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Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds

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Summary

1. Plant-mediated interactions between co-occurring herbivores play an important role in insect herbivore communities. Although induced resistance pathways associated with jasmonic acid and salicylic acid are often implicated in such plant-mediated interactions, there are few examples from non-model systems involving specialized herbivores that regularly interact in nature.

2. Here, we tested reciprocal impacts between co-occurring specialist herbivores from two feeding guilds, monarch caterpillars *Danaus plexippus* and oleander aphids *Aphis nerii,* on two co-occurring and closely related, but defensively contrasting milkweeds, *Asclepias syriaca* and *A. tuberosa*.

3. Larvae grew 38% faster on aphid-infested *A. syriaca* compared to controls. Reciprocally, aphid growth was >50% lower on caterpillar-damaged *A. syriaca* compared to controls. While caterpillar feeding on *A. syriaca* induced a jasmonate burst and higher defensive end products (cardenolides and latex), this induction was substantially attenuated in the presence of aphids. We found a negative correlation between jasmonic acid and salicylic acid only on *A. syriaca* infested by both caterpillars an aphids.

4. *Asclepias tuberosa* displayed distinct hormonal dynamics and lacked induction of defensive end products. Accordingly, we found no evidence for plant-mediated interactions between monarchs and aphids on *A. tuberosa*.

5. Thus, *A. syriaca* has specific responses to each herbivore, but if challenged simultaneously, the outcome is asymmetric: monarchs benefit from defence attenuation by aphids, while aphids are impaired by monarch feeding.

6. Our results suggest phytohormonal trade-offs induced by two feeding guilds can differ between closely related plant species, and our notion of trade-offs in defence based on phytohormonal pathways would improve with further comparative designs from both model and non-model systems.

Key-words: cardenolides, herbivores, induced plant resistance, interspecific competition, jasmonic acid, latex, salicylic acid, specialist

Introduction

Plants are confronted with a diverse array of biotic attackers and have apparently evolved sets of inducible defences as means to maximize fitness (Karban & Baldwin 1997; Agrawal 2011; Mouttet *et al.* 2013). Because the chemical phenotype of a plant can change in highly specific ways in response to herbivore feeding, subsequent species interactions may result from altered insect preference and performance (Kessler & Baldwin 2002; Viswanathan, Narwani & Thaler 2005; Dicke, van Loon & Soler 2009). Indeed, plant-mediated interactions between insect herbivores are recognized as a major structuring force in herbivore communities, where competition and facilitation can occur (Denno, McClure & Ott 1995; Kaplan & Denno 2007).

Competition theory predicts that two organisms engaged in a reciprocal struggle for resources are most intense with co-occurrence and similarity in feeding guild (Janzen 1973; Denno, McClure & Ott 1995). Furthermore, knowledge of biochemical pathways suggests that herbivores differing in their feeding guild will induce distinct defence pathways, often to the benefit or detriment of other attackers (Zarate,

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Kempema & Walling 2007; Walling 2008; Thaler, Agrawal & Halitschke 2010; Bidart-Bouzat & Kliebenstein 2011). Insect damage triggers plant defence signalling pathways in which the plant hormones jasmonic acid (JA) and salicylic acid (SA) play important roles (Pieterse & Dicke 2007). Many studies have suggested the involvement of SA in response to phloem-sucking insects (Walling 2000; Mewis *et al*. 2006; Goggin 2007; Broekgaarden *et al*. 2011), whereas plant response to chewing herbivores (mainly Lepidopterans) is typically induced via JA (and often ethylene, ET) (Kessler & Baldwin 2002; De Vos *et al*. 2005). The role SA has in plants fed on by aphids may have direct defence effects from end products that SA induces or play an alternative beneficial role due to signal interactions (i.e. 'crosstalk') with JA (Goggin 2007).

Crosstalk between these hormone signal-transduction pathways is thought to equip the plant with the regulatory capacity to finely tune its responses (Thaler, Humphrey & Whiteman 2012). However, there is evidence that the induction of the salicylate pathway by phloem-feeding insects effectively suppresses plant responses to caterpillar feeding (Walling 2008), and studies have demonstrated that plant resistance to caterpillars is weakened when in the presence of aphids (Stout *et al*. 1998; Rodriguez-Saona, Crafts-Brandner & Cañas 2003; Rodriguez-Saona *et al*. 2005; Schwartzberg, Böröczky & Tumlinson 2011). Such responses may be maladaptive for the plant if caterpillars impose stronger fitness losses than aphids. The reciprocal effect of caterpillar feeding and associated JA induction appears to often be negative for aphids (Agrawal 1998; Thaler *et al*. 2001; Cooper & Goggin 2005; Walling 2008). Accordingly, the relationship between aphid feeding and induction of the SA pathway may be beneficial to the aphid. There is growing evidence of genetic variation within a plant species allowing for altered induced responses (Agrawal 2002; Wu *et al*. 2008; Bingham & Agrawal 2010; Uesugi, Poelman & Kessler 2013), thus suggesting the potential for plant-mediated interactions between herbivores to select for alternative defence strategies.

Remarkably, most studies to date documenting plant specificity to insects in different feeding guilds have neither rigorously examined reciprocal interactions (Heidel & Baldwin 2004; Mewis *et al*. 2006; but see: Rodriguez-Saona *et al*. 2010; Stout *et al*. 1998; Erb *et al*. 2011) nor tested for specificity among related plants (Ali & Agrawal 2012). Furthermore, despite the often-accepted paradigm of a JA-SA trade-off, there are still substantial gaps in our ability to predict the outcome of inter-guild interactions mediated through phytohormones. First, studies often lack natural phylogenetic contrast species which may show divergent relationships of plants with herbivores. Closely related but contrasting plant species, or genotypes from highly divergent populations of the same species, may be informative about the importance of signal interactions for ecological outcomes (Schuman *et al*. 2009; Uesugi, Poelman & Kessler 2013). Secondly, despite the reciprocal antagonism of JA and SA often being observed in well-studied model systems (i.e. *Arabidopsis*, tomato, tobacco), many studies only measure differential plant responses (e.g. volatiles, transcription, defence compounds) to generalist herbivores and lack bioassays of naturally co-occurring, specialized insects. Plant variation in specificity can affect relative competitive strengths among herbivore species on individual plants and may promote coexistence or conflict between herbivore species (Erb *et al*. 2011; Uesugi, Poelman & Kessler 2013). Here, we utilize two specialist herbivores attacking milkweed, *Asclepias* spp., to specifically address plant-mediated interactions between a chewing and sucking herbivores on two closely related plant species.

