

Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*

Peter A. Van Zandt and Anurag A. Agrawal

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Induced plant responses to herbivory appear to be universal, yet the degree to which they are specific to sets of herbivores is poorly understood. The generalist/specialist hypothesis predicts that generalist herbivores are more often negatively affected by host plant defenses, whereas specialists may be either unaffected by or attracted to these same “plant defenses”. Therefore, specialists should be less predictable than generalists in their responses to induced plant resistance traits. To better understand the variation in plant responses to herbivore attack, and the impacts these responses have on specialist herbivores, we conducted a series of experiments examining pairwise interactions between two specialist herbivores of the common milkweed (*Asclepias syriaca*). We damaged plants mechanically, with swamp milkweed beetles (*Labidomera clivicollis*), or with monarchs (*Danaus plexippus*), and then assessed specificity of elicitation, both by measuring a putative defensive trait (latex volume) and by challenging plants with insects of both species in bioassays. Latex production increased by 34% and 13% following beetle and monarch herbivory, respectively, but only beetles significantly elevated latex production compared to undamaged controls. While beetle growth was negatively affected by latex across all experiments, beetles were not affected by previous damage caused by conspecifics or by monarchs. In contrast, monarchs feeding on previously damaged plants were 20% smaller, and their response was the same on plants damaged mechanically or by either herbivore. Therefore, these specialist herbivores exhibit both specificity of elicitation in plant responses and specificity of effects in response to prior damage.

P. A. Van Zandt and A. A. Agrawal, Dept of Botany, Univ. of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2, Canada. Present address for P. A. Van Zandt: Dept of Biology, 1 Brookings, Campus Box 1137, Washington Univ., St. Louis, MO 63130, USA (vanzandt@biology2.wustl.edu).

Induced responses to herbivory are a ubiquitous phenomenon (Karban and Baldwin 1997), with plants exhibiting both heightened (Agrawal 1998, Stout et al. 1998, Traw and Dawson 2002a) and reduced (Carroll and Hoffman 1980, Danell and Huss-Danell 1985, Karban and Niiho 1995) resistance following damage, depending on the species and environmental conditions. Modifications in plant defensive phenotypes following herbivory can take several forms, including changes in toxic or antinutritive compounds, protein or nutritional constituents, leaf toughness, or density of thorns, spines,

or trichomes (Karban and Baldwin 1997). These changes in plant phenotype may induce resistance or susceptibility to subsequent herbivores. Resistance and susceptibility are defined from the herbivore’s perspective and are functions of both the types of plant changes following damage and the insects’ responses to those changes (Karban and Baldwin 1997). Consequently, specific changes in plant and insect responses can determine whether a damaged plant is resistant or susceptible, and therefore have the potential to alter the identity of the resultant herbivore community.

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Specificity in plant and insect responses may be categorized in two types: specificity of elicitation and specificity of effect (Karban and Baldwin 1997, Stout et al. 1998, Stout and Bostock 1999). Specificity of elicitation occurs when plants exhibit unique responses depending on the identity of the initial damaging herbivore. Such specificity can be assessed by measuring changes in chemical profiles following damage (Stout et al. 1998, Cipollini and Sipe 2001), or by measuring differences in performance in bioassays using a given species of challenge insect across plants damaged independently by two or more herbivore species (Stout et al. 1998, Agrawal 2000b, Agrawal and Karban 2000). In contrast, specificity of effect occurs when two or more insect species exhibit different preference for or performance on plants expressing a given induced phenotype, and is usually assessed with bioassays (Stout et al. 1998, Messina et al. 2002, Traw and Dawson 2002b).

