76 (127.9 - 1033) in grasslands. The association between incidence of herbivory

and adult seed production was significantly positive in both forest understory and grasslands ($P < 0.01$; Fig. 3). The mean $\pm$ sd proportion of rosette survival per quadrat was 0.28 ± 0.17 ($0.01 - 0.88$) in forest understory and 0.47 ± 0.23 ($0.01 - 0.85$) in grasslands. The association between incidence of herbivory and rosette survival was not statistically significant in either habitat (Table 1). Conspecific plant density had significant negative effects on rosette survival in forest understory ($P = 0.03$) and significant positive effects on adult seed production in grasslands ($P < 0.01$). Soil moisture showed significant positive effects on rosette survival in grasslands ($P < 0.01$) and on adult seed production in forest understory (Fig. 3, Table 1). Light had a significant positive effect on rosette survival in grasslands ($P < 0.01$). The effects of environmental conditions, conspecific plant density and incidence of herbivory on adult seed production and rosette survival were mostly direct. Only indirect effects of soil moisture and plant density on adult seed production were statistically significant (Fig 3, Table 1).

Palatability of leaf tissue (experiment)

Leaves showed obvious signs of herbivory (Fig. 4), and the loss of weight per unit leaf biomass was significantly higher in herbivore-treated leaves than in control leaves ($P < 0.001$; Table 2), indicating that leaf tissue was consumed by *Trichoplusia*. In both forest understory and grasslands, the weight loss was significantly lower in leaves of rosettes than adults ($P < 0.001$; Fig. 5). Though leaves from grasslands lost less weight than leaves from forest understory, after accounting for the marginal effects of life stage and habitat type, herbivory effects depended on the life stage, but not on habitat type, and the three-way interaction was not significant (Table 2). This means that leaf palatability varied with plant life stage: rosette leaves were less palatable than adult leaves. In fact, while *Trichoplusia* fed on adult leaves to a variable degree, they did

not feed on rosette leaves at all (Fig. 4): weight loss in rosette leaves was due solely to loss of leaf moisture through evaporation, as for the controls.

Discussion

Patterns of herbivory

Palatability of plant tissue is a key determinant of herbivory. Our laboratory experiment showed that rosette leaves were much less palatable to a generalist herbivore than adult leaves (Figs 4 and 5). Our result of lower juvenile than adult herbivory is unexpected because adult leaves often experience less herbivory due to higher lignin content, at least in the case of mammalian herbivores (Loney et al. 2006, Barton and Koricheva 2010); as well, in Europe, rosettes of *A. petiolata* are heavily attacked by the weevil *Ceutorhynchus scrobicollis* (Davis et al. 2006). This may explain the findings of higher herbivory in adults than rosettes in the field study (Fig. 2), where generalists are likely to be the herbivores most commonly encountered by *A. petiolata*. While we did not examine leaf chemistry, rosette leaves may contain higher concentrations of herbivore-repellent toxic allelochemicals—such as glucosinolates and apigenin flavonoids—than adult leaves (Haribala and Renwick 2001). Adult plants must allocate finite resources among growth, reproduction, persistence and defense, so that defensive and reproductive allocations may compete (Bazzaz et al. 1987). In contrast, a rosette does not allocate resources to reproduction and it may thus invest more in herbivory defense by producing more allelochemicals, so that rosettes would experience less herbivory than adults. As well, rosettes may represent an especially important life-history stage, as suggested by demographic elasticity analyses (Davis et al. 2006; Evans et al. 2012); in contrast, once an adult has begun to

set seed, there may be little or no selection pressure for further foliar defense in a biennial species.

The incidence of herbivory was higher in grasslands than forest understory (Fig 2). Environmental site conditions (e.g., canopy openness, dense ground-level vegetation or lack of bare ground, and especially moisture) in grasslands may be more conducive to gastropods than forest understory (moisture range: 22.33 - 47.40% in grasslands and 8.30 – 29.70% in forest understory). Thus, abundance of generalist herbivores may be higher in grasslands, which would explain the incidence of higher herbivory in grasslands than in forest understory.

