

## PALATABILITY AND TOLERANCE TO SIMULATED HERBIVORY IN NATIVE AND INTRODUCED POPULATIONS OF *ALLIARIA PETIOLATA* (BRASSICACEAE)<sup>1</sup>

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The European herb garlic mustard (*Alliaria petiolata*) is a serious invader of North American deciduous forests. One explanation for its success could be that in the absence of specialized herbivores, selection has favored less defended but more vigorous genotypes. This idea was addressed by comparing offspring from several native and introduced *Alliaria* populations with respect to their palatability to insect herbivores and their tolerance to simulated herbivory. Feeding rates of a specialist weevil from the native range were significantly greater on American plants, suggesting a loss of resistance in the introduced range. In contrast, there was significant population variation but no continent effect in the feeding rates of a generalist caterpillar. After simulated herbivory, *A. petiolata* showed a substantial regrowth capacity that involved changes in plant growth, architecture, and allocation. Removal of 75% leaf area or of all bolting stems reduced plant fitness to 81% and 58%, respectively, of the fitness of controls. There was no indication of a difference in tolerance between native and introduced *Alliaria* populations or of a trade-off between tolerance and resistance.

**Key words:** biological invasions; *Ceutorhynchus scrobicollis*; compensatory regrowth; EICA hypothesis; microevolution; plant–herbivore interactions; plant resistance; *Spodoptera littoralis*.

One explanation for the success of invasive plants is that they are released from their native specialist herbivores (Maron and Vilà, 2001; Keane and Crawley, 2002). Resources normally lost to these enemies are allocated to growth and reproduction, thereby increasing plant vigor and abundance in the new range. Invoking optimal defense theory and the possibility of rapid evolutionary change, Blossey and Nötzold (1995) recently expanded this idea to propose the “evolution of increased competitive ability” (EICA) hypothesis: if there is a trade-off between growth and defense, then selection should favor less defended but more competitive genotypes in the new range. Although several tests of the EICA hypothesis have been carried out recently (Daehler and Strong, 1997; Willis et al., 1999, 2000; Siemann and Rogers, 2001), two points have received little attention: the distinctions between specialist and generalist herbivores and between resistance and tolerance components of plant defense. A reasonable next step would be to include these into the conceptual framework of the EICA hypothesis and to adjust its predictions accordingly.

Plant defense includes a range of strategies that are commonly divided into those associated with resistance, i.e., any trait that reduces the preference or performance of herbivores, and those associated with tolerance, i.e., the degree to which plant fitness is affected by herbivory relative to fitness in the undamaged state (Strauss and Agrawal, 1999). Because specialist insects often adapt to or even utilize plant toxins (Ber-

enbaum and Zangerl, 1992a), these are assumed to be primarily a defense against generalists (Van der Meijden, 1996), whereas the effect of specialists may be reduced by quantitative deterrents or by tolerance.

Even if invasive plants are released from their specialist enemies, generalists may have similar attack rates in the new range (Jobin et al., 1996; Memmott et al., 2000). Thus, there is little reason to expect an invader to be less defended overall. In contrast, one might expect the following: (1) If the primary defense against specialists is tolerance and tolerance is costly, then there might be selection against it in the new range. Moreover, if there is a trade-off between resistance and tolerance (Van der Meijden et al., 1988), then resources might even be reallocated to chemical defense, so that one would expect lower tolerance and equal or even higher resistance to generalists in invasive genotypes. (2) If the primary defense against specialists is a class of chemicals other than those acting against generalists, then selection might reduce the former, but, again, resources might be reallocated to growth or generalist defense. As a result, invasive genotypes should be less resistant against specialists, but equally or more resistant to generalists.

We addressed these ideas in a series of experiments with native and introduced populations of *Alliaria petiolata*, a European crucifer that has become a serious pest in North American deciduous forests. We used palatability tests to estimate plant resistance to a native specialist and to a common bioassay generalist herbivore. In addition, we carried out clipping experiments in which we estimated plant tolerance to different types of simulated herbivory.

