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Population and leaf-level variation of iridoid glycosides in the invasive weed *Verbascum thapsus* L. (common mullein): implications for herbivory by generalist insects

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Abstract Plant–insect interactions, which are strongly mediated by chemical defenses, have the potential to shape invasion dynamics. Despite this, few studies have quantified natural variation in key defensive compounds of invasive plant populations, or how those defenses relate to levels of herbivory. Here, we evaluated variation in the iridoid glycosides aucubin and catalpol in rosette plants of naturally occurring, introduced populations of the North American invader, *Verbascum thapsus* L. (common mullein; Scrophulariaceae). We examined two scales that are likely to structure interactions with insect herbivores—among populations and within plant tissues (i.e., between young and old leaves). We additionally estimated the severity of damage incurred at these scales due to insect chewing herbivores (predominantly grasshoppers and caterpillars), and evaluated the relationship between iridoid glycoside content and leaf damage. We found significant variation in iridoid glycoside concentrations among populations and between young and old leaves, with levels of herbivory strongly tracking leaf-level investment in defense. Specifically, across populations, young leaves were highly defended by iridoids (averaging 6.5× the concentration present in old leaves, and containing higher proportions of the potentially more toxic iridoid, catalpol) and suffered only minimal damage from generalist herbivores. In contrast, old leaves were significantly less defended and accordingly more substantially utilized. These findings reveal that quantitative variation in iridoid glycosides is a key feature explaining patterns of herbivory in an introduced plant. In particular, these data support the hypothesis that defenses limit the ability of generalists to feed on mullein's well-defended young leaves, resulting in minimal losses of high-quality tissue, and increasing performance of this introduced species.

Keywords Aucubin · Catalpol · Plant–insect interactions · Plant invasions

Introduction

The long-standing interest in how plant defenses influence herbivory and thus plant performance has become an important aspect of research on biological invasions. For example, the *novel chemistry*, *evolution of increased competitive ability*, and *resource-enemy release* hypotheses propose that the unique or optimal deployment of plant defenses facilitate invasion (Müller-Schärer et al. 2004; Blumenthal 2006; Cappuccino and Arnason 2006). Yet, while much research on invasions assigns a central role to the chemical ecology of plant–insect interactions, few studies have quantified natural variation in defense compounds in introduced plant populations (but see Darrow and Bowers 1997; Zangerl et al. 2008; Barto et al. 2010; Jamieson and Bowers 2010), and even fewer have related quantitative variation in invasive species' defenses with herbivore damage in the field (but see Funk and Throop 2010; Jamieson and Bowers 2012; Alba et al. 2012).

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The rationale that phytochemical uniqueness of introduced plants facilitates invasion assumes that naïve herbivores do not recognize invaders as potential hosts (Strong et al. 1984; Cappuccino and Arnason, 2006; but see Lind and Parker 2010). However, herbivores often successfully feed on invasive plants if, for example, they are taxonomically or chemically similar to co-occurring native plants (e.g., Feeny 1976; Thomas et al. 1987; Bowers et al. 1992; Courant et al. 1994), or if herbivores can rapidly adapt to overcome their unique defenses (Karowe 1990). Indeed, several herbivorous insects in North America have successfully incorporated introduced plants into their diets (e.g., Strong et al. 1984; Singer et al. 1993; Graves and Shapiro 2003). Given that novel interactions such as these have the potential to shape plant performance, and by extension, invasion dynamics, it is critical to quantify the amount, distribution, and ecological relevance of chemical defenses in introduced plants. Hence, in this study, we (1) evaluated phenotypic variation in the iridoid glycosides aucubin and catalpol in introduced populations of the North American invader, *Verbascum thapsus* L. (common mullein; Scrophulariaceae) and (2) related variation in defense investment to damage by chewing herbivores in the field.

Common mullein, a biennial forb introduced to North America more than 400 years ago (Mitich 1989), contains two common iridoid glycosides as a main chemical defense, aucubin and catalpol. These iridoid glycosides, a group of cyclopentanoid monoterpene-derived compounds, occur in more than 50 plant families of widespread geographic distribution (including Caprifoliaceae, Dipsacaceae, Gentianaceae, Plantaginaceae, Scrophulariaceae, Valerianaceae, and Verbenaceae; Bowers 1991) and have been demonstrated to mediate plant–insect interactions (e.g., L'Empereur and Stermitz 1990a, b; Biere et al. 2004; De Deyn et al. 2004; Beninger et al. 2008). In particular, aucubin and catalpol can act as deterrents to generalist herbivores (e.g., *Spodoptera eridania*, Puttick and Bowers 1988) as well as attractants to specialist herbivores (e.g., *Euphydryas chalcedona* and *Junonia coenia*, Bowers 1983; Bowers and Puttick 1988). Additionally, because catalpol can be more toxic to some generalists than aucubin (Bowers and Puttick 1988; Bowers 1991), the proportion of iridoids composed of catalpol may influence herbivore feeding preferences in addition to the total amount of aucubin and catalpol that is present.