The community of insects attacking milkweed have evolved various mechanisms to cope with the array of defensive traits expressed by this plant. For example, milkweed exudes sticky latex following leaf damage by chewing herbivores as a physical barrier, yet chewing herbivores can circumvent latex exudation by trenching the laticifers (Dussourd & Eisner 1987). Similarly, cardenolides are produced and stored throughout the plant, but specialist herbivores have evolved various physiological adaptations to tolerate these toxic compounds (Rasmann, Johnson & Agrawal 2009; Agrawal *et al*. 2012a; Dobler *et al*. 2012). We chose two members of the natural community of milkweed that commonly feed simultaneously on the same plant, the monarch butterfly, *Danaus plexippus*, and the oleander aphid, *Aphis nerii*, to quantify their impact on plant physiology and on each other in a set of bioassays. We compared their interactions on two closely related *Asclepias* spp. (*A. syriaca* and *A. tuberosa*) that differ in levels of cardenolides, latex exudation (Malcolm 1991; Agrawal & Fishbein 2006), and have different hormonal dynamics (A. A. Agrawal, unpublished manuscript).

Specifically, we asked the following questions: (i) Are there reciprocal plant-mediated interactions between monarchs and aphids on milkweed? (ii) To what extent are these effects mediated by specific plant defensive responses (including interactions between biochemical signalling pathways) to the two herbivores? And, (iii) are the plant-mediated effects concordant or distinct on two milkweeds with divergent defensive strategies?

Materials and methods

PLANTS

Bioassay experiments were carried out on common milkweed (*Asclepias syriaca* L.; Apocynaceae) and butterfly weed (*A. tuberosa* L.). Both *A. syriaca* and *A. tuberosa* are native perennial plants that occur throughout eastern North America in open habitats such as roadsides, pastures and abandoned fields. When damaged, milkweed plants typically exude sticky latex that serves as a physical barrier to herbivores (Zalucki, Brower & Alonso 2001). This latex, along with all other plant parts, contains cardenolides of varying polarity and effectiveness against both specialist and generalist herbivores (Malcolm & Zalucki 1996; Zalucki, Brower & Alonso 2001). Cardenolides are bitter-tasting steroids that have

toxic effects on most animals by disrupting the sodium and potassium flux in cells (Malcolm 1991; Agrawal *et al.* 2012a). Cardenolides are present constitutively in milkweed, and their production is also known to be induced following damage by several species of foliage-chewing herbivores (Agrawal *et al.* 2012a). *Asclepias tuberosa* is a closely related but contrasting species to *A. syriaca*, known to have little if any cardenolides or latex exudation (Malcolm & Brower 1989; Agrawal & Fishbein 2006; Zehnder & Hunter 2007), and shows weak phytohormonal induction following herbivory (A. A. Agrawal, unpublished manuscript).

All bioassay experiments were conducted on young plants (30–45 days old) grown from seed. Seeds of *A. syriaca* and *A. tuberosa* (collected from Tompkins County, NY, USA) were germinated at room temperature after being stratified at 4 °C on moist filter paper for 2 weeks. Seedlings were planted into potting soil (10-cm-diameter pots) and grown completely randomized in a growth chamber (14-h daylight, 26 °C day: 20 °C night). Plants were fertilized [N : P : K 21 : 5 : 20, 150 ppm N (μg g^{-1})] once, 1 week after planting.

CATERPILLARS

Monarch larvae *D. plexippus* feed on *A. syriaca*, as well as most of the other common North American milkweed species. Despite being milkweed specialists (and having the ability to sequester cardenolides), monarchs are sensitive to both the presence of latex and cardenolides (Zalucki, Brower & Alonso 2001; Agrawal *et al. in press*). For the bioassays, we used freshly hatched larvae from eggs collected from a large colony maintained on greenhouse-grown *A. curassavica* in Florida, USA.

APHIDS

Aphis nerii feeds broadly within the Apocynaceae (Blackman & Eastop 2006) and is known to sequester cardenolides (Rothschild, Euw & Reichstein 1970; Mooney, Jones & Agrawal 2008). *Aphis nerii* feeds in dense aggregations on apical leaves, but also feeds on the underside of lower leaves when apical leaves are crowded and thus unavailable. Aphids used for bioassays were maintained in a laboratory colony on greenhouse-grown *A. syriaca* from aphids collected in Minnesota, USA. Aphids and we maintained on milkweed plants in a growth chamber (14-h daylight, 26 °C day: 20 °C night) with new milkweed seedlings introduced as needed.

MONARCH BIOASSAY

At the beginning of the experiment, six aphids were placed on the most apical leaves of 4-week-old plants of either *A. syriaca* ($n = 15$) or *A. tuberosa* ($n = 15$) and allowed to reproduce for 5 days, while a second equivalent set of plants received no aphids and served as the experimental controls. All treatments were intermixed and randomized in the same growth chamber. To prohibit insect movement between treatments, each plant was encased with a clear acetate film (0·005 inch thickness, Grafix®, Cleveland, OH, USA, 10 cm diameter) on which one end had been sealed off by a mesh cap to ensure sufficient ventilation. Aphids grew well on both plant species and reached population densities of 50–100 individuals before caterpillars were introduced. Five days after aphid introduction, single assay larvae of *D. plexippus* were placed on the undamaged apical leaves of all experimental plants and were allowed to move and feed freely for 5 days on each plant along with aphids. Larvae from all plants were then removed, dried at 50 °C and weighed on a Mettler-Toledo UMT-2 balance (Hightstown, NJ). Caterpillar damage was measured on ten plants per treatment, using a clear acetate sheet with a 2 mm^2.

APHID BIOASSAY

For the aphid growth bioassay, monarch larvae were placed on half of the experimental plants ($n = 15$ *A. syrica*; $n = 12$ *A. tuberosa*) and were allowed to feed for 5 days and removed the day after aphid application; the remaining plants received no monarch damage and served as our experimental controls ($n = 15$ *A. syrica*; $n = 12$ *A. tuberosa*). To initiate the bioassay, six aphids were added to all plants (as described above), and the aphid density per plant was recorded every 4 days for 16 days. Insects were contained on plants using acetate cylinders as described above.