### MATERIALS AND METHODS

**Study species**—Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara and Grande] is a hexaploid ( $2n = 42$ ) member of the mustard family (Brassicaceae) native to the Eurasian temperate zone. Plants typically germinate in

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TABLE 1. Native (Europe) and introduced (USA) populations of *Alliaria petiolata* that were used in this study. MON, HF, and HW were used only in the palatability tests. OHA was used only in the stem removal experiment.

ID	Location	Latitude	Longitude
Europe			
BRU	Bruck, Austria	47°18' N	12°49' E
BUD	Budweis, Czech Republic	48°58' N	14°29' E
HAL	Halle, Germany	51°28' N	11°58' E
IAS	Iasi, Romania	47°09' N	27°38' E
KOP	Copenhagen, Denmark	55°43' N	12°34' E
MON	Montpellier, France	43°36' N	03°53' E
PAR	Ascot, UK	51°25' N	00°41' W
SOY	Soyhières, Switzerland	47°24' N	07°22' E
USA			
CAS	Ipswich, Massachusetts	42°41' N	70°51' W
FF	McLean, Illinois	40°29' N	89°00' W
HF	Petersham, Massachusetts	42°54' N	72°17' W
HW	Mahomet, Illinois	40°23' N	88°09' W
OHA	Athens, Ohio	39°20' N	82°83' W
OHB	Athens, Ohio	39°19' N	82°07' W
VRO	Danville, Illinois	40°09' N	87°37' W

early spring, form a rosette in the first year, overwinter as a rosette, develop flowering stems in the following spring, produce seeds in June/July, and die. Selfing seems to be very frequent (Anderson et al., 1996; Cruden et al., 1996). In Europe, garlic mustard occurs in mesic semi-shade habitats such as forest edges and moist woodlands. Introduced to North America in the 19th century, the species has continuously expanded its range and is now present in 34 of the United States and four Canadian provinces (V. Nuzzo, Nature Conservancy, unpublished report). Garlic mustard invades the understory of North American deciduous forests where it may displace native plant species (McCarthy, 1997), disrupt plant–insect associations (Porter, 1994; Huang et al., 1995) and eventually collapse native food webs (B. Blossey, Cornell University, personal communication). As a consequence, garlic mustard has become the target of a recently established biocontrol research program (Blossey et al., 2001).

Previous ecological studies on garlic mustard either described life-history variation in its new range (Anderson et al., 1996; Byers and Quinn, 1998; Meekins and McCarthy, 2002) or focused on its reproductive biology (Baskin and Baskin, 1992; Cruden et al., 1996; Susko and Lovett-Doust, 1999, 2000), its potential for competitive and allelopathic interference (Meekins and McCarthy, 1999; Vaughn and Berhow, 1999; Roberts and Anderson, 2001), or its response to environmental variation (Meekins and McCarthy, 2000, 2001). There is a lack of research in two areas: (1) comparisons of native and introduced populations testing for genetic differentiation in invasion-related traits and (2) the ecology of plant–herbivore interactions and plant defense.

*Alliaria* contains glucosinolates, particularly sinigrin and its breakdown products (Larsen et al., 1983; Vaughn and Berhow, 1999), which are part of its defense chemistry but also act as feeding stimulants for specialist weevils (Nielsen et al., 1989) and *Pieris* larvae (Renwick and Lopez, 1999). Other compounds that may play a defensive role include flavonoids that appear to be feeding deterrents for *Pieris* larvae (Haribal and Renwick, 1998, 2001; Renwick et al., 2001). Little is known about the herbivore communities on natural *Alliaria* populations. A recent literature survey found 69 insect species associated with *Alliaria* in Europe (Hinz and Gerber, 1998). No surveys have been made yet in invasive populations.