Mullein has a long residence time in its introduced North American range, and its chemical defenses are quite common, being present in a number of co-occurring native plant species (e.g., *Besseya* spp., *Castilleja* spp., *Penstemon* spp.; Stermitz et al. 1986, L'Empereur and Stermitz 1990a, b) as well as other introduced species (e.g., *Linaria* spp., *Lonicera* spp., *Plantago* spp., and *Verbascum* spp.; Tatli and Akdemir 2004; Darrow and

Bowers 1997; Jamieson and Bowers 2010). Given this, we expect that insect herbivores present in mullein's introduced range have incorporated mullein into their diet and therefore, have the potential to affect plant performance. In this case, variation in defensive phenotypes should explain variation in the distribution and severity of plant damage.

Previous research has shown that introduced mullein populations perform better than their native counterparts (Alba and Hufbauer 2012) and that within-plant variation in plant defenses can partially explain patterns of tissue damage in the field (Alba et al. 2012). Yet, those studies either lack an assessment of mullein's chemical defenses (Alba and Hufbauer 2012) or quantified chemical defenses during the bolting stage (Alba et al. 2012), when plants tend to reallocate resources to reproductive tissues, potentially altering patterns of defense investment (Zangerl and Bazzaz 1992). Additionally, younger developmental stages (e.g., the rosette stage in biennials such as mullein) are often more vulnerable to herbivore damage (Moles and Westoby 2004) and are exposed to damage for extended periods of time. Further, the value of their tissues is high given that the resources captured and stored during the juvenile through mature (but pre-reproductive) stages strongly determine overall plant fitness (Chapin et al. 1990). It is thus important to assess variation in defense phenotypes and herbivore damage across all developmental stages, including the most vulnerable stage(s), and to establish comparisons across stages.

As such, we here build on previous research on bolting mullein plants (Alba et al. 2012) by assessing variation in iridoid glycoside content in rosette-stage plants. We focus on two distinct scales that have been shown to structure ecological interactions with insect herbivores in this and other systems (e.g., van Dam et al. 1995; Bidart-Bouzat and Kliebenstein 2008; Alba et al. 2012): among populations and within individual plants. At the population level, we examined five populations located in semi-arid, high-elevation regions of the introduced range where mullein is particularly problematic (Alba and Hufbauer 2012) and where generalists had previously been observed feeding on mullein (Alba, pers. obs.). At the within-plant level, we compared young and old leaves. Young leaves are critical to the future growth of a plant (i.e., they are highly valuable to the plant), and tend to be well-defended, while older leaves are typically less valuable and less well-defended (e.g., McKey 1979; Coley 1983; Krischik and Denno 1983). We predicted that rosettes would exhibit significant variation in iridoid glycoside investment across populations and that young leaves would contain more iridoid glycosides than old leaves. We further predicted that if aucubin and catalpol effectively protect rosette plants against generalist herbivores, then (1) patterns of chewing damage would track differential investment in

iridoid glycosides between young and old leaves, and (2) increasing levels of iridoid glycosides would be associated with decreasing levels of attack.

Methods and materials

Study system

Common mullein is a typically biennial forb that was repeatedly introduced to the US and Canada by European settlers who used it for its medicinal properties and as a piscicide (Wilhelm 1974; Gross and Werner 1978; Turker and Gurel 2005). Introductions date back to the early 1600s (Mitich 1989), and populations are now established in all 50 states, with noxious status in Colorado, South Dakota, and Hawaii. Mullein has a large native range, with populations present throughout the British Isles and Europe (including Scandinavia), to the east in Russia and China, and to the south in the Caucasus Mountains, Western Himalayas, and North Africa (Clapham et al. 1952; Gross and Werner 1978). Plants in both the native and introduced ranges typically grow in dry, sandy soils and often require canopy-opening disturbance to facilitate recruitment (Gross 1980). Individuals form a basal rosette in the first year and overwinter before developing a large flowering stalk that can produce up to 175,000 seeds (Gross and Werner 1978).