Previous studies on *A. petiolata* herbivory did not distinguish incidence of herbivory in different habitat types, and methodological details vary between studies. We quantified incidence of herbivory as a binary variable based on a cut-off of approx. 3% of total leaf area, assessed by the presence of 2 - 3 holes, spots or signs of herbivory per leaf. Based on these data, the overall incidence of herbivory observed in this study is within the range of findings from previous studies. Riper et al. (2010) found herbivory removed an average of 2% of leaf area (range of means: 1.5 – 3%) in 12 *A. petiolata* populations in Minnesota, USA. Evans and Landis (2007) found an average leaf area removal of 2.3% [range: 0.6 – 9.8%] in spring and 4.5% [range: 1.8 – 6.7%] in fall in 88.2% of their 1280 sampled quadrats in 8 *A. petiolata* population in Michigan, USA. In contrast, in native European populations (Hungary), Lewis et al. (2006) found that herbivory removed >10% leaf area. Collectively, these studies and ours suggest enemy release has occurred in North America.

Determinants of herbivory

A variety of biotic and abiotic factors may modulate herbivory in natural landscapes; we focused on conspecific plant density, soil moisture and light. In adults, we found positive plant-density dependence of herbivory in grasslands but significantly negative plant-density dependence of herbivory in forest understory (Fig. 3). In rosettes, there was no statistically significant association between plant density and herbivory incidence. Herbivores may be more abundant in densely-vegetated grasslands than in sparsely vegetated forest understory, which could explain the observed reversal of the sign of density dependence of herbivory from positive in moist grassland to negative in mesic forest understory. Similarly, density-dependent herbivory might be less pronounced for rosettes simply because they are avoided by herbivores. This could be verified experimentally in future studies by quantifying intensity of herbivory along with densities of host plants and herbivores.

Regarding abiotic effects, in moist grasslands, we found significant positive effects of soil moisture on herbivory incidence in rosettes; soil moisture and canopy openness likely are conducive to some herbivores such as gastropods (slugs) or meadow spittlebugs. However, like Lewis et al (2006), we did not detect any significant effect of light on the incidence of herbivory (Table 1). We suspect that spatial and temporal heterogeneity may have masked any potential light effects on herbivory incidence; for instance, foliage may produce small-scale heterogeneity in light intensity not captured by our canopy measurements, while our snapshot measurements ignored temporal and seasonal variation in sun angle. With the caveat that our environmental variables are only snapshot measurements, we suggest that both environmental site conditions and host plant density are important determinants of herbivory incidence, but their effect may

further depend on plant life stage and abundance of herbivores (Scholwaller and Lowman 1999; Lewis et al. 2006).

Demographic consequences of herbivory

While herbivory is usually damaging to plants, the overall demographic consequences of herbivory may be positive, negative or neutral (Huntly 1991). In rosettes, herbivory effects were neutral —i.e., there was no statistically significant effect of incidence of herbivory on rosette survival (Table 1). Although the observed incidence of herbivory (binary) was high, the actual tissue removal may be too low to affect the plant (Evans and Landis 2007; Riper et al. 2010). In adults however, the association between incidence of herbivory and adult fitness (seed production) was significantly positive (Fig. 3). Due to the concurrent sampling of herbivory incidence and adult fitness we cannot establish whether the observed high adult fitness was due to high herbivory incidence or whether high herbivory incidence was due to plants of high quality (Price 1991). For instance, larger plants may be more attractive to herbivores, so that herbivores accumulated on these plants, which already have set seed and thus have less herbivore defense. However, field observations suggested that in grasslands, where herbivory incidence was high, plants generally had multiple shoots, which was likely induced by herbivore damage to apical shoots in the early growing season (Bossdorf et al. 2004a). These findings are consistent with Evans and Landis (2007) and Bossdorf et al. (2004a). Evans and Landis (2007), in a four-year field-level demographic monitoring in *A. petiolata*, reported significant positive association between the incidence of herbivory and adult fecundity. Bossdorf et al. (2004a) experimentally showed that *A. petiolata* recovered greatly from leaf damage (after 75% damage to the leaf area,

regrowth was almost unaffected) and plants that had experienced stem damage were more branched than undamaged plants.