In this study we used seeds from eight European and seven American populations (Table 1). As in other highly selfing species, most of the genetic variation in *Alliaria* appears to be between rather than within populations (Meekins et al., 2001). The populations were not selected for specific habitat criteria but chosen among those available to cover a reasonable geographic range. We regard them to be random samples within continents. In fall 2000, mature siliques were collected from several mother plants in each population. The seeds were cleaned and stored under cold, dry conditions.

**Palatability tests**—In January 2002, seeds from several maternal families in each of eight European and six American populations (Table 1) were placed in petri dishes filled with a sterilized 1 : 1 mixture of sand and seeding compost (COMPOSANA Anzuchterde, COMPO GmbH, Münster, Germany) and dark stratified at 4°C for 100 d. The petri dishes were then placed in a climate chamber with a 12/12 h light/dark cycle at 8°/12°C, where germinated seedlings were transferred to planting trays filled with a 1 : 1 mixture of sand and potting soil (LATerra Typ P, HAWITA-Gruppe GmbH, Vechta, Germany). They were kept in the chamber for several weeks, until no further germination was observed. At the end of May, six replicates from four families per population, altogether 336 plants, were planted into 0.5-L pots filled with the same substrate described, plus 1 g slow-release fertilizer (Osmocote Exact 8-9 M Standard, Scotts International BV, Geldermalsen, The Netherlands). The pots were placed in an unheated greenhouse and watered as needed. To prevent the spread of an aphid infestation in July, all plants were treated three times with an organic insecticide (Neudosan, W. Neudorff GmbH KG, Emmerthal, Germany), a potassium soap solution that suffocates the aphids but does not leave any residues in the plant. Very few plants had been colonized by aphids. None were observed afterwards. Two no-choice palatability tests were done 2–3 mo later with new leaves that developed after the insecticide treatment. In each test we used three replicates from each *Alliaria* family, so that each plant and insect were used only once.

Half of the replicates from each *Alliaria* family were used in a test with *Ceutorhynchus scrobicollis* (Coleoptera: Curculionidae), a monophagous weevil that is currently tested as a biocontrol agent for garlic mustard (Blossey et al., 2001). The weevil has been found in several native Central European *Alliaria* populations, with great abundance in some of them (Hinz and Gerber, 2000). The larvae of *C. scrobicollis* mine the root crowns of garlic mustard, while adult beetles feed on the leaves in the fall. In October 2002, adult weevils were collected in natural populations in northeastern Germany, brought to a climate chamber where they were kept at 15°C and a 12/12 h light/dark cycle and fed with *Alliaria* leaves from a nearby population not included in this study. The palatability tests were done in this chamber, using male beetles only. Prior to the experiment, each beetle was placed in a petri dish with moist filter paper and starved for 24 h. Two identical 4-mm discs were taken from the youngest fully expanded rosette leaf of each plant. In pilot feeding trials with a range of disc sizes, 4 mm was optimal for maximizing the effect size relative to measurement error, given the small size of the weevils. In each case, we determined the fresh masses of the two leaf discs and immediately dried the control disc to a constant mass at 80°C. The test disc was randomly assigned to one of the petri dishes where a weevil fed on it for 24 h. After that, the remainder of the test disc was also dried. We calculated the dry mass by fresh mass ratio of the control disc and used this ratio to estimate dry mass of the test disc before feeding. This value minus dry mass after feeding estimated the leaf mass consumed by each beetle. We used this estimate as a direct measure of plant palatability.

The second test was done with third-instar larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae), a widely employed generalist herbivore known to feed on plants in over 40 families worldwide. Laboratory strains of *S. littoralis* had been provided by Gero Eck (University of Düsseldorf, Düsseldorf, Germany) and bred at our institute since May 2002. The larvae were raised on artificial diet at 26°C and a 12/12 h light/dark cycle. The experiment was identical to the one with *C. scrobicollis*, except that, because the caterpillars are larger, 13-mm leaf discs were used. Also, there was no initial starvation period, because, unlike in weevils, starvation is not needed to stimulate feeding of caterpillars, but may even kill them.