Ecological differences exist between native and introduced mullein, with introduced mullein exhibiting increased population- and plant-level performance (Alba and Hufbauer 2012). This increased performance is associated with a shift in the prevalence and identity of herbivore enemies on introduced relative to native mullein populations (Alba and Hufbauer 2012). In particular, introduced populations have partially or fully escaped from

several insect guilds including caterpillars, weevils, leafhoppers, aphids, and snails across a widespread portion of their introduced range (sensu the *enemy release* hypothesis, Elton 1958; Keane and Crawley 2002). Additionally, introduced mullein incurs significantly less damage by chewing herbivores than its native counterparts (Alba and Hufbauer 2012). This reduced herbivory in part reflects the fact that introduced mullein has escaped from the specialist caterpillar *Cucullia verbasci* L. (Noctuidae), which causes substantial damage in the native range (Maw 1980; Alba, pers. obs.). However, introduced plants still incur damage from generalist grasshoppers (Woodman and Fernandes 1991; Alba and Hufbauer 2012) and generalist Noctuids [e.g., *Autographa californica* (alfalfa looper) and unidentified cutworms; Alba, pers. obs.]. In fact, grasshoppers are more common on introduced plants than native plants (Alba and Hufbauer 2012), suggesting that this guild is of particular importance in the new range.

Collection of leaf tissue for iridoid glycoside analysis

We harvested young and old leaves from ten randomly chosen, overwintered rosettes from each of five mullein populations in Utah and Colorado (see Table 1 for sampling dates and population characteristics) for analysis of iridoid glycoside concentrations. Populations were sampled over a span of less than 2 weeks to minimize differences in phenology. Young leaves were positioned at the 2nd or 3rd rank out from the center of the rosette and old leaves were positioned at the 2nd or 3rd rank in from the outermost whorl of the rosette. Leaves were harvested after herbivory scores were assigned (see below). In many cases, we had to harvest more young leaves than were used to estimate herbivory to ensure that we had enough tissue for chemical analysis. In those cases, we harvested leaves from adjacent leaf ranks, which typically had similar levels of herbivory. All plant tissues were oven-dried at 50 °C to a constant

Table 1 Summary of sampling locations for herbivory and iridoid glycoside content of *Verbascum thapsus* (mullein) plants

Location	State	Site ID	Sample date	Latitude (N). longitude (W)	Elevation (m)	Site characteristics
Beaver 1	UT	B1	6.1.2010	38.304111, 112.837642	2,043	Burned (2007) area on BLM land; sagebrush-dominated uplands; sedge and forb-rich lowlands
Beaver 2	UT	B2	6.1.2010	38.419186, 112.713906	2,043	Burned (2007) area on BLM land; steep slope with weedy plants including *Bromus inermis* and *Bromus tectorum*.
Strawberry Reservoir	UT	ST	6.2.2010	40.182913,111.050444	2,355	Roadside population; highly disturbed with minimal vegetation
Logan	CO	LG	5.22.2010	40.650061, 102.741167	1,269	Conservation Reserve Program land; seeded with erosion-controlling bunchgrasses; weedy plants present (e.g., *Bromus tectorum, Medicago sativa*)
Hewlett Gulch	CO	HG	5.27.2010	40.701733, 105.314656	1,791	Trailside population on burned (2005) National Forest Service land: mixture of weedy plants (e.g., *Bromus tectorum*) and native species

mass and weighed to the nearest 0.01 g. To assess variation in concentrations of iridoid glycosides, young and old leaves from each plant were separately ground into a fine powder from which we removed leaf trichomes by passing samples over a mesh screen. We then prepared 50-mg subsamples for chemical extraction and analysis by gas chromatography following previously described methods (e.g., Bowers and Stamp 1993). Briefly, the subsamples were extracted overnight in methanol and the extract was filtered off of the remaining tissue under a vacuum. We added an internal standard (phenyl-β-D-glucopyranoside) to the remaining sample and partitioned the extract between water and ether to remove chlorophyll and hydrophobic compounds. An aliquot of the remaining solution was removed, evaporated, and derivatized with Tri-Sil-Z^{TM} (Pierce Chemical Company) and injected into a HP 7890A gas chromatograph (Agilent Technology) using an Agilent DB-1 column (30 m, 0.320 mm, 0.25 μm particle size). Concentrations of aucubin and catalpol were quantified using ChemStation B-03-01 software and they are presented as percent dry weight for comparative purposes (e.g., Fuchs and Bowers 2004; Barton 2007; Jamieson and Bowers 2010).

Herbivory estimates

We estimated chewing damage by insect herbivores on the same leaves that were collected for iridoid glycoside analysis (although, as noted above, in some cases we harvested additional young leaves to ensure that we had enough tissue for chemical analysis). Damage estimates were made on three young and three old leaves per plant using the following scoring system: 0 = no leaf tissue missing, 1 = 1–10 % of tissue missing, 2 = 11–50 % of tissue missing, 3 = 51–75 % of tissue missing, and 4 = greater than 75 % of tissue missing. Because we sampled the plants in late May and early June at relatively high-altitude locations, the current season's herbivores were still scarce and the majority of damage recorded was from the previous season. We interpret zero damage as indicating avoidance behaviors rather than a lack of natural enemies in the area. Given that our sampling captured the chewing damage of highly mobile herbivores (mostly grasshoppers; cf. Alba and Hufbauer 2012) accumulated over an entire growing season, we believe this assumption is justified.