Indirect effects of biotic and abiotic factors may amplify or mask the demographic consequences of herbivory. We found two significant indirect effects in adults: moisture effects on adult seed production through plant density and herbivory in mesic forest understory, and plant density effects on adult seed production through herbivory in moist grasslands; both indirect effects amplified the effects of herbivory (Table 1). From the low *pseudo-R*² values (range <1 to 30%; see Fig. 3) in the path analyses we suspect that possible other indirect effects were masked by unmeasured biotic and/or abiotic factors. Adaptations of *A. petiolata* to diverse environmental conditions (Cavers et al. 1979; Nuzzo 2000; Meekins and McCarthy 2001) in terms of variable demographic parameters (Evans et al. 2012), competitive ability (Meekins and McCarthy 2002; Bossdorf et al. 2004b) or enemy defense (Haribala and Renwick 2001; Lewis et al. 2006; Lankau et al. 2009) may contribute to such variability. Nonetheless, by combining the results from both rosette and adult life stages over two different habitats, it seems that invasive *A. petiolata* may be very tolerant to herbivory damage (Bossdorf et al. 2004a; Haribala and Renwick 2001).

Conclusions

We suggest that patterns, determinants and demographic consequences of herbivory for invasive plants are highly context-dependent, especially in heterogeneous natural landscapes (Giffard et al. 2012). These results indicate that even for a highly invasive species such as *Alliaria*, which apparently has escaped many of its native-range herbivores, enemy release (as identified by herbivory damage) may be inconsistent; conversely, high tolerance may reduce the

importance of any damage which does occur. Our striking finding of life stage-dependent herbivory (Boege and Marquis 2005, Massad 2013), which is possibly related to a defense–fitness trade off, suggests that *A. petiolata* may employ its defense strategies judiciously (McKey 1974; Bazzaz et al. 1987; McCall and Fordyce 2010). This also implies that there may be a dependency of the effect of enemy release on the plant’s ontogenetic stage (Massad 2013). Investigation on the generality of this strategy in other biennial or perennial invasive species may help understand ecological strategies of a successful invader.

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Table 1 Results of path analysis of biotic and abiotic effects on rosette survival and adult seed production of *A. petiolata*. Shown are decomposition of factor level total effects into direct and indirect components; statistically significant ($\alpha = 0.05$) effects are shown in boldface.

Habitat	Life stage	Definition of effect	Direct	Indirect	Total effect
Forest understory	Rosette	Light → density	-0.016	-	-0.016
		Light → herbivory	-0.099	+0.001	-0.098
		Light → survival	+0.030	+0.009	+0.039
		Moisture → density	-0.020	-	-0.020
		Moisture → herbivory	-0.099	+0.001	-0.098
		Moisture → survival	+0.126	+0.008	+0.134
		Density → herbivory	-0.071	-	-0.071
		Density → survival	-0.174	+0.001	-0.173
		Herbivory → survival	-0.063	-	-0.063
	Adult	Light → density	-0.113	-	-0.113
		Light → herbivory	-0.056	+0.017	-0.039
		Light → seed production	+0.031	-0.032	-0.001
		Moisture → density	-0.264	-	-0.264
		Moisture → herbivory	-0.066	+0.040	-0.026
		Moisture → seed production	+0.217	+0.016	+0.233
		Density → herbivory	-0.153	-	-0.153
		Density → seed production	+0.096	-0.042	+0.054
		Herbivory → seed production	+0.373	-	+0.373
		Light → density	+0.078	-	+0.078