**Clipping experiments**—To estimate plant tolerance, herbivory was simulated by manual clipping in two experiments. The first experiment was a defoliation study that imposed damage typical of generalist herbivores such as snails in natural *Alliaria* populations. Seeds were germinated in 2001 and plants cultivated in a climate chamber as described above. In June 2001, we planted four seedlings from each of eight European and eight American populations in 1.5-L containers filled with a 1 : 1 mixture of sand and potting soil. The plants were placed in an unheated greenhouse and watered as needed. In the fall, all plants received 2 g slow-release fertilizer (Osmocote Plus

3-4 M, Scotts International BV) and were treated against aphids with a systemic insecticide (Bi-58, BASF AG, Ludwigshafen, Germany). To ensure plant vernalization under realistic conditions, the first-year rosettes were brought to an experimental garden in November, where they were packed into bark mulch and overwintered. A harsh frost period killed some rosettes, so that in March 2002, when the plants were returned to the greenhouse, 52 of 64 had survived. These consisted of 25 European and 28 American plants. On 10 April, we recorded the diameter of each rosette. We randomly selected half of the European and the American plants and removed 75% of the area of each leaf with scissors. The others were left as controls. On 24 April, we clipped newly produced leaves. The plants were randomized on a greenhouse bench, watered as needed, and harvested at fruit maturity. On each plant, we counted the numbers of stems and siliques. Siliques and remaining vegetative aboveground parts were separately dried to a constant mass at 80°C and weighed. Total aboveground biomass was calculated as the sum of the two fractions, and reproductive allocation was the ratio between reproductive and vegetative biomass. We used the number of siliques and their biomass as estimates of plant fitness.

In the second experiment, we simulated herbivory by removing all apical meristems, which has a fundamentally different effect than leaf removal (Tiffin and Rausher, 1999). In *Alliaria*, similar damage is caused by the root crown-mining larvae of *C. scrobicollis*, which often kill entire developing stems (Blossey et al., 2001). The plants were raised and overwintered as described, with 20 seedlings from each of eight European and eight American populations grown in 1-L pots. Unfortunately, winter mortality was much higher in these plants, with only 146 survivors of 320. Some additional plants died before the start of the experiment. We used only populations that could still be replicated in each treatment, so that eventually our experiment consisted of 113 plants from seven European and five American populations (Table 1), with 4–19 replicates per population. From each population, half of the plants were randomly chosen to be clipped; the others were used as controls. There was great phenological variation among populations, so we clipped at a common phenological stage rather than at the same time. Plants were clipped when they had developed five immature fruits at least 1 cm long. The first flowers and fruits of *Alliaria* appear when bolting starts, so the clipping took place at an early stage of flowering. We recorded the initial diameters of each plant and cut off all flowering stems above their second cauline leaf. The first plants were clipped on 12 April, the last on 21 May. Control plants were measured at the same stage. The plants were harvested at fruit maturity. For each, we recorded the numbers of stems and siliques, dried the biomass fractions to a constant mass at 80°C, and calculated total aboveground biomass and reproductive allocation as described.

**Statistical analyses**—The palatability data were standardized to zero mean and unity variance and analyzed as nested ANOVA models using the GLM procedure in SAS (SAS Institute, 2001). Each model included continent, population nested within continent, family nested within population, and herbivore mass as a covariate. We used a sequential (type I sum of squares) model in which the covariate was fitted first. As a surrogate for the genetic correlation between resistance to specialist and generalist herbivores, we calculated Pearson's product-moment correlations between the feeding rates of the two herbivores. These correlations were done across continents, both at the level of family and population means.