Statistical analysis

All statistical analyses were conducted in SAS (v. 9.1, Cary, NC). Because aucubin and catalpol were correlated (Pearson's correlation coefficient = 0.86; $P < 0.0001$), we initially assessed population and leaf-age variation in the percent dry weight of both iridoids using multivariate

ANOVA (proc glm), with a repeated measures statement to account for young and old leaves collected from the same plant. We included population, leaf age, and a population × leaf age interaction as fixed effects. All effects were significant using MANOVA (between-subject effects: population, age, and population × age, $P < 0.0001$; within-subject effects: plant, plant × population, plant × age, and plant × population × age, $P < 0.0001$). As such, we continued with univariate ANOVA for each iridoid, as well as for the total percent dry weight of iridoids, and the proportion of iridoids composed of catalpol (cf. Jamieson and Bowers 2010). All univariate ANOVAs included population, leaf age, and a population × leaf age interaction as fixed effects, and treated young and old leaves from the same plant as repeated measures (see Littell et al. (1996) for a description of how to construct repeated measures using the glm procedure). We did not include plant diameter or number of leaves as a covariate in the models because there was no relationship between plant size and investment in iridoid glycosides (Pearson's correlation coefficient for diameter = −0.09, $P = 0.51$; leaves = 0.22, $P = 0.12$). We used least mean squares with a Tukey adjustment to test all pairwise comparisons of the fixed effects. All data were arcsine-square root transformed to meet assumptions of normality.

The herbivory data contained only zeros for young leaves at site B2 and for young and old leaves at site ST, and did not meet assumptions of normality following data transformation. We therefore used the raw herbivory scores to create two categories: undamaged leaves (herbivory score of zero) and damaged leaves (herbivory scores of 1–4). Treating the data this way allowed us to use a generalized linear mixed model (GLMM) with a binomial distribution and logit link function (proc glimmix) to test for differences in herbivory among populations and between young and old leaves (Schabenberger 2005). The GLMM procedure uses "events per trial" syntax, where an event is damage to a leaf (scores 1–4) and trials are the number of leaves sampled. Therefore, each of the ten plants sampled per population had three trials for young leaves and three trials for old leaves, giving 30 trials for each leaf age in each population. We included population, leaf age, and a population × leaf age interaction as fixed effects in the model, and treated young and old leaves from the same plant as repeated measures (see Schabenberger (2005) for a description of how to construct repeated measures using the glimmix procedure). In order to allow the model to converge on a solution, we included one event in the B2 and ST datasets for young leaves (i.e., 1 herbivory event out of 30 trials, rather than 0 events out of 30 trials), and one event in the ST dataset for old leaves. This change is conservative with respect to our analyses, as it reduces differences in herbivory between young and old leaves. We

used least square means with a Tukey adjustment to test all pairwise comparisons of the fixed effects.

Finally, we explored whether plant size (number of leaves) and iridoid glycoside content explain variation in the severity of damage to young and old leaves using partial regression models. We included plant size because this can influence herbivore foraging decisions (Price 1991; Cornelissen et al. 2008) in addition to the defense phenotypes of interest. We treated plant size, total percent dry weight of iridoid glycosides, and the proportion of iridoids composed of catalpol as independent variables and leaf damage as the dependent variable. We generated partial residual plots (see "Results") that illustrate the relationship between each independent variable and leaf damage when controlling for the effects of the remaining independent variables. Damage levels consisted of the average herbivory scores (0–4) for the group of leaves for which iridoid content was measured. A square-root transformation of the damage scores improved normality and homogeneity of variance.

Results

Iridoid glycoside content

Chemical defenses of rosette plants, measured as the concentration of aucubin plus catalpol, as well as separately for aucubin, catalpol, and the proportion of iridoids made up of catalpol, significantly varied among populations and as a function of leaf age (Figs. 1, 2; Table 2). When averaging over leaf age, population-level variability in the mean (±SE) concentration of total iridoid glycosides ranged from 1.63 ± 0.43 % dry weight in population LG to 5.03 ± 1.12 % dry weight in population B2. Additionally, leaf age was an important source of variation, with young leaves containing on average $6.5\times$ more iridoid glycosides than old leaves (Table 2). This general pattern held for every population except ST (Fig. 1). There was also significant variability in iridoid concentrations within age classes (with young leaves averaging from 3 to almost 10 % dry weight, and old leaves ranging from 0.2 to 2 % dry weight; Fig. 1) depending on the population of origin (cf. the significant population × age interaction, Table 2). Finally, the proportion of the potentially more toxic iridoid catalpol was significantly greater in young leaves (0.75–0.85) than that in old leaves (0.48–0.58) across all populations (note the lack of a population × age interaction, Table 2).