LATEX EXUDATION

We measured the amount of latex exuded at the end of each bioassay on all plants by cutting the first 5 mm off the tip of the youngest fully expanded and intact leaf. Latex stopped flowing after ≈10 s, and all latex was absorbed onto a 1-cm-diameter pre-weighed filter paper. This disc was placed in a pre-weighed microcentrifuge tube and weighed to quantify wet latex exudation per plant.

HPLC ANALYSIS OF CARDENOLIDES

After collecting latex, we harvested all above-ground plant tissue and immediately frozen in liquid nitrogen, to stop further metabolic processes before cold storage −80 °C. Collected plant material was oven-dried at 50 °C for 3 days and ground. Fifty milligrams of ground material, spiked with 20 μg of digitoxin (Sigma, St. Louis, MO, USA) as internal standard, was extracted with 1·9 mL of 95% ethanol in a sonicating water bath at 55 °C for 20 min. Ethanol was evaporated, and the residue was dissolved in 0·5 mL methanol. Samples were analyed by HPLC using a Gemini C18 reversed-phase column (3 μm, 150 × 4·6 mm, Phenomenex, Torrance, CA, USA) and an Agilent 1100 series instrument with diode array detection. The 15 μL injection was eluted at a constant flow of 0·7 mL min^{-1} with a gradient of acetronile and 0·25% phosphoric acid in water as follows: 0–5 min 20% acetonitrile; 20 min 70% acetonitrile; 20–25 min 70% acetonitrile; 30 min 95% acetonitrile; 30–35 min 95% acetonitrile. Peaks were detected by a diode array detector at 218 nm, and absorbance spectra were recorded from 200 to 400 nm. Peaks showing a characteristic symmetrical absorption band with a maximum between 217 and 222 nm were recorded as cardenolides (Malcolm & Zalucki 1996). Concentrations were calculated by relating peak areas to the internal standard.

PHYTOHORMONE ANALYSIS

Jasmonic acid and SA were extracted at the end of each bioassay from the plant leaves as described in Thaler, Agrawal & Halitschke (2010) with the following modification of the extraction procedure. Frozen samples were transferred into 2-mL screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartelsville, OK, USA) and 1 mL extraction buffer. d4-SA and d5-JA (CDN isotopes, Point-Claire, QC, Canada) were added to each sample as internal standards, and samples were homogenized on a FastPrep homogenizer (MP Biomedicals, Solon, OH, USA) at 6 m s^{-1} for 45 s. Samples were dissolved in 200 μL methanol after extraction with dichloromethane and solvent evaporation, and 15 μL was analysed on a triple-quadrupole LC-MS/MS system (Quantum Access; Thermo Scientific, Waltham, MA, USA). Analytes were separated on a C18 reversed-phase HPLC column (Gemini-NX, 3 l, 150 3 2·00 mm; Phenomenex, Torrance, CA, USA) using a gradient of 0·1% formic acid in water (solvent A) and 0·1% formic acid in acetonitrile (solvent B) at a flow rate of

300 L min^{-1}. The initial condition of 10% solvent B was kept constant for 2 min and then increased to 100% over a time of 20 min. Phytohormones were analysed by negative electrospray ionization (spray voltage: 3·5 kV; sheat gas: 15; auxiliary gas: 15; capillary temperature: 350 °C), collision-induced dissociation [argon CID gas pressure 1·3 mTorr (1·3 micron Hg), CID energy 16 V] and selected reaction monitoring (SRM) of compound-specific parent/product ion transitions: SA137- > 93; d4-SA141- > 97; JA209- > 59; d5-JA 214 ->62.

STATISTICAL ANALYSES

All statistical analyses were conducted using JMP (Version 9·0·2, SAS Institute Inc.). A two-way ANOVA was used to analyse the impacts of monarchs, aphids and their interaction on each of the plant responses (latex, cardenolides, JA and SA). To test for monarch effects on aphids, we employed a repeated-measure MANOVA on aphid number over time. *T*-tests were used to compare means from larval mass and plant damage. A correlation analysis was used to test for a relationship between the amount or JA and SA in plant tissues from each treatment. Data were log-transformed where necessary to improve homogeneity of variances and normality of residuals; back-transformed least squared means and standard errors (SEs) are reported in the figures. Minor differences in degrees of freedom between treatments and response variables result from differential recovery of bioassay insects or lost plant samples.

Results

ASCLEPIAS SYRIACA

Danaus plexippus larvae feeding on plants together with *A. nerii* weighed 37·7% more compared to larvae on control plants without aphids ($t_{24} = 2{\cdot}65$, $P = 0{\cdot}014$, Fig. 1a). However, the presence of aphids did not influence amount of leaf damage caused by monarchs (monarchs: 300 ± 39·1 mm^2, monarchs + aphids: 252 ± 34·1 mm^2 (mean ± SE); $t_{18} = 1{\cdot}05$, $P = 0{\cdot}300$), suggesting that monarch feeding behaviour was not impacted by the presence of aphids, and increased weight was not simply due to greater feeding. Reciprocally, aphid growth rate was negatively affected by monarch feeding, and this effect persisted over 16 days (monarch treatment: $F_{1,28} = 9{\cdot}26$, $P = 0{\cdot}005$, time: $F_{4,25} = 24{\cdot}58$, $P < 0{\cdot}001$, time-by-treatment interaction: $F_{4,25} = 9{\cdot}079$, $P < 0{\cdot}001$, Fig. 1b).

Feeding by *D. plexippus* induced a > 70% increase in cardenolides (Fig. 2a) and a > 60% increase in latex exudation compared to controls (Fig. 2b). Aphid feeding alone did not induce cardenolides, but attenuated cardenolide induction by monarchs when both herbivores were feeding together on a plant (Fig. 2a, Table 1). Conversely, aphids had no impact on latex exudation in any treatment (Fig. 2b, Table 1). Nonetheless, we found that latex exudation negatively correlated with monarch mass, but only in the absence of aphids (latex: $F_{1,23} = 0{\cdot}695$, $P = 0{\cdot}414$, aphids: $F_{1,23} = 9{\cdot}424$, $P = 0{\cdot}006$, interaction: $F_{1,23} = 6{\cdot}841$, $P = 0{\cdot}016$; Fig. 3).

Effects on JA paralleled those on cardenolides, with plants fed on by monarchs showing a 10-fold JA burst in the absence of aphids, but this effect being less than half as strong in their presence (Fig. 2c, Table 1). For SA, effects were reversed, with aphids increasing SA levels by 132%, and no independent or interactive effect of monarchs (Fig. 2d, Table 1). We found that SA and JA had a significant negative correlation, but only when both herbivores were feeding on the plant ($r_{10} = -0{\cdot}60$, $P = 0{\cdot}036$).