The leaf removal data were analyzed as a two-factorial ANOVA with clipping, continent, and their interaction as fixed factors. Due to the low sample size, we did not include a population level in this analysis. Prior to the analyses, stem numbers, silique numbers, and silique biomass were square-root transformed. The stem removal data were analyzed as a nested ANOVA with clipping, continent, and their interaction as fixed factors. Populations and their interaction with clipping were random factors nested within continent and continent  $\times$  clipping, respectively. Silique numbers were square-root transformed prior to analyses. In both analyses, we used type III sum of squares to account for the unbalanced design, and we included plant diameter as a covariate to control for the effect of plant size at the beginning of the experiment. Differences among continents or populations in their tolerance to her-

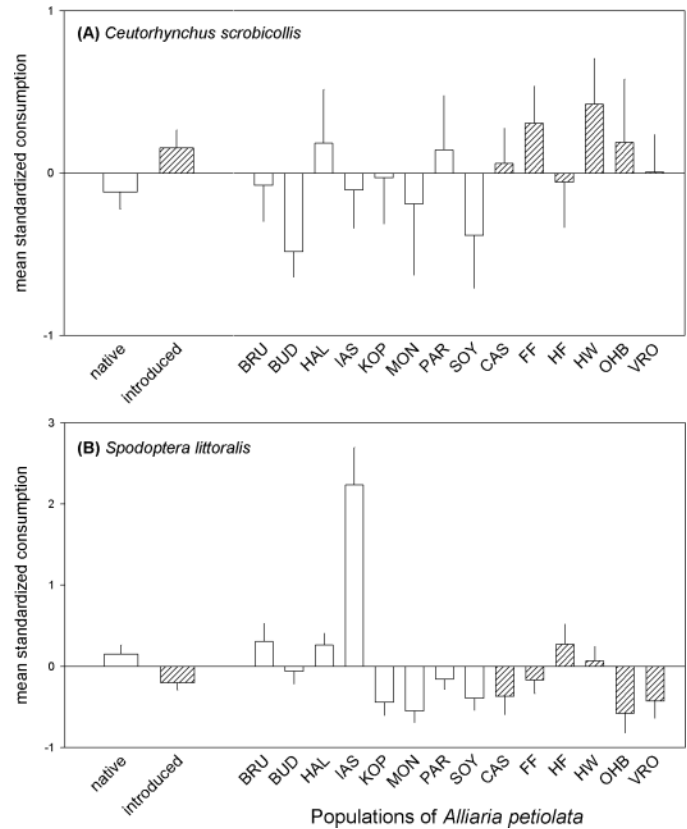


Fig. 1. Palatability of native European (white bars) and invasive American (hatched bars) populations of *Alliaria petiolata* to a specialist weevil, *Ceutorhynchus scrobicollis* (A), and a generalist caterpillar, *Spodoptera littoralis* (B). Plants were raised from seed in a common environment, and their leaves were used for no-choice palatability tests in a climate chamber. The values are continental (left) and population (right) means ( $\pm$  SE) of standardized consumption estimates. See Table 1 for population details and Table 2 for the associated analyses of variance.

bivory are indicated by significant continent  $\times$  clipping and population  $\times$  clipping interactions, respectively (Stowe et al., 2000).

In addition, the stem removal data were used to examine costs of tolerance and the relationship between resistance and tolerance. Costs of tolerance were analyzed as a regression of fitness in the damaged vs. undamaged state (Strauss and Agrawal, 1999). We used a major axis (model II) regression (Legendre, 2001) on log-transformed population means, so that costs of tolerance were indicated by a deviation of the slope from unity. The 11 populations that had been used both in the palatability tests and the stem removal experiment (Table 1) were used to test for a relationship between resistance and tolerance. We calculated population-level tolerance as the ratio between damaged and undamaged trait means. A ratio of unity indicates perfect compensation, whereas  $<1$  indicates under- and  $>1$  overcompensation. Using these indices, we calculated the correlations between standardized specialist and generalist feeding rates and tolerance in terms of silique numbers, silique biomass, and total biomass.