Herbivory

The proportion of rosette leaves damaged by generalist herbivores varied significantly as a result of population and
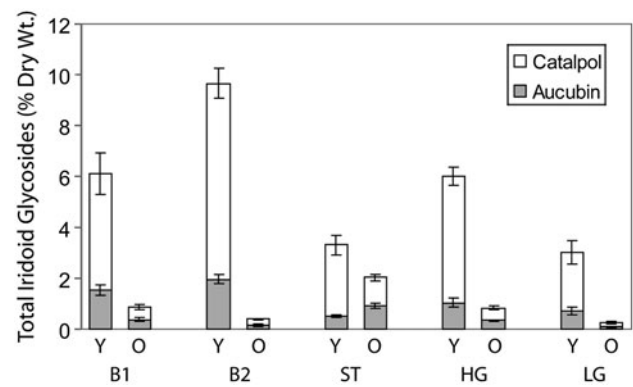

Fig. 1 Mean percent dry weight (±SE) of total percent iridoid glycosides (aucubin + catalpol) in the young (Y) and old (O) leaves of plants ($n = 10$) from five mullein populations in Utah and Colorado, USA. Population abbreviations as in Table 1. See Table 2 for ANOVA results
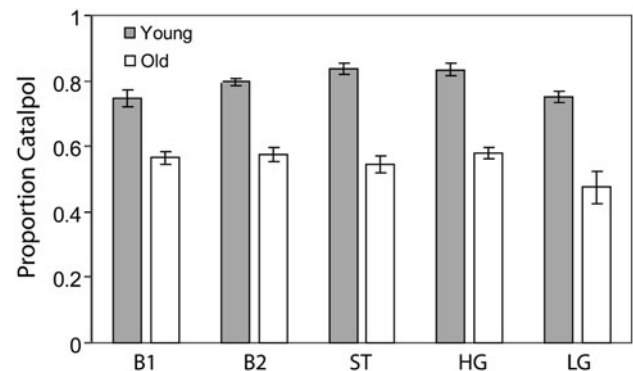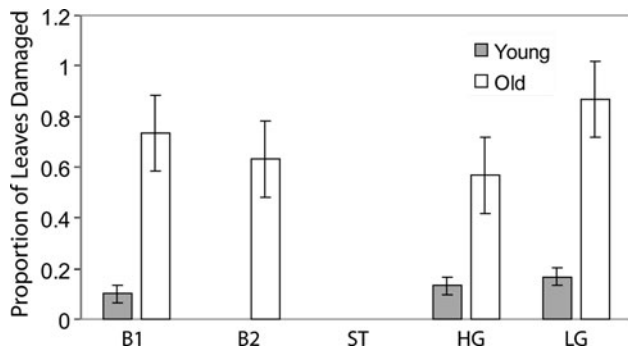

Fig. 2 Mean proportion of catalpol (±SE) present in young and old leaves of plants ($n = 10$) from five mullein populations in Utah and Colorado, USA. Population abbreviations as in Table 1. See Table 2 for ANOVA results

leaf age (Table 2). The main source of variation among populations was the lack of damage to any plant in population ST. Across populations, the proportion of young leaves with chewing damage was typically zero and never exceeded 17 % (population HG) of the leaves sampled in a given population. Conversely, the proportion of old leaves damaged was substantial, ranging from 57 % (population LG) to 87 % (population HG). As such, the proportion of young leaves damaged was significantly less than that of old leaves in all populations except ST, where none of the leaves were attacked, regardless of age (Fig. 3; Table 2). There was not a significant interaction between population and leaf age (Table 2), suggesting that overall, the distribution of damage between young and old leaves was similar across populations (with the only significant pairwise differences being that old leaves of population ST were significantly less damaged than old leaves of all other populations).