ASCLEPIAS TUBEROSA

We found no evidence for plant-mediated interactions between aphids and monarchs on *A. tuberosa*. The presence of aphids neither affected *D. plexippus* growth ($t_{27} = 0{\cdot}384$, $P = 0{\cdot}703$, Fig. 1c) nor leaf damage

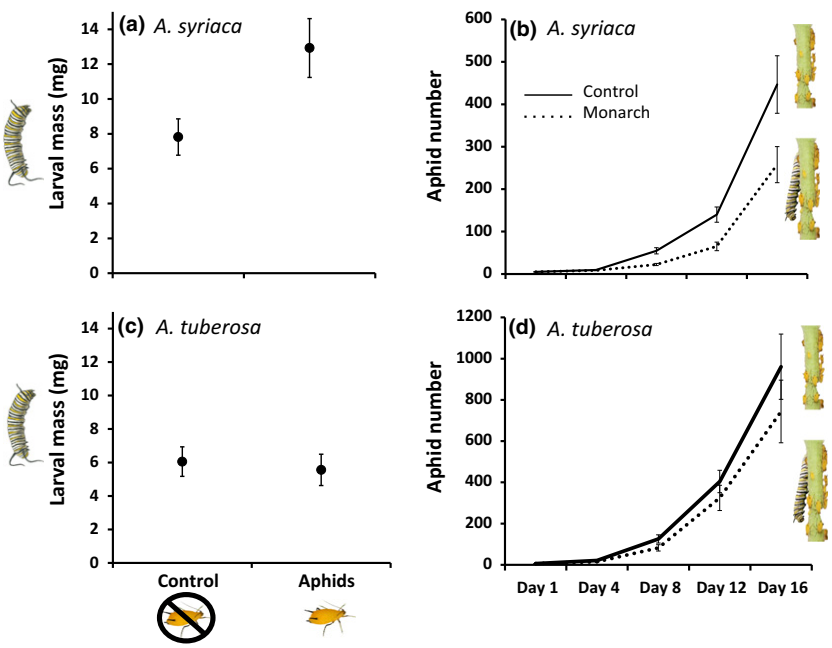

Fig. 1. Means ± SE (a) *Danaus plexippus* larval mass feeding on *Asclepias syriaca* without aphids present (control) or with aphids present ($n = 15$), (b) *Aphis nerii* population size feeding on control plants (solid line) or plants with previous monarch damage (dashed line) ($n = 15$) and (c) *D. plexippus* larval mass feeding on *Asclepias tuberosa* without aphids present (control) or with aphids present ($n = 15$), (d) *Aphis nerii* population size feeding on control plants (solid line) or plants with previous monarch damage ($n = 12$).

Table 1. Two-way factorial ANOVAS testing the effects herbivore feeding (monarch or aphids) on each of the traits sampled in the induction experiments

Source	Effect	DF	*F* ratio	*P*
Asclepias syriaca				
Cardenolides	Monarch	1,48	0·5513	0·462
	Aphids	1,48	0·027	0·870
	MxA	1,48	4·6791	**0·036**
Latex	Monarch	1,47	5·7062	**0·021**
	Aphids	1,47	0·0928	0·762
	MxA	1,47	0·2836	0·597
Jasmonic acid	Monarch	1,53	99·7654	**< 0·0001**
	Aphids	1,53	2·6122	0·112
	MxA	1,53	4·0222	**0·050**
Salicylic acid	Monarch	1,53	0·3854	0·538
	Aphids	1,53	5·2641	**0·026**
	MxA	1,53	1·6586	0·204
Asclepias tuberosa				
Latex	Monarch	1,49	0·3584	0·5523
	Aphids	1,49	0·7622	0·3872
	MxA	1,49	2·9648	0·0918
Jasmonic acid	Monarch	1,50	36·596	**< 0·0001**
	Aphids	1,50	4·8837	**0·0320**
	MxA	1,50	0·1082	0·7436
Salicylic acid	Monarch	1,50	2·9935	0·0902
	Aphids	1,50	2·3491	0·1321
	MxA	1,50	0·9306	0·3396

Bold values represent P < 0.05.

(monarchs: 318 ± 45·7 mm^2, monarchs + aphids: 287 ± 29·7 mm^2 (mean ± SE); $t_{18} = 0·56$, $P = 0·577$) when feeding on *A. tuberosa*. Also in contrast to *A. syriaca*, aphid growth rate was not affected by monarch feeding (monarch treatment: $F_{1,22} = 1·020$, $P = 0·3234$, time: $F_{4,19} = 17·349$, $P < 0·001$, time-by-treatment interaction: $F_{4,19} = 1·034$, $P = 0·4155$, Fig. 1d).

We found no effects of the herbivores on latex (cardenolides are essentially absent in *A. tuberosa*), despite the fact that monarch feeding increased JA >4-fold, while aphid feeding had a negative impact, decreasing it by 39% (Table 1, Fig. 2e, see also SI: *A. tuberosa* Latex) (Fig. S1, Table S1, Supporting information). For SA, we only found a modest effect of *D. plexippus* feeding, which increased it by 15% (Table 1, Fig. 2f). Finally, there were no correlations between SA and JA in any of the treatments (all Ps >> 0·10).

Discussion

We present evidence that reciprocal plant-mediated interactions can occur between monarchs and oleander aphids feeding on common milkweed, *A. syriaca*, but that these interactions are drastically different on the closely related butterfly weed, *A. tuberosa*. In the field, these two herbivores substantially overlap in phenology and can often be found feeding on the same host plant. Our evaluation of