## RESULTS

The feeding rates of *C. scrobicollis* were more variable than those of *S. littoralis* (Fig. 1). As a result, the full ANOVA model explained 67% of the variance in *S. littoralis* feeding, but only 30% in the weevil data (Table 2). Native and introduced *Alliaria* populations differed in their palatability to *C. scrobicollis*, which had higher feeding rates on leaf discs from



TABLE 4. Tolerance to removal of all apical meristems in native European (EU) and invasive American (US) populations of *Alliaria petiolata*. Plants of different origin were raised from seed in a common environment and subjected to simulated herbivory at the beginning of the flowering period. For each trait, the treatment by continent means ( $\pm$  SD) are given, followed by its analysis of variance. Effects with  $P < 0.05$  are in boldface type.

Treatment	No. stems		No. siliques		Silique biomass (g)		Total biomass (g)		Reproductive allocation	
EU Control ( $n = 31$ )	3.26 $\pm$ 1.37		196.1 $\pm$ 40.8		13.48 $\pm$ 2.77		26.67 $\pm$ 6.10		1.07 $\pm$ 0.22	
US Control ( $n = 28$ )	2.57 $\pm$ 1.69		188.5 $\pm$ 98.1		10.71 $\pm$ 4.85		22.74 $\pm$ 9.90		0.89 $\pm$ 0.28	
EU Clipped ( $n = 32$ )	6.00 $\pm$ 2.33		130.9 $\pm$ 40.9		7.75 $\pm$ 2.60		17.13 $\pm$ 5.22		0.84 $\pm$ 0.20	
US Clipped ( $n = 22$ )	5.27 $\pm$ 2.64		126.3 $\pm$ 43.3		6.12 $\pm$ 3.18		13.96 $\pm$ 6.20		0.75 $\pm$ 0.24	
Analyses of variance										
Source (df)	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Plant diameter (1)	90.91	<0.001	16.37	<0.001	30.08	<0.001	58.61	<0.001	0.46	0.500
Clipping (1)	63.39	<0.001	30.42	<0.001	73.73	<0.001	74.71	<0.001	15.11	<0.001
Continent (1)	1.44	0.258	0.03	0.863	6.28	0.031	2.33	0.158	2.88	0.120
Population(C) (10)	1.66	0.104	1.38	0.204	1.40	0.196	2.59	0.009	2.04	0.039
Clip $\times$ Continent (1)	0.20	0.663	1.12	0.314	1.41	0.262	0.54	0.479	0.05	0.823
Clip $\times$ Population(C) (10)	0.64	0.773	0.54	0.855	0.74	0.688	0.83	0.597	0.71	0.715
Error (87)										

sin inhibitor, in particular, is a toxin and feeding deterrent to *S. littoralis* (De Leo and Gallerani, 2002). Cipollini (2002) found significant environmental variation for trypsin inhibitor levels in a natural *Alliaria* population.

Little is known about the chemical defense of *Alliaria* against specialist herbivores, so we can only speculate about the increased feeding rates of *C. scrobicollis* on American populations. Glucosinolates, again, could be involved if *C. scrobicollis* is affected by types other than *S. littoralis*. In most Brassicaceae, there is a complex system of glucosinolates (Fahey et al., 2001). At least some also have negative effects on specialists (Siemens and Mitchell-Olds, 1996; Stowe, 1998a; Li et al., 2000). Kliebenstein et al. (2001) examined 34 glucosinolates in 39 *Arabidopsis* ecotypes and found a limited set of characteristic glucosinolate profiles. Such a modular system may allow differential responses to various herbivores. Alternatively, another class of chemicals might be involved. Flavonoids, for instance, inhibit feeding by larvae of the oligophagous *Pieris napi oleracea* on *Alliaria* (Renwick et al., 2001). They could play a role in the resistance against other specialist herbivores, too.