Table 2 ANOVA results of the effect of population, leaf age. and the interaction of population and leaf age on the percent dry weight of the indoid glycosides (IG) aucubin and catalpol, the total percent dry weight of IGs, the proportion of IGs composed of catalpol, and the proportion of leaves damaged by herbivores

Source	Aucubin		Catalpol		Total IG		Proportion Catalpol		Herbivory	
	F (df)	P	F (df)	P	F (df)	P	F (df)	P	F (df)	P
Fixed effects										
Population	14.5 (4)	<0.0001	24.5 (4)	<0.0001	23.2 (4)	<0.0001	8.5 (4)	<0.0001	2.5 (4)	0.05
Leaf age	198.9 (1)	<0.0001	767.1 (1)	<0.0001	625.1 (1)	<0.0001	434 (1)	<0.0001	17.1 (1)	<0.0001
Population × leaf Age	38.7 (4)	<0.0001	31.3 (4)	<0.0001	35.9 (4)	<0.0001	3.2 (4)	0.02	0.82 (4)	0.52

Fig. 3 Mean proportion (±SE) of young and old leaves damaged by chewing herbivores in five mullein populations in Utah and Colorado, USA. Population abbreviations as in Table 1. See "Methods and materials" for details of how damage was scored and analyzed

We note here that although it is possible that the observed differences in chewing damage between young and old leaves reflect that old leaves were simply exposed to herbivory for a longer period of time, there is compelling evidence that suggests otherwise. First, as part of a related project, an additional 26 introduced (US) and 21 native (European) mullein populations were surveyed for damage using the same basic protocol as detailed above, beginning 1 week after the completion of the surveys discussed here. We found that more than half of young leaves on European plants were damaged, in large part by the specialist herbivore *Cucullia verbasci* (Noctuidae), relative to only 16 % of young leaves on introduced plants (Alba et al. 2012). This provides evidence that low herbivory levels on young leaves in the introduced range reflect true avoidance behaviors by generalist herbivores. Second, in laboratory feeding trials, the generalist *Trichoplusia ni* (Noctuidae) significantly preferred old mullein leaves to young when given a choice (Alba, unpublished data; $n = 20$; $P = 0.02$).

Relationship between iridoid glycoside content and herbivory

In young leaves, plant size ($\beta = 0.01$; $t = 3.1$; $P = 0.003$), percent total iridoid glycoside content ($\beta = -1.5$;

$t = -2.6$; $P = 0.01$), and the proportion of iridoids composed of catalpol ($\beta = -1.3$; $t = -2.3$; $P = 0.02$) were all significantly correlated with leaf damage (Fig. 4a–c). Collinearity diagnostics indicated that the percent total iridoids was moderately collinear with the proportion of iridoids composed of catalpol (data not shown); however, we retained both predictors to achieve higher explanatory power ($R^2 = 0.23$) than possible with either of the single predictors (percent iridoids only, $R^2 = 0.13$; proportion catalpol only, $R^2 = 0.11$). For old leaves (model $R^2 = 0.42$), there was a positive relationship between plant size and leaf damage ($\beta = 0.01$; $t = -2.3$; $P = 0.02$), and a strongly negative relationship between percent total iridoids and leaf damage ($\beta = -7.8$; $t = -5.2$; $P < 0.0001$; Fig. 5). In contrast, there was no relationship between the proportion of iridoids composed of catalpol and damage ($\beta = -0.05$; $t = -0.07$; $P = 0.94$), and this term was removed from the final model.