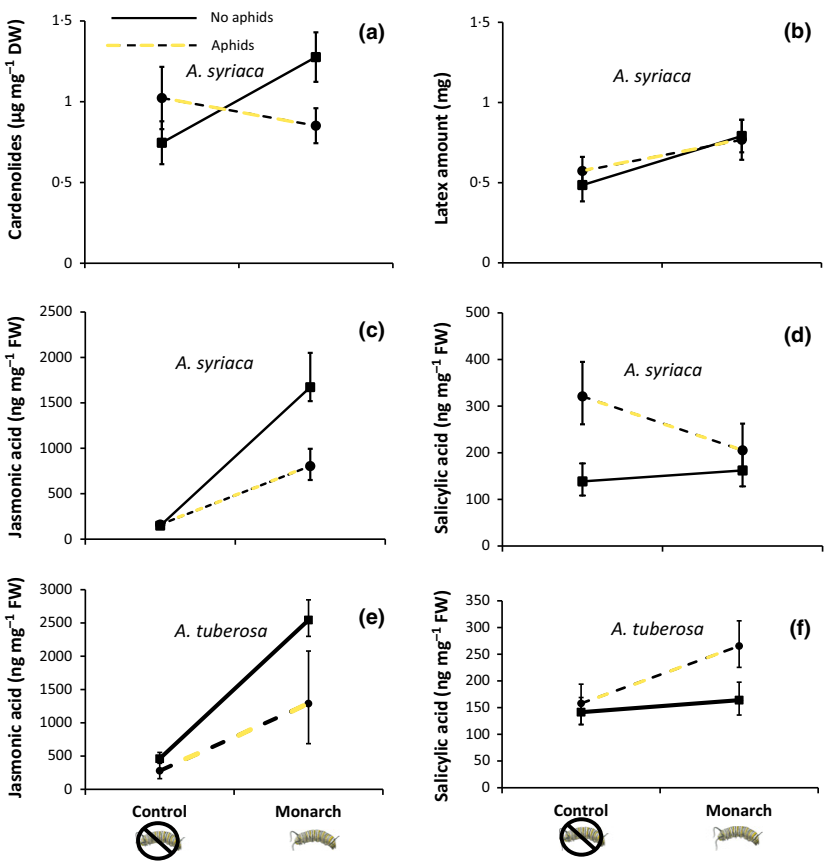

Fig. 2. Impacts of *Aphis nerii* or *Danaus plexippus* feeding either separately or together on levels of (a) cardenolides, (b) latex, (c) jasmonic acid, (d) salicylic acid on *Asclepias syriaca* plants, (e) jasmonic acid and (f) salicylic acid on *A. tuberosa*. Shown are Means ± SE; values are back-transformed for phytohormones. See Table 1 for corresponding statistical analysis.

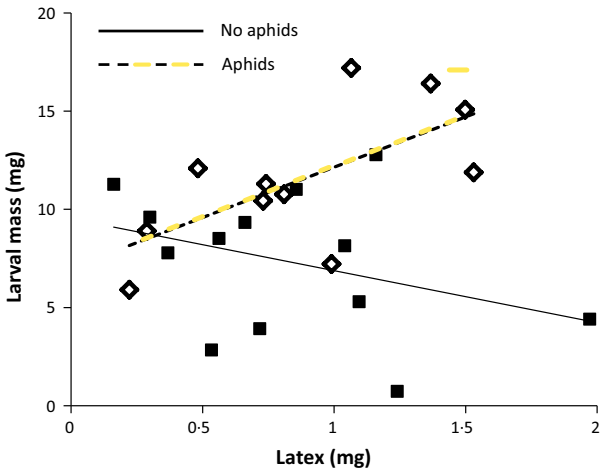

Fig. 3. Relationship between latex production and *Danaus plexippus* larval mass feeding on *Asclepias syriaca* plants either with (dashed line) or without (solid line) *Aphis nerii* present.

plant responses suggests that *A. nerii* infestation on common milkweed attenuated defensive responses to monarch damage, while aphid population growth was reduced after monarch-induced responses. Of the other studies that have examined aphid-caterpillar interactions that included bioassays, most report that aphids benefit caterpillar larvae either by increasing growth and survival (Stout *et al.*1998; Rodriguez-Saona *et al.* 2010; Soler *et al.* 2012) or by reducing herbivore-induced volatiles and thereby making caterpillars less apparent to their parasitoids (Turlings *et al.* 1998; Rodriguez-Saona, Crafts-Brandner & Cañas 2003; Schwartzberg, Böröczky & Tumlinson 2011).

We found evidence for differential milkweed responses to the two herbivores. The levels of both cardenolides and JA induced after monarch herbivory were substantially reduced when aphids were present on *A. syriaca*. Although the level of latex was not influenced by aphids, we did observe that the presence of aphids reversed the effect of latex on monarch growth (Fig. 3). This result suggests that aphid feeding modifies the properties of latex without affecting its quantity, rendering it a less effective defence against caterpillars. It is also possible that aphid feeding attenuates other stress factors that have an interactive effect on monarch performance or reduce the effectiveness of latex. Given its high mobility within the plant, latex could be involved in the rapid redistribution of cardenolides upon defence induction. The reduced negative effect of latex on caterpillars when aphids are present may therefore be another consequence of suppressed cardenolide induction (or an unknown additional JA-mediated plant response).

Reduction in foliar cardenolides has implications for the plant that are not easily interpretable, as the lowering of defences in the presence of herbivores should not be evolutionarily advantageous. We recognize that for the specialist insects on milkweeds, cardenolides are not strictly detrimental or beneficial, but likely a double-edged sword. Indeed monarchs can benefit from cardenolide sequestration, using the toxic compounds as their own defensive strategy (Malcolm & Brower 1989; Malcolm 1991), and it has recently been demonstrated that the aphid-induced changes lessen the defensive quality of plant cardenolides in a manner which increases virulence of monarch pathogens (de Roode *et al.* 2011). Nonetheless, even highly specialized herbivores are not completely immune to the negative effects of cardenolides, which are expected to act in a dose-dependent manner even on specialized insects (Agrawal *et al.* 2012a). It may be that the benefit cardenolides play in larval defences trade-off with caterpillar growth rate. Indeed, our previous work and that of others have shown negative effects of cardenolides both on monarchs (Zalucki, Brower & Alonso 2001; Agrawal 2005; Rasmann, Johnson & Agrawal 2009) and *A. nerii* (Agrawal 2004).