**Plant tolerance**—*Alliaria petiolata* had a great capability to tolerate damage. After a 75% loss of leaf area or removal of all bolting stems, damaged plants regained 81% and 58%, respectively, of the fitness of controls (Tables 3, 4). Regrowth of *Alliaria* involved increased growth, activation of dormant meristems, as well as changes in plant architecture and allocation pattern. After stem removal, damaged plants quickly produced new stems from lateral buds, which caused a more branchy growth form than in undamaged plants (Table 4). Changes in allocation pattern depended on the type of damage: leaf removal increased but stem removal decreased reproductive allocation (Tables 3, 4). This diversity of mechanisms possibly indicates past selection by herbivores unaffected by resistance (Stowe et al., 2000). Specialist herbivores may have been important in the evolution of *A. petiolata*.

Apart from two comparisons of a native and an invasive species (Schierenbeck et al., 1994; Rogers and Siemann, 2002), plant tolerance has received little attention in the context of plant invasions. To our knowledge, no one has ever tested the possibility that tolerance may have evolved during an invasion. Here we have made a first attempt to address this

question. However, we found no differences between native and introduced populations (Tables 3, 4). One reason for this could be that the reduced number of samples caused by winter mortality made the detection of presumably small continent effects difficult. Alternatively, tolerance might not have evolved in the new range because it has functions other than defense against specialists (Strauss and Agrawal, 1999), or it may not have evolved yet, given a timescale of 150 yr ( $\sim$ 75 generations).

A plant that has maximal resistance will not be damaged. A highly tolerant one will not need to be resistant. If resistance and tolerance serve the same function and both have a cost, then we should expect a trade-off between the two (van der Meijden et al., 1988; Herms and Mattson, 1992). The empirical evidence for this trade-off, however, is ambiguous (e.g., Simms and Triplett, 1994; Fineblum and Rausher, 1995; Mauricio et al., 1997; Stowe, 1998b; Strauss and Agrawal, 1999; Tiffin and Rausher, 1999). There was no indication of a trade-off in our data, and it is unlikely that this was due to low statistical power, because there was even a marginally significant positive correlation between tolerance and resistance to *S. littoralis*. Resistance and tolerance might not be mutually exclusive, but to some extent, be complementary (Mauricio et al., 1997, "less-than-additive") mechanisms of the defense of *Alliaria*. Also, we found a positive relationship between fitness in the damaged vs. undamaged state in the stem removal experiment, so the assumption of costs of tolerance might not be true.

**Implications for biocontrol**—One of the basic assumptions of biocontrol theory is that invasive plants have been released from their specialist enemies (van Driesche and Bellows, 1996). If defense against specialists has become redundant, selection might act against costly defense mechanisms (Blossey and Nötzold, 1995). This would make invasive genotypes even more vulnerable to biocontrol. Our data on *A. petiolata* support this hypothesis: leaves from invasive populations were more palatable to the specialist weevil and potential biocontrol agent *C. scrobicollis*. Still, the plant populations varied greatly in their resistance to both herbivores (Fig. 1). Many biocontrol studies are done on a single target population, although genetic variation for defense traits is common in natural plant populations (e.g., Fritz and Price, 1988; Berenbaum and Zangerl,

1992b). Biocontrol programs should greatly profit from taking this genetic variation of their targets into account.

**Garlic mustard, plant defense, and the EICA hypothesis—**

The EICA hypothesis makes two major predictions. First, plants from the invasive range should be defended less. Second, they should have a higher fitness in the absence of herbivores. Here, we have found experimental support for the first prediction, but we do not know yet whether this translates into greater fitness of invasive *Alliaria* genotypes. The significant continent effect in the stem removal experiment (Table 4) suggests the opposite, but clearly further work that explores this EICA prediction is needed. Also, we have no quantitative data yet on the actual extent of an enemy release in American populations of *Alliaria*. Wolfe (2002) recently showed that both specialists and generalists were reduced in the invasive range of *Silene latifolia*. Hence, the assumption that only specialists are absent may not always be true. Finally, we do not know enough about the defense chemistry of *Alliaria*, in particular about its defense against specialists such as *C. scrobicollis*. Closing these gaps in our knowledge would enable us to test more precise hypotheses about the evolution of defense in garlic mustard invasions.

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