An intuitively appealing hypothesis predicts that specialist and generalist herbivores have divergent reactions to induced plant responses (Carroll and Hoffman 1980, Dussourd 1993, Karban and Baldwin 1997, Agrawal and Karban 1999). Several lines of reasoning support this hypothesis. First, plant defenses in general are frequently more effective against generalists than specialists (Jermy 1984, Krischik et al. 1991, Rausher et al. 1993, Bolser and Hay 1998, Agrawal 1999, Agrawal 2000a). Presumably, this difference arises because of a long coevolutionary history between specialists and their host plants (Ehrllich and Raven 1964, Rausher 1996), whereby reciprocal evolution has resulted in specialists that are more often able to tolerate or detoxify their host's defensive compounds (Rhoades 1979, Berenbaum and Zangerl 1998, Stout and Bostock 1999), or even sequester them for their own defenses (Nishida 2002). Often, plant defenses are feeding or oviposition stimulants for specialists (Giamonstaris and Mithen 1995, Agrawal and Sherriffs 2001), reflecting the ecological costs of defensive chemicals (Simms 1992, Agrawal et al. 1999, Agrawal et al. 2002, Strauss et al. 2002). Finally, most cases of induced susceptibility involve specialist herbivores (Karban and Baldwin 1997), indicating that they are the most likely group of herbivores to benefit from prior herbivore attack. However, some specialists are also negatively impacted by plant defensive traits (Malcolm and Zalucki 1996, Van Der Meijden 1996, Agrawal and Kurashige 2003). Therefore, while generalists will be more often negatively affected by induced plant responses, specialists should vary more in their responses to induced plant traits (plant responses will often lead to either susceptibility or no adverse effect on specialists). This prediction has only been tested in one instance (Agrawal 2000b), and was not supported. In the present study, we examine two specialist herbivores of milkweed and test for specificity of elicitation and effect following initial herbivory. In particular, we take a

bioassay approach focusing on net effects on the herbivores and also assay one putative resistance trait: the volume of pressurized latex produced by the plant.

Methods

Natural History

These experiments used the common milkweed (*Asclepias syriaca* L.; Apocynales, Asclepiadaceae), and two of its specialist herbivores, larval monarch (*Danaus plexippus* L.; Lepidoptera, Danaidae) and swamp milkweed beetles (*Labidomera clivicollis* Kirby; Coleoptera, Chrysomelidae). The common milkweed is a native weedy plant that occurs throughout eastern North America in open habitats such as roadsides, pastures, and abandoned fields (Uva et al. 1997). It is the major food plant for migrating monarch adults and their larvae (Seiber et al. 1986, Malcolm et al. 1989). When damaged, milkweed plants immediately exude white latex that serves as a physical barrier to herbivores (Zalucki et al. 2001a). This latex, along with all other plant parts, contains cardenolides of varying polarity and effectiveness against both specialist and generalist herbivores (Malcolm and Zalucki 1996, Zalucki et al. 2001a,b). Cardenolide concentrations increase quickly (reaching a maximum at 24 hours) following mechanical damage, and return to constitutive levels by 6 days (Malcolm and Zalucki 1996).

The swamp milkweed beetle occurs in moist to mesic habitats in eastern and central North America where *Asclepias* species are present (Eickwort 1977, Price and Willson 1979, Palmer 1985). It typically occurs in very low numbers (Eickwort 1977), but can reach moderate densities where it has been observed to cause substantial damage on *A. syriaca* and *A. incarnata* (N. Cappuccino, pers. comm., and Eickwort 1971, Price and Willson 1979). Both larval and adult *L. clivicollis* feed exclusively on foliar tissue of Asclepiads (Price and Willson 1979, Palmer 1985). Although it is unknown whether milkweed beetles are sensitive to milkweed resistance traits, their characteristic trenching behavior (Dussourd and Eisner 1987) suggests that latex is detrimental to growth.

Monarchs have a more cosmopolitan distribution and a slightly broader host plant range within the *Asclepiadaceae*. Larvae feed on *A. syriaca*, as well as most of the other common North American milkweed species (Urquhart 1987, Zalucki et al. 2001a). Despite being milkweed specialists, monarchs are sensitive to both the presence of latex as well as the concentration and polarity of cardenolides (Malcolm and Zalucki 1996, Zalucki et al. 2001b), and also disarm plant latex supply through notching of leaves (Dussourd and Eisner 1987).