Of the studies that compare plant responses to insects in different feeding guilds, many show distinct plant responses, yet interpreting these responses as advantageous to the plant or to the herbivore is difficult. There have been studies that show phloem-feeding insects can suppress defences that typically deter other phloem feeders or induce changes that make plants more attractive to conspecifics (Prado & Tjallingii 2007; Mann *et al.* 2012). Although SA and JA defence pathways have been found to have varying effects on aphid populations, defences associated with the JA pathway appear to be important in reducing aphid population growth on tomato, *Arabidopsis*, sorghum and *Medicago* (Thaler *et al.* 2001; Ellis, Karafyllidis & Turner 2002; Zhu-Salzman *et al.* 2004; Gao *et al.* 2007). We show that caterpillar feeding, which induces JA, reduced aphid growth on *A. syriaca,* but not on *A. tuberosa.*

EXPLAINING DIVERGENT PLANT-MEDIATED EFFECTS ON PLANTS WITH DIFFERING DEFENSIVE STRATEGIES

Many of the phytohormonal dynamics in *A. syriaca* fit the typical pattern of assumptions based on trade-offs observed in model systems. For *A. syriaca*, JA and SA levels were negatively correlated in our treatment where both herbivores were feeding together (see SI: Hormones). Similarly, previous work on *A. syriaca* and phytohormone-induced changes found that cardenolides were increased by 33% with application of JA or monarch herbivory; however, aphids reduced cardenolides by 14% (Mooney, Jones & Agrawal 2008; see also Martel & Malcolm 2004). Abiotic factors such as shade, which also reduce JA induction, have been shown to suppress latex induction and increase monarch performance (Agrawal *et al.* 2012b). Further, studies that include the application of hormones to plants might help resolve the role crosstalk plays in this system by isolating responses the hormones induce.

While *A. syriaca* followed the typical pattern of phytohormonal crosstalk (Fig. S2, Supporting information), *A. tuberosa* exhibited a very different response, as there was a reduction in JA after aphid feeding, but no corresponding induction of SA, and no evidence of a trade-off

with SA levels in any of the treatments. The mechanism for aphid-induced reduction in JA in *A. tuberosa* is unclear, and the lack of an effect of aphids on caterpillars may be linked to (i) the overall lack of an effect the aphids had on the plants' SA production, (ii) that there was no negative relationship between SA and JA, (iii) the extent of JA reduction caused by aphids on *A. tuberosa* was not comparable to the aphid reduction in JA on *A. syriaca* or (iv) that the overall defensive strategy of *A. tuberosa* is quite different from that of *A. syriaca*. It is important to note that temporal kinetics of hormone induction were not examined. Furthermore, future examination of these interactions in the field on larger plants would test whether the faster growth of these caterpillars feeding with aphids translates into increased survival on *A. syrica*, even if *A. tuberosa*'s hormone signature eventually mirrored the effects we found in *A. syrica*.

Our observation that even closely related congeners have very different SA-JA responses to their specialist herbivores is perhaps consistent with the vulnerability inherent to plants for maintaining such trade-offs, which can be exploited by attackers (Cui *et al*. 2005; Zarate, Kempema & Walling 2007; Weech *et al*. 2008; Diezel *et al*. 2009; Pieterse *et al*. 2012). Indeed, in *A. tuberosa,* we found that caterpillar growth, both with and without aphids, was maintained at the same level as in the non-compromised *A. syriaca* controls (Fig. 1). Furthermore, *A. tuberosa* may present alternative defensive strategies against herbivores that do not involve cardenolides or latex (Agrawal & Fishbein 2006). Perhaps the mechanisms linked to the production of cardenolides, which are sequestered by specialists, are linked to other qualities associated with herbivore manipulations (i.e. SA-JA trade-offs), and *A. tuberosa* avoids this. Indeed, in the field, *A. tuberosa* receives far less damage by monarchs and oleander aphids, on average, than *A. syriaca*.

SYNTHESIS

We have yet to unravel the ecological drivers of the different phytohormonal dynamics in *A. syriaca* and *A. tuberosa*, but our findings suggest that defensive strategies may differ dramatically between closely related species. The JA-SA trade-off paradigm has been established from a very small group of plant taxa, predominantly Brassicaceae and Solanaceae, and largely using generalist insect inducers (particularly the generalist aphid, *Myzus persicae*). The role these dynamics have in natural ecological systems may help us understand relationships between phytohormones and defence in an evolutionary context. Further work should aim at screening for and quantifying the expression of conserved marker genes associated with plant hormonal pathways in non-model systems. We find the outcome of a relationship between two specialists herbivores is highly dependent on the species of plant they find themselves on and that our typical assumptions of JA-SA trade-offs do not always hold, even on closely related congeners. Understanding divergence in such basal mechanisms may help us resolve how different defensive syndromes arise between closely related species in natural populations.

For *A. tuberosa*, defence appears to be rather non-dynamic, and herbivores largely stay at bay. In contrast, for *A. syriaca*, monarchs benefit from defence attenuation by aphids, while aphids are impaired by monarch feeding. This asymmetric interaction between insects in two feeding guilds is consistent with the monarchs being important selective agents and aphids being stealthy herbivores.

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References

Agrawal, A.A. (1998) Induced responses to herbivory and increased plant performance. *Science*, **279**, 1201–1202.

Agrawal, A.A. (2002) Commentary: optimal foraging and phenotypic plasticity in plants. *Trends in Ecology & Evolution*, **17**, 305.

Agrawal, A.A. (2004) Plant defense and density dependence in the population growth of herbivores. *The American Naturalist*, **164**, 113–120.

Agrawal, A.A. (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*, **7**, 651–667.

Agrawal, A.A. (2011) Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, **25**, 420–432.

Agrawal, A.A. & Fishbein, M. (2006) Plant defense syndromes. *Ecology*, **87**, S132–S149.

Agrawal, A.A., Petschenka, G., Bingham, R.A., Weber, M.G. & Rasmann, S. (2012a) Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytologist*, **194**, 28–45.

Agrawal, A.A., Kearney, E.E., Hastings, A.P. & Ramsey, T.E. (2012b) Attenuation of the jasmonate burst, plant defensive traits, and resistance to specialist monarch caterpillars on shaded common milkweed (*Asclepias syriaca*). *Journal of Chemical Ecology*, **38**, 893–901.

Agrawal, A.A., Ali, J.G., Rasmann, S. & Fishbein, M. (in press) Macroevolutionary trends in the defense of milkweeds against monarchs: latex, cardenolides, and tolerance of herbivory. *Monarchs in a Changing World: Biology and Conservation of an Iconic Insect*, (edS. K. Oberhauser, S. Altizer & K. Nail), Cornell University Press, Ithaca, NY.

Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, **17**, 293–302.

Bidart-Bouzat, M.G. & Kliebenstein, D. (2011) An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. *Oecologia*, **167**, 677–689.

Bingham, R.A. & Agrawal, A.A. (2010) Ecological genetics of herbivore-specific induced defenses in common milkweed. *Journal of Ecology*, **98**, 1014–1022.

Blackman, R.L. & Eastop, V.F. (2006) *Aphids on the World's Herbaceous Plants and Shrubs*, pp. 1439. Wiley, London.