Discussion

Here, we showed that percent total iridoid glycosides in rosette mullein plants can vary up to three times among populations when averaging over leaf age (Fig. 1; Table 2) and that the proportion of iridoids composed of the potentially more toxic compound catalpol significantly varies among populations as well (Fig. 2; Table 2). Such among-population variation is common across different plant species and defensive compounds (e.g., Krischik and Denno 1983; Zangerl and Berenbaum 1990) and has been demonstrated in other invasive weeds that contain iridoid glycosides (e.g., *Plantago lanceolata*, Adler et al. 1995; Darrow and Bowers 1997; Barton 2007; and *Linaria dalmatica*, Jamieson and Bowers 2010), as well as in bolting (reproductive) mullein plants, which can vary up to seven times in total iridoid glycoside content in the introduced range. (Alba et al. 2012). Chemical defenses can vary among populations for a variety of reasons ranging from underlying genetics to any number of environmental factors that elicit a plastic response in plants (e.g., Coley
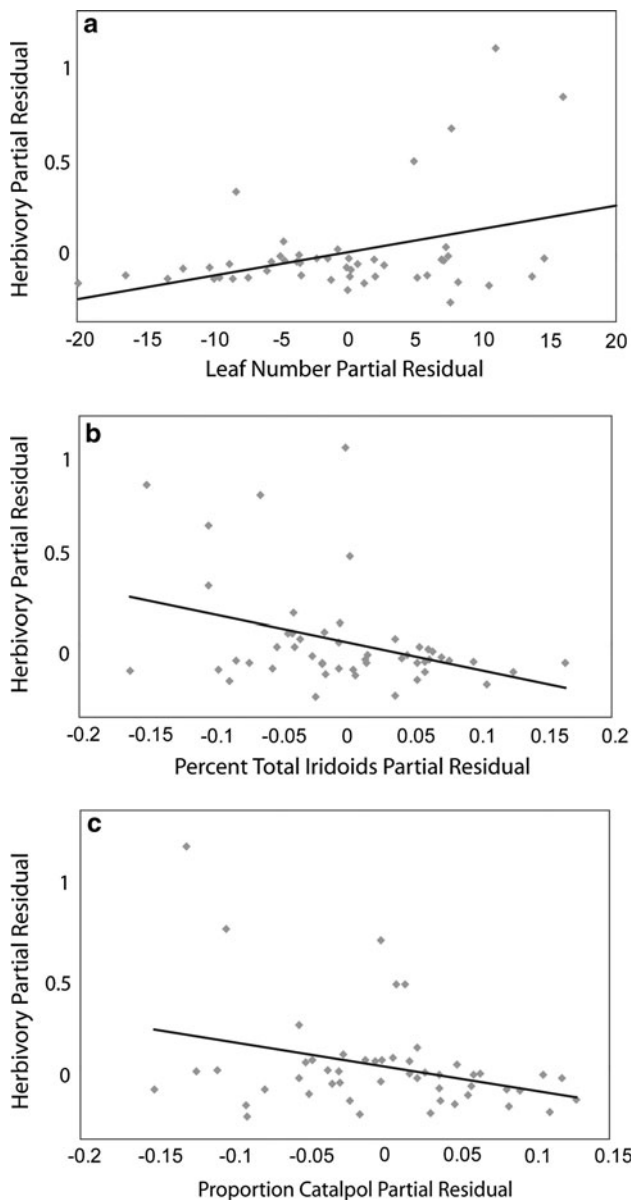
Fig. 4 Partial regression plots illustrating the relationship between herbivory by generalist herbivores and **a** plant size, **b** total percent iridoid glycosides (aucubin + catalpol), and **c** the proportion of iridoids composed of catalpol in young leaves of mullein plants. See text for associated results
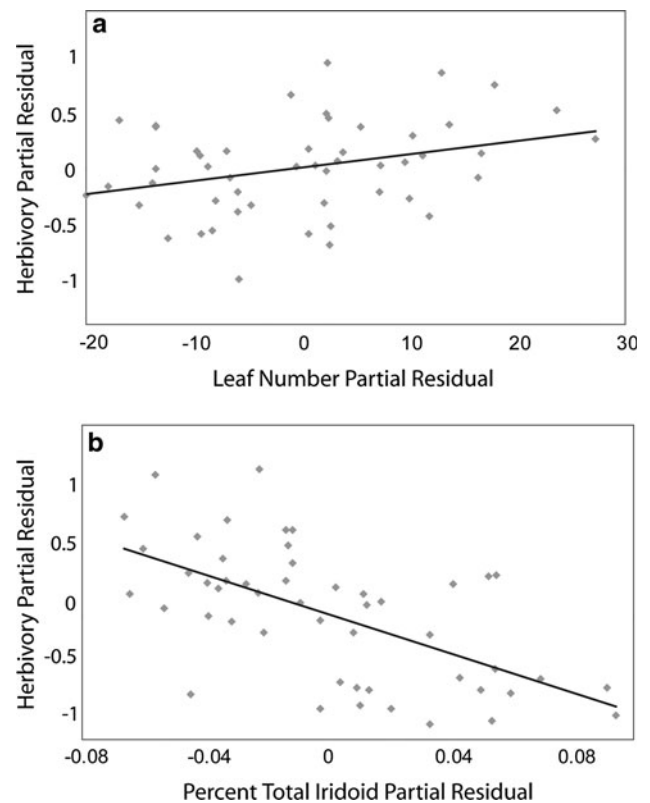

Fig. 5 Partial regression plots illustrating the relationship between herbivory by generalist herbivores and **a** plant size and **b** total percent iridoid glycosides (aucubin + catalpol) in old leaves of mullein plants. There was not a significant relationship between herbivory and the proportion of iridoids composed of catalpol. See text for associated results

1983; Coley et al. 1985; Fritz and Simms 1992; Karban and Baldwin 1997; Boege and Marquis 2005). It is likely that many of these sources contributed to the variability in percent total iridoids that we observed across our study locations. For example, mullein populations exhibit genetically based variation in percent total iridoids, with average concentrations across 14 populations ranging from <1 to ~2.5 % in plants grown from seed under green-house conditions (Alba et al. 2011). Additionally, differences in certain environmental factors such as insect attack, soil nutrient availability, UV light, and temperature

can alter iridoid content (Darrow and Bowers 1999; Jar-zomski et al. 2000; Tamura 2001; Fuchs and Bowers 2004; Wurst et al. 2008). Regardless of the source of variation, our results indicate that introduced mullein represents a heterogeneous food source for generalist herbivores in terms of iridoid glycoside content.