General methodology

We conducted a series of ten independent experiments (henceforth, trials) to test the hypothesis that initial herbivory causes induced plant responses that affect subsequent herbivores, and that these effects are dependent on the identity of the damaging herbivore and the assay herbivore (Table 1). We conducted a series of multiple trials because of limitations in the number of insects and growth space. Although conditions varied slightly between trials, they all tested a set of closely related hypotheses using standard methods, thus the consistency of our results demonstrates an element of robustness across conditions.

In each trial, an equal sub-set of plants were treated with 3rd or 5th instar monarch or beetle larvae (Table 1), which were allowed to damage plants for 3–9 days depending on their rate of damage (damage rates were lower in trials 1 and 2 due to cool evening temperatures). Another sub-set of plants was left as undamaged controls. The target level of damage was removal of approximately 20% of leaf area, as estimated visually on entire plants (mean percent damage imposed across all trials \pm SEM = 16.8 ± 0.72). Plants were allowed to recover from treatment damage for 2–5 days, and then were measured for latex production and challenged with assaying insects (beetle or monarch larvae).

For the insect bioassays, we used larvae from maintained colonies, and all bioassays were conducted with

neonates that had not fed prior to being placed on experimental plants. For each plant, a single assay insect was placed on the undamaged apical leaves and was allowed to move and feed freely for 3–4 days on the plant. They were then removed, dried at 60°C, and weighed to the nearest microgram on a Mettler-Toledo UMT-2 balance (Hightstown, NJ). In one trial (trial 4), we assessed the specificity of elicitation and specificity of monarch response in a factorial design with monarch and mechanical damage as the two treatments. For the mechanical damage treatment, we took one 5 cm² core out of a single leaf with a cork borer. We mechanically damaged plants on the same day that damaging herbivores were removed to synchronize plant responses between the treatments.

To estimate latex production, we selected the youngest fully expanded, undamaged apical leaf for consistency and pricked one primary laticifer on each side of the midvein. We then soaked up all the exuded latex with a 1 cm diameter filter paper disc (No. 1 Whatman International, Maidstone, U.K.), dried them at 60°C, and weighed each disc to the nearest microgram. This method mimics the vein cutting behavior of adult and larval beetles, as well as 3rd and 4th instar monarchs, and has shown consistent differences in latex production between full sibling plant families in both the field and laboratory (Van Zandt and Agrawal, unpubl.). This method of assessing latex production is a reasonable

Table 1. Summary of experimental trials. Treatments included undamaged plants (control), monarchs, beetles, or mechanical damage, and the number of plants treated in each category is listed in parentheses. Total N = the number of plants initiated in each trial, but recovery of bioassay insects was always lower. In trial 4, mechanical damage and monarch damage were crossed factorially; therefore N for all treatments does not equal total N.

Trial	Location	Treatments	Assay insects	Latex assessed?	Total N
1	field	beetle (44) control (44)	beetles monarchs	no	88
2	field	beetle (39) control (39)	beetles	yes (78)	78
3	chamber	monarch (36) control (37)	monarchs	no	73
4	chamber	monarch (68) mechanical (68) control (34)	beetles monarchs	yes (68)	136
5	chamber	monarch (46) control (41)	beetles monarchs	no	87
6	chamber	monarch (27) beetle (26) control (24)	beetles	yes (40)	77
7	chamber	monarch (11) beetle (14) control (12)	beetles	yes (15)	37
8	chamber	monarch (37) beetle (37) control (37)	beetles	yes (51)	111
9	chamber	monarch (16) beetle (15) control (16)	beetles	yes (47)	47
10	chamber	beetle (23) control (22)	beetles	yes (45)	45

index of what herbivores encounter, and correlates with herbivore growth (Agrawal, unpubl., this study).