Broekgaarden, C., Voorrips, R.E., Dicke, M. & Vosman, B. (2011) Transcriptional responses of *Brassica nigra* to feeding by specialist insects of different feeding guilds. *Insect Science*, **18**, 259–272.

Cooper, W.R. & Goggin, F.L. (2005) Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomologia experimentalis et applicata*, **115**, 107–115.

Cui, J., Bahrami, A.K., Pringle, E.G., Hernandez-Guzman, G., Bender, C.L., Pierce, N.E. *et al*. (2005) *Pseudomonas syringae* manipulates systemic plant defenses against pathogens and herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 1791–1796.

De Vos, M., Van Oosten, V.R., Van Poecke, R.M.P., Van Pelt, J.A., Pozo, M.J., Mueller, M.J. *et al*. (2005) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Molecular Plant-Microbe Interactions*, **18**, 923–937.

Denno, R.F., McClure, M.S. & Ott, J.R. (1995) Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology*, **40**, 297–331.

Dicke, M., van Loon, J.J.A. & Soler, R. (2009) Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology*, **5**, 317–324.

Diezel, C., von Dahl, C.C., Gaquerel, E. & Baldwin, I.T. (2009) Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. *Plant Physiology*, **150**, 1576–1586.

Dobler, S., Dalla, S., Wagschal, V. & Agrawal, A.A. (2012) Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na, K-ATPase. *Proceedings of the National Academy of Sciences*, **109**, 13040–13045.

Dussourd, D.E. & Eisner, T. (1987) Vein-cutting behavior: insect counterploy to the latex defense of plants. *Science*, **237**, 898–901.

Ellis, C., Karafyllidis, I. & Turner, J.G. (2002) Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. *Molecular Plant-Microbe Interactions*, **15**, 1025–1030.

Erb, M., Robert, C.A., Hibbard, B.E. & Turlings, T.C. (2011) Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology*, **99**, 7–15.

Gao, L.L., Anderson, J.P., Klingler, J.P., Nair, R.M., Edwards, O.R. & Singh, K.B. (2007) Involvement of the octadecanoid pathway in bluegreen aphid resistance in *Medicago truncatula*. *Molecular Plant-Microbe Interactions*, **20**, 82–93.

Goggin, F.L. (2007) Plant–aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology*, **10**, 399–408.

Heidel, A.J. & Baldwin, I.T. (2004) Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. *Plant, Cell & Environment*, **27**, 1362–1373.

Janzen, D.H. (1973) Host plants as islands. 2. Competition in evolutionary and contemporary time. *American Naturalist*, **107**, 786–790.

Kaplan, I. & Denno, R.F. (2007) Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, **10**, 977–994.

Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*, 1st edn. The University of Chicago Press, Chicago, IL, USA.

Kessler, A. & Baldwin, I.T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, **53**, 299–328.

Malcolm, S.B. (1991) Cardenolide-mediated interactions between plants and herbivores. *Herbivores: Their Interactions with Secondary Plant Metabolites*, **1**, 251–296.

Malcolm, S.B. & Brower, L. (1989) Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. *Experientia*, **45**, 284–295.

Malcolm, S.B. & Zalucki, M.P. (1996) Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomologia Experimentalis et Applicata*, **80**, 193–196.

Mann, R.S., Ali, J.G., Hermann, S.L., Tiwari, S., Pelz-Stelinski, K.S., Alborn, H.T. *et al.* (2012) Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. *PLoS Pathogens*, **8**, e1002610.

Martel, J.W. & Malcolm, S.B. (2004) Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. *Journal of Chemical*, **30**, 545–561.

Mewis, I., Tokuhisa, J.G., Schultz, J.C., Appel, H.M., Ulrichs, C. & Gershenzon, J. (2006) Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry*, **67**, 2450–2462.

Mooney, K.A., Jones, P. & Agrawal, A.A. (2008) Coexisting congeners: demography, competition, and interactions with cardenolides for two milkweed-feeding aphids. *Oikos*, **117**, 450–458.

Mouttet, R., Kaplan, I., Bearez, P., Amiens-Desneux, E. & Desneux, N. (2013) Spatiotemporal patterns of induced resistance and susceptibility linking diverse plant parasites. *Oecologia*, **173**, 1379–1386.

Pieterse, C.M.J. & Dicke, M. (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends in Plant Science*, **12**, 564–569.

Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S.C. (2012) Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, **28**, 489–521.

Prado, E. & Tjallingii, W.F. (2007) Behavioral evidence for local reduction of aphid-induced resistance. *Journal of Insect Science*, **7**, 48.

Rasmann, S., Johnson, M.D. & Agrawal, A.A. (2009) Induced responses to herbivory and jasmonate in three milkweed species. *Journal of Chemical Ecology*, **35**, 1326–1334.

Rodriguez-Saona, C.R., Crafts-Brandner, S.J. & Cañas, L.A. (2003) Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology*, **29**, 2539–2550.

Rodriguez-Saona, C.R., Chalmers, J.A., Raj, S. & Thaler, J.S. (2005) Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia*, **143**, 566–577.

Rodriguez-Saona, C.R., Musser, R.O., Vogel, H., Hum-Musser, S.M. & Thaler, J.S. (2010) Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. *Journal of Chemical Ecology*, **36**, 1043–1057.

de Roode, J.C., Rarick, R.M., Mongue, A.J., Gerardo, N.M. & Hunter, M.D. (2011) Aphids indirectly increase virulence and transmission potential of a monarch butterfly parasite by reducing defensive chemistry of a shared food plant. *Ecology Letters*, **14**, 453–461.

Rothschild, M., Euw, J.V. & Reichstein, T. (1970) Cardiac glycosides in Oleander aphid, A*phis nerii*. *Journal of Insect Physiology*, **16**, 1141–1145.

Schuman, M.C., Heinzel, N., Gaquerel, E., Svatos, A. & Baldwin, I.T. (2009) Polymorphism in jasmonate signaling partially accounts for the variety of volatiles produced by *Nicotiana attenuata* plants in a native population. *New Phytologist*, **183**, 1134–1148.

Schwartzberg, E.G., Böröczky, K. & Tumlinson, J.H. (2011) Pea aphids, *Acyrthosiphon Pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. *Journal of Chemical Ecology*, **37**, 1055–1062.

Soler, R., Badenes-Pérez, F.R., Broekgaarden, C., Zheng, S.J., David, A., Boland, W. *et al.* (2012) Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Functional Ecology*, **26**, 156–166.