Habitat	Life stage	Definition of effect	Direct	Indirect	Total effect
Grasslands	Rosette	Light → herbivory	+0.094	+0.013	+0.107
		Light → survival	+0.278	-0.008	+0.270
		Moisture → density	-0.048	-	-0.048
		Moisture → herbivory	+0.338	-0.008	+0.330
		Moisture → survival	+0.353	-0.016	+0.337
		Density → herbivory	+0.163	-	+0.163
		Density → survival	-0.069	-0.002	-0.071
	Adult	Herbivory → survival	-0.024	-	-0.024
		Light → density	-0.032	-	-0.032
		Light → herbivory	-0.092	-0.008	-0.100
		Light → seed production	-0.054	-0.038	-0.092
		Moisture → density	-0.008	-	-0.008
		Moisture → herbivory	+0.061	-0.002	+0.059
		Moisture → seed production	+0.145	+0.016	+0.161
		Density → herbivory	+0.255	-	+0.255
		Density → seed production	+0.325	+0.010	+0.335
		Herbivory → seed production	+0.302	-	+0.302

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582

Table 2 Results of a fixed-effects factorial ANOVA of data from a palatability experiment in which leaves of *A. petiolata* were exposed to larvae of *Trichoplusia ni* in petri dishes. Analyses indicate whether palatability (per unit weight loss) varied with herbivory treatment (larva addition vs. control), plant life stage, and habitat type.

	Sum of Squares (Type III)	df	F value	Pr (>F)
Herbivory treatment	0.0067	1	9.10	0.003
Plant life stage	0.0253	1	34.31	<0.001
Habitat type	0.0444	1	60.20	<0.001
Herbivory treatment x Plant life stage	0.0093	1	12.72	<0.001
Herbivory treatment x Habitat type	0.0008	1	1.11	0.295
Plant life stage x Habitat type	0.0175	1	23.79	<0.001
Herbivory treatment x Plant life stage x Habitat type	0.0011	1	1.53	0.219
Error	0.0531	72		

List of Figures

Fig. 1 Schematic diagram of hypothesized causal relationships among biotic and abiotic factors that may govern the incidence of herbivory and factors that may modulate plant demography (juvenile survival and adult seed production). It is anticipated that (i) incidence of herbivory may be affected by environmental conditions (light and soil moisture) and conspecific (host) plant density, where conspecific plant density itself may be affected by environmental conditions, and that (ii) plant demographic traits may be affected by incidence of herbivory, conspecific plant density and environmental conditions. The direction of direct effects, as indicated with arrows and marked with path coefficients (p_{1-9}), may be positive, negative or neutral (see *text*).

Fig. 2 Interaction plot showing the dependency of incidence of herbivory (logit scale) on plant life stages in forest understory and grasslands.

Fig. 3 Results of spatial path analysis showing direct relationships among variables. Only significant relationships ($\alpha = 0.05$) are shown (see Table 1 for complete table of direct and indirect effects including statistically non-significant trends). Solid lines indicate positive effects and dotted lines indicate negative effects, ϵ indicates independent errors associated with each relationship and λ indicates the coefficient of the spatial error term. Standardized path coefficients (direct effects; p_1 - p_9) are presented alongside each line. Different sample sizes (N) were due to variation in abundance of rosette or adults.

Fig. 4 Representative images showing a selection of the herbivore treated leaves at the end of the palatability experiment. Leaves were exposed to larvae of *Trichoplusia ni* in petri dishes.

612 **Fig. 5** Interval plots (mean $\pm$ 95% confidence interval) showing the dependence of leaf
613 palatability (higher weight loss per unit biomass means higher palatability) of *A. petiolata* on
614 plant life stage (rosette vs. adult) and habitat

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5