Plant rearing

All trials used plants derived from seeds from 7–13 milkweed plants from the University of Toronto's Koffler Scientific Reserve at Joker's Hill, near Newmarket, Ontario, Canada (44°03'N, 79°29'W). Germinated seedlings for each trial were planted in 500 ml pots in Pro-Mix BX soil (Red Hill, PA) supplemented with approximately 0.6 g of slow-release Nutricote fertilizer pellets (13:13:13 N:P:K, Vicksburg Chemical, Vicksburg, MS). For trials 2–10, plants were enclosed in spun bond polyester fiber bags (Rockingham Opportunities Corporation, Reidsville, NC) to keep assay herbivores on individual plants and exclude other insects.

Two trials were performed at Joker's Hill, one of which (trial 1) was conducted on plants in 500 ml plots that were housed outside in a 4 × 2 × 3 m screened enclosure to exclude herbivores and predators. For this trial, we used 160 5-week-old plants that had an average of 10 true leaves. Plants were not bagged, but were arranged in eight spatial blocks (trays). Treatments and plants were randomized within the enclosure. Beetles moved among plants within blocks in this trial, therefore, we averaged masses for assaying beetles for all plants within each block (n = 8 replicate blocks for beetles). Monarchs remained on individual plants, so we treated each plant as a replicate for these assays. For the second field trial (trial 2), we randomly planted 82 5-week-old plants in a tilled, unweeded field, enclosed them in bags, and allowed them to grow undamaged for 3 months. In this trial, we utilized 12 full-sibling families of milkweed so that we could assess family level variation in latex production and its genetic correlation with herbivore growth. The remaining trials (3–10) were carried out in growth chambers on the University of Toronto campus, with each trial utilizing 5-week-old plants fertilized as above and grown in 16:8 day:night cycle at 26°:24°C (Table 1).

Statistical methods

We assessed the effects of treatment and trials on bioassay insect biomass with PROC GLM utilizing type III sums of squares and assessed treatment effects on larval recovery with PROC LOGISTIC using SAS version 8.2 (SAS 2001). We compared least squared means using Tukey-Kramer multiple post-hoc comparisons to assess differences between treatments. Trials were considered as blocks in each analysis because they were designed to test comparable hypotheses and were conducted similarly. This partitioned spatial and temporal variation among trials into the block term. The

trials assessing the response of monarchs to beetle (trial 1), monarch (trials 3–5), and mechanical damage (trial 4) were not conducted factorially with all treatments at the same time. Therefore, they were analyzed and presented separately. Conversely, trials assessing beetle performance were conducted with monarch and beetle damaged plants. Data were log transformed where necessary to improve homogeneity of variances and normality of residuals, but untransformed least squared means and standard errors (SEs) are reported in the figures. Differences in degrees of freedom between trials and treatments result from differential recovery of bioassay insects across trials and treatments.

Not all plants were assayed for latex production; consequently, this variable could not be used as a covariate in an analysis of all the data. Since control plants lacked any damage, we did not use damage level as a covariate. Because of the problems associated with performing regressions on residuals (Garcia-Berthou 2001), we were unable to account for the effects of latex production or previous damage with a model that included treatment and trial effects. Therefore, after separate ANOVAs on the complete data-set for each insect responder, we performed simple linear regressions of latex and prior damage on insect mass.

Results

The insects fed on different parts of milkweed plants: while monarch larvae tended to remain on apical leaves, beetle larvae moved immediately to the most basal leaves to feed. Initial herbivory in the beetle and monarch treatments did not differ (mean percentage of plant damage ± SE: beetles 20.2 ± 1.15, monarchs 19.0 ± 1.34; $F_{1,382} = 0.39$, $P = 0.56$). We found a trend for plants damaged by either monarchs or beetles to have higher induced latex production, although only those plants damaged by beetles produced significantly more latex than controls in post-hoc comparisons (Table 2, Fig. 1). Monarch damaged plants had 13% more latex than controls, while those damaged by beetles exhibited a 34% increase compared to undamaged plants, suggesting specificity of elicitation. Latex production across all treatments was positively correlated with the level of plant damage, both with (n = 343, $r = 0.13$, $P = 0.016$)

Table 2. Two-way ANOVA for effects of beetle and monarch damage on log transformed latex production. The analysis was conducted on seven trials (2, 4, 6–10).