Stout, M.J., Workman, K.V., Bostock, R.M. & Duffey, S.S. (1998) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. *Oecologia*, **113**, 74–81.

Thaler, J.S., Agrawal, A.A. & Halitschke, R. (2010) Salicylate-mediated interactions between pathogens and herbivores. *Ecology*, **91**, 1075–1082.

Thaler, J.S., Humphrey, P.T. & Whiteman, N.K. (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, **17**, 260–270.

Thaler, J.S., Stout, M.J., Karban, R. & Duffey, S.S. (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology*, **26**, 312–324.

Turlings, T.C., Bernasconi, M., Bertossa, R., Bigler, F., Caloz, G. & Dorn, S. (1998) The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological Control*, **11**, 122–129.

Uesugi, A., Poelman, E.H. & Kessler, A. (2013) A test of genotypic variation in specificity of herbivore-induced responses in *Solidago altissima* L. (Asteraceae). *Oecologia*, **173**, 1387–1396.

Viswanathan, D.V., Narwani, A.J.T. & Thaler, J.S. (2005) Specificity in induced plant responses shapes patterns of herbivore occurrence on *Solanum dulcamara*. *Ecology*, **86**, 886–896.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195–216.

Walling, L.L. (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859–866.

Weech, M.-H., Chapleau, M., Pan, L., Ide, C. & Bede, J.C. (2008) Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botany*, **59**, 2437–2448.

Wu, J., Hettenhausen, C., Schuman, M.C. & Baldwin, I.T. (2008) A comparison of two *Nicotiana attenuata* accessions reveals large differences in signaling induced by oral secretions of the specialist herbivore *Manduca sexta*. *Plant Physiology*, **146**, 927–939.

Zalucki, M.P., Brower, L.P. & Alonso, A. (2001) Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology*, **26**, 212–224.

Zarate, S.I., Kempema, L.A. & Walling, L.L. (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology*, **143**, 866–875.

Zehnder, C.B. & Hunter, M.D. (2007) Interspecific variation within the genus *Asclepias* in response to herbivory by a phloem-feeding insect herbivore. *Journal of Chemical Ecology*, **33**, 2044–2053.

Zhu-Salzman, K., Salzman, R.A., Ahn, J.E. & Koiwa, H. (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiology*, **134**, 420–431.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Impacts of *Aphis nerii* or *Danaus plexippus* feeding either separately or together on levels of latex in *Ascelpias tuberosa*.
Fig. S2. Relationship between salicylic acid (SA) and jasmonic acid (JA) when monarch and aphids were feeding together on *Asclepias syriaca*.
Table S1. Two-way factorial ANOVAS testing the effects herbivore feeding (monarch or aphids) on latex in *Acselpias tuberosa*.

Supporting Information

Submission to: Functional Ecology

Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds (*Ascelpias syriaca* and *A. tuberosa*)

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Two Figures:

1) Latex for *A. tuberosa*
2) SA-JA relationship

One Table:

1) Two-way ANOVA

Figures:

Supporting Figure 1. Impacts of *Aphis nerii* or *Danaus plexippus* feeding either separately or together on levels of latex in *Ascelpias tuberosa.* (see SupportingTable 1)

Supporting Figure 2. Relationship between salicylic acid (SA) and jasmonic acid (JA) when monarch and aphids were feeding together on *Asclepias syriaca*.

Tables:

Supporting Table 1. Two-way factorial ANOVAs testing the effects herbivore feeding (monarch or aphids) on latex in *Acselpias tuberosa*

Effect	**DF**	**F Ratio**	**Prob > F**
Monarch	1	0.4015	0.529
Aphids	1	0.4828	0.4901
M*A	1	3.388	0.0712

Figure 1

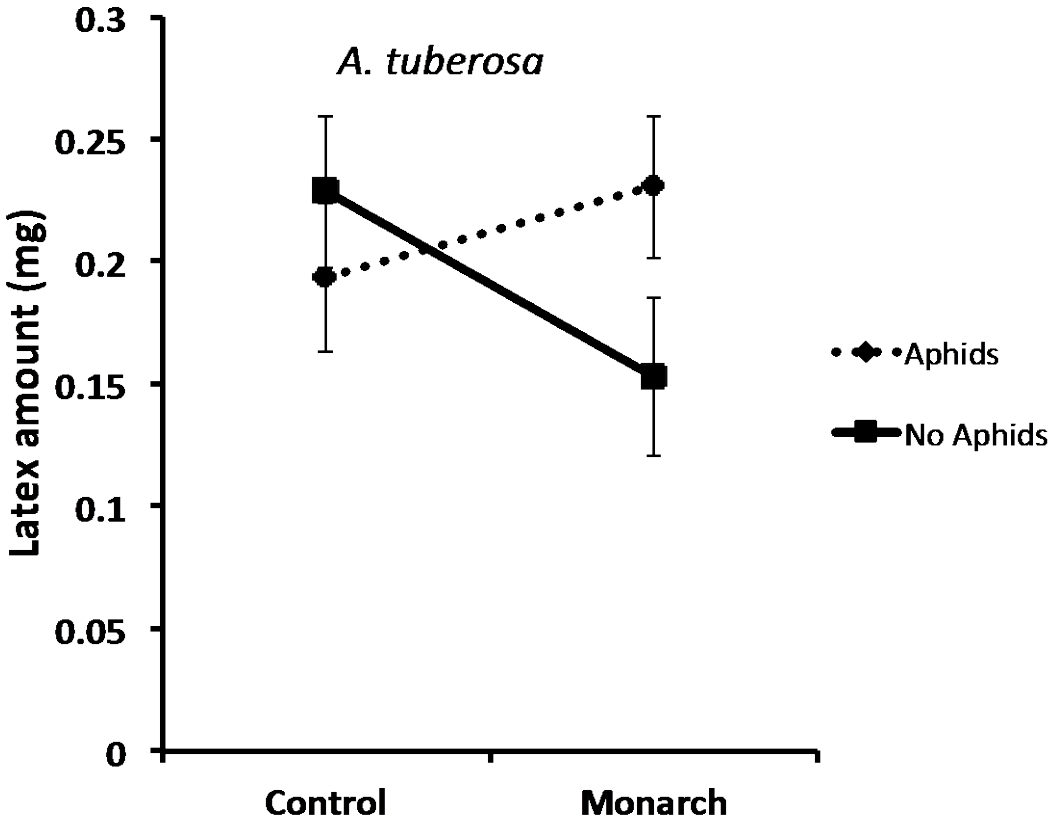