At the within-plant scale, young rosette leaves contained, on average, 6.5× more total iridoids and almost double the proportion of catalpol than older rosette leaves. Highly defended young leaves are typical of several other systems (e.g., Rhoades and Cates 1976; Coley 1983), which is consistent with the fact that young leaves are often more valuable to a plant than old leaves (sensu Optimal Defense Theory; McKey 1979; Feeny 1976). Young leaves typically contain more nitrogen or are more highly photo-synthetic than old leaves, and thus make a potentially greater contribution to future fitness than do old leaves (reviewed in Denno and McClure 1983). Given that dam-age to expanding (meristematic) tissue can be particularly problematic if it depresses subsequent growth (Ehrlen 1995), highly defended young leaves may represent a critical trait in mullein's invasion success in the introduced

range where generalists, which generally avoid high levels of iridoids, predominate. In contrast, in the native range, the presence of specialists that use iridoids as feeding and oviposition cues could render such high levels of iridoids a detriment (Alba et al. 2012).

Accordingly, as predicted, the distribution of herbivore damage clearly tracked within-plant investment in defense, with damage being skewed toward old leaves that had low concentrations of iridoid glycosides and lower proportions of catalpol (Figs. 1, 2, 3). This same pattern of highly defended young leaves with low levels of damage was also observed in bolting mullein plants from a different set of introduced populations. Indeed, the within-plant distributions of defense were identical, with young leaves containing 6.5× more total iridoids than old leaves (Alba et al. 2012). Taken together these findings suggest that within-plant variation and overall investment in defense is remarkably consistent across developmental stages in mullein, as opposed to other iridoid-containing plant species (Bowers and Stamp 1993; Darrow and Bowers 1997; Beninger et al. 2007, 2009; Jamieson and Bowers 2010). They further indicate that in the introduced range where generalist chewers predominate, plants avoid damage to valuable young leaves over the majority of their life cycle.

Furthermore, our data suggest a negative dosage-dependent effect of iridoid glycosides on herbivore feeding decisions, as predicted for systems where damage is caused mostly by generalist herbivores. Specifically, in the infrequent cases that generalists did feed on young leaves, increasing percent total iridoid glycosides and the proportion of iridoids composed of catalpol were both associated with decreasing amounts of damage (Fig. 4). In more commonly damaged older leaves, there was again a negative relationship between percent total iridoids and damage, while the proportion of catalpol did not correlate with feeding behavior (Fig. 5). Plant size was also correlated with herbivore feeding decisions, with larger plants incurring more damage to both young and old leaves (Figs. 4, 5). These findings indicate that generalist herbivores may strike a balance between avoiding high levels of iridoids, while at the same time foraging on more vigorous and potentially apparent plants, a phenomenon reported in several other systems (Price 1991; Cornelissen et al. 2008; Wilbur 2011).

Although it is difficult to assign causality based on field data, we contend that chemical defenses influenced herbivory and not otherwise (i.e., herbivory did not induce plant defenses). First, young mullein leaves have higher percent total iridoid glycosides and higher proportions of catalpol than old leaves when plants are grown in the absence of herbivory in the greenhouse (Alba, unpublished data), suggesting that the within-plant distribution in defenses that we observed in the field are constitutively

expressed and genetically based. Second, very few young leaves were damaged, and yet, they contained high levels of iridoids, a pattern inconsistent with induction as the driver of high levels of iridoids. Third, induction following damage usually alters the relative proportion of synthesized individual compounds (e.g., the ratio of catalpol to total iridoid glycosides; Bowers and Stamp 1993; Quintero and Bowers 2011). However, in this case, despite wide variation in levels of damage among and within populations, the proportion of iridoids composed of catalpol was similar across the landscape (Fig. 2).

In sum, expanding previous research on bolting mullein plants (Alba et al. 2012), we demonstrated that iridoid glycosides in rosettes of the invasive weed *Verbascum thapsus* vary significantly across populations and plant tissues (i.e., leaf age), and that within-plant variation in iridoids is a key feature explaining patterns of generalist herbivory. In addition, despite the long residence time of this introduced species, and the commonality of its chemical defenses in its introduced North American range, specialist chewing herbivores have not incorporated introduced mullein into their diets. Instead, we observed ecological interactions between introduced plants and generalist chewing herbivores, which have the potential to affect plant performance, and potentially, invasion dynamics.

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