Source	DF	MS	F	P
Model	8	2.93	27.88	< 0.0001
Treatment	2	0.63	5.98	0.0028
Trial	6	2.97	28.33	< 0.0001
Error	336	0.10		

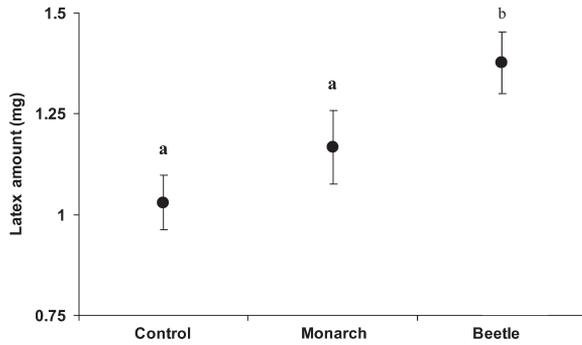


Fig. 1. Plant latex volume from all seven trials where latex volume was assessed for undamaged controls or following damage by monarch, or *Labidomera* larvae (Table 1). Data are least squared means ± 1 SE, and unique letters above symbols represent groups that differ significantly following Tukey-Kramer adjusted post-hoc contrasts.

and without ($n = 239$, $r = 0.29$, $P < 0.001$) the inclusion of undamaged plants in the correlation.

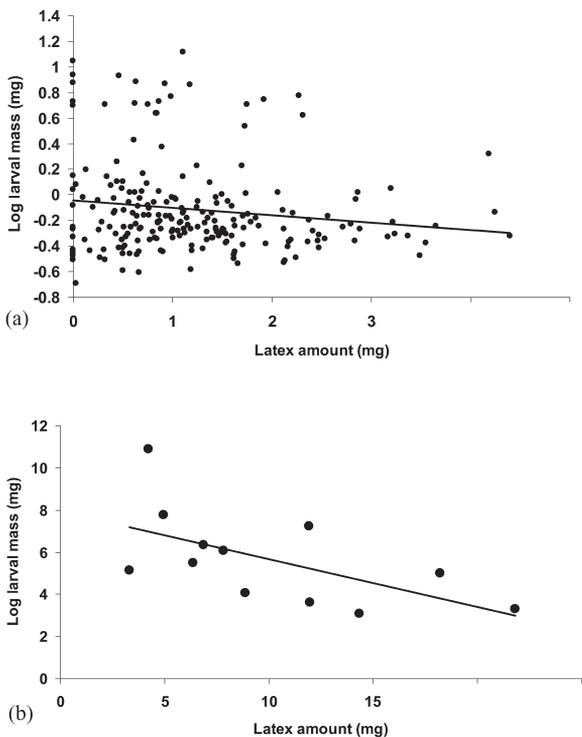


Fig. 2. (a) Phenotypic and (b) genotypic (milkweed full-sib family means) correlations between *Labidomera* dry larval biomass and plant latex production. Data for (a) are from trials 2, 4, and 6–10, and for (b) are from trial 2.

Across all trials, beetle mass decreased with increasing latex production in plants (Fig. 2a, $n = 214$, $r = -0.35$, $P = 0.03$). In trial 2, beetles also grew the slowest on milkweed families that produced the highest amounts of latex (Fig. 2b, Pearson $r = -0.65$, $P = 0.024$). However, beetles did not exhibit altered growth on plants previously damaged by monarchs or beetles relative to controls ($F_{2,365} = 0.37$, $P = 0.69$). The level of plant damage did not correlate with beetle growth ($n = 367$, $r = -0.002$, $P = 0.96$). Beetle recovery was very low on average (54%), but there was no effect of damage type on the recovery rate ($n = 189$; $df = 2$, Wald $\chi^2 = 1.50$, $P = 0.47$).

In contrast to the results for beetle growth, monarchs grew approximately 20% less on plants damaged by beetles (Fig. 3a, $F_{1,69} = 6.83$, $P = 0.011$) or by monarchs (Fig. 3b, $F_{1,110} = 11.45$, $P = 0.001$), compared to control plants. Monarch recovery was very high from both damaged and control plants, averaging 92%, and there was no difference in recovery based on prior herbivory type ($n = 219$, $df = 2$, Wald $\chi^2 = 3.87$, $P = 0.14$). In the experiment crossing mechanical and monarch damage, monarch larvae again grew best on undamaged plants

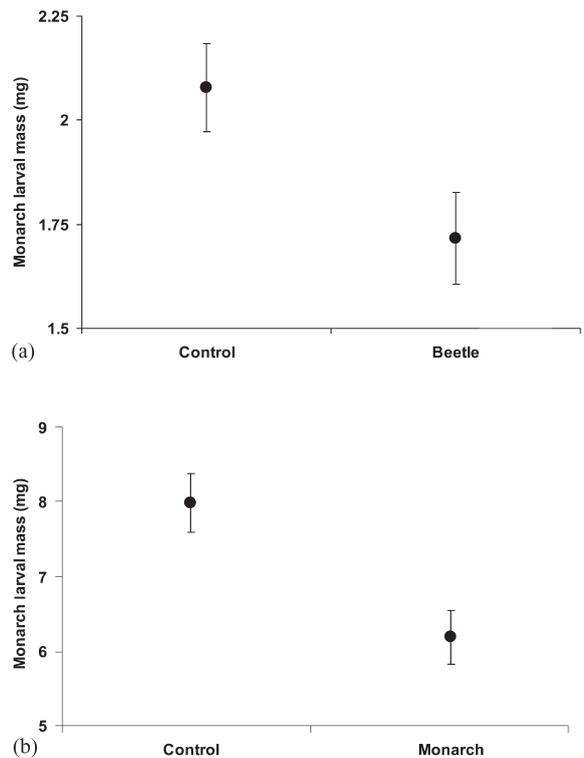


Fig. 3. Induced resistance measured by monarch larval growth on milkweed plants damaged by (a) *Labidomera* beetles (total $n = 71$; trial 1) and (b) monarch larvae (total $n = 112$; trials 3 and 5). Data are least squared means ± 1 SE.

Table 3. Two-way ANOVA for effects of monarch and mechanical damage on monarch larval mass. Data are from trial 4.

Source	DF	MS	F	P
Model	3	0.80	3.38	0.024
Mechanical	1	1.11	4.72	0.034
Monarch	1	0.06	0.25	0.62
Mechanical \times monarch	1	1.18	4.99	0.029
Error	63	0.24		

(Table 3), but there was no difference among any of the damaged treatments (Fig. 4), indicating that instantaneous mechanical damage and initial monarch feeding have the same consequences for monarchs. Monarch growth was not correlated with levels of latex production in plants ($n = 36$, $r = -0.09$, $P = 0.68$), or with levels of plant damage ($n = 182$, $r = -0.04$, $P = 0.30$).

Discussion

Induced responses of milkweed

The common milkweed alters its phenotype after both insect herbivory and mechanical damage, consistent with findings for several other plant species (Malcolm and Zalucki 1996, Karban and Baldwin 1997). While we did not measure changes in cardenolide profiles, Malcolm and Zalucki (1996) have shown that cardenolides increase 4-fold following mechanical damage, and preliminary results show that cardenolides are inducible following herbivory by monarchs (Agrawal, unpubl.). We show that plant latex production increases up to 34% following insect damage, and that this latex negatively affects beetles as indicated in both phenotypic (Fig. 2a) and genetic (Fig. 2b) correlations. However, changes in plant traits did not affect the growth rate of first instar beetle larvae in a consistently negative way. In fact, two trials indicated near significant induced susceptibility when analyzed separately (results not shown). This apparent inconsistency may be because the relatively weak relationship between induced latex production and beetle growth (Fig. 2a) was outweighed by other factors.

It appears that although milkweed defensive traits change following herbivory, beetles are not strongly affected, while monarch larvae are sensitive to these changes. Contrary to the response of beetles, we did not show a significant affect of latex on monarch performance. It should be noted, however, that the current test of the effects of latex on monarchs is preliminary due to relatively small sample size. In an experiment assessing monarch performance across 30 species of *Asclepias*, monarch growth was negatively correlated with latex production (Agrawal, unpubl.). Additionally, others have shown that constitutive production of latex, cardenolides, and other undetermined factors all negatively affect first instar monarch survival (Zalucki et al.

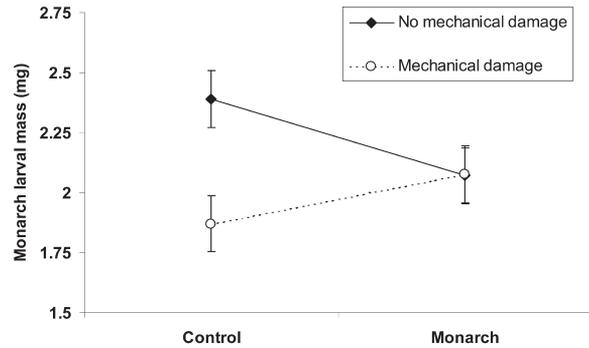


Fig. 4. Induced plant resistance measured by monarch larval growth in a factorial trial with mechanical damage and previous damage by 3rd instar monarch larvae as main effects ($n = 67$; trial 4). Data are least squared means ± 1 SE.

1990, Malcolm 1995, Malcolm and Zalucki 1996, Zalucki et al. 2001a, b), and miring in latex is often observed in first instar monarchs (Zalucki et al. 2001b, Agrawal and Van Zandt, pers. obs.), but rarely for later instars.

Specificity of elicitation and effect

Plants show an amazing ability to detect specific stimuli, including caterpillar footsteps (Bown et al. 2002), oviposition (Wegener et al. 2001, Hilker and Meiners 2002), and volatiles from neighboring plants (Karbon et al. 2000). Specificity of elicitation has also been demonstrated in response to different salivary constituents (reviewed by Dicke 1999, Felton and Eichenseer 1999). Most studies that have attempted to characterize the chemical responses of plants following herbivore or pathogen attack have also demonstrated changes unique to the species of attacker (De Moraes et al. 1998, Stout and Bostock 1999, Traw and Dawson 2002b). Given the broad array of plant changes to unique stimuli, it is likely that specificity of elicitation will at some level be a universal phenomenon (Stout et al. 1998, Stout and Bostock 1999).

Specificity of elicitation was indicated in this study by the differential induction of latex by milkweed herbivores, where latex production was significantly higher in beetle-damaged plants than either monarch damaged plants or controls. This difference was not caused by dissimilar amounts of initial (treatment) damage, as beetle and monarch treatments had similar levels of initial damage. Therefore, it appears that milkweed latex production is more responsive to the type, rather than the extent of damage. The proximate cues responsible for this differentiation may include salivary constituents or the location that each insect feeds. Further work will be

required to characterize plant responses to each of these cues.

While plants can clearly exhibit a wide range of specific responses to stimuli, specificity in elicitation is neither necessary nor sufficient for specificity of effect on herbivores, which occurs when different herbivores have differential sensitivity to plant defenses. Consequently, despite much evidence for specificity in plant elicitation, the current belief is that specificity in effect is uncommon, since most anti-herbivore defenses are rather broad in their mode of action (Karban and Baldwin 1997, Karban and Kuć 1999, Stout and Bostock 1999). For example, cucurbitacins (bitter terpenoid compounds present in the Cucurbitaceae), glucosinolates (characteristic glycosides of the Brassicaceae), and cardenolides are generalized feeding deterrents for most herbivores. However, the fact that these compounds are attractive to specialist herbivores (Hicks 1974, Blau et al. 1978, Carroll and Hoffman 1980, Giamonstaris and Mithen 1995, McCloud et al. 1995, Tallamy et al. 1997), suggests at minimum that specificity in effect for constitutive defenses may be widespread.

Several cases of specificity of effect in induced plants have recently been documented (Stout et al. 1998, Agrawal and Karban 2000), further extending our recognition of the extent of specificity in plant/herbivore interactions. In studies explicitly examining specificity of effect, induced plants often vary in resource quality for subsequent herbivores. For example, Agrawal (2000b) showed that wild radish plants damaged by each of four lepidopteran herbivore species differentially induced resistance for the same four species, showing both specificity of elicitation and effect. Similarly, Messina et al. (2002) found that two aphid species feeding on wheat exhibited varying responses to prior feeding by aphids, also showing specificity of elicitation and effect.

Some authors have shown that specialist and generalist herbivores may differ in their responses to previously damaged plants (Carroll and Hoffman 1980, Agrawal and Karban 1999, Agrawal et al. 1999). Presumably this is because specialists have evolved counter-defenses to plant resistance traits (Dussourd and Eisner 1987, Dussourd 1993), or have used plant defensive compounds as feeding or oviposition stimulants (Apriyanto and Potter 1990, Giamonstaris and Mithen 1995). To date, the only study to compare the specificity of effect for generalist and specialist herbivores on induced plants has found that the generalist/specialist dichotomy did not accurately predict herbivore responses (Agrawal 2000b).

In the current study, beetles responded as predicted for specialists, while monarchs acted more like generalists in response to changes in plant quality following prior damage. This finding demonstrates that even specialist herbivores of chemically defended plants can vary in their susceptibility to these defenses. The fact

that both herbivores responded uniquely to plant induction is still consistent with predictions based on feeding specialization, since the expectation is that on average, generalists will be strongly affected by plant compounds while specialists will be more variable in their sensitivity. Despite differences in methodology (e.g. mechanical damage applied at one instance compared to 3–4 day damage by herbivores), responses of monarchs were consistently negative following all types of damage. This suggests that early instar monarchs are sensitive to plant responses following damage and that the stimuli required to decrease monarch performance can be quite varied. Possibly, this is because monarchs have a slightly broader diet range, thus making them more susceptible to variation in cardenolide levels as first instar larvae. This explanation arises from the observation that despite their use of high cardenolide *Asclepiads* (Malcolm 1991), monarchs are strongly impacted by both latex and cardenolide concentrations (Zalucki and Malcolm 1999, Zalucki et al. 2001b).

Community predictions

Changes in plant defensive traits are commonly detected in controlled experiments; however, it remains a challenge to demonstrate their importance at the population and community levels (Karban and Carey 1984, Hartley and Lawton 1987, Thaler et al. 2001, Underwood and Rausher 2002). Induced plant responses may affect the growth rate of subsequent herbivores, but if their effects are not detectable beyond the individual herbivore, then they are not likely to play significant ecological or evolutionary roles. One way that induced responses may be important is if they alter the composition of the herbivore community on previously damaged plants relative to undamaged plants, but the feasibility of this scaling is dependent on whether there is specificity of elicitation or effect; i.e. whether insect species are entirely interchangeable in their interactions as mediated through the host plant (no specificity of elicitation or effect). In the current study, specialist herbivores of milkweed responded differently to previously damaged host plants. Scaling the effects of induced plant responses to changes in the herbivorous insect community, therefore, is more difficult in this system, given the variability that these herbivores display. This difficulty echoes similar concerns that exist for the accuracy of a priori quantification of the consequences of indirect effects in other communities (Wootton 1994).

In summary, our study demonstrates three levels of specificity in the milkweed system. First, damage by beetles and monarchs differentially alter latex production, resulting in specificity of elicitation. Second, these two herbivores differ in their response to latex, with beetles exhibiting more sensitivity than monarchs. Fi-

nally, divergent responses of monarchs and beetles to previously damaged plants demonstrate specificity of effect for these two specialist herbivores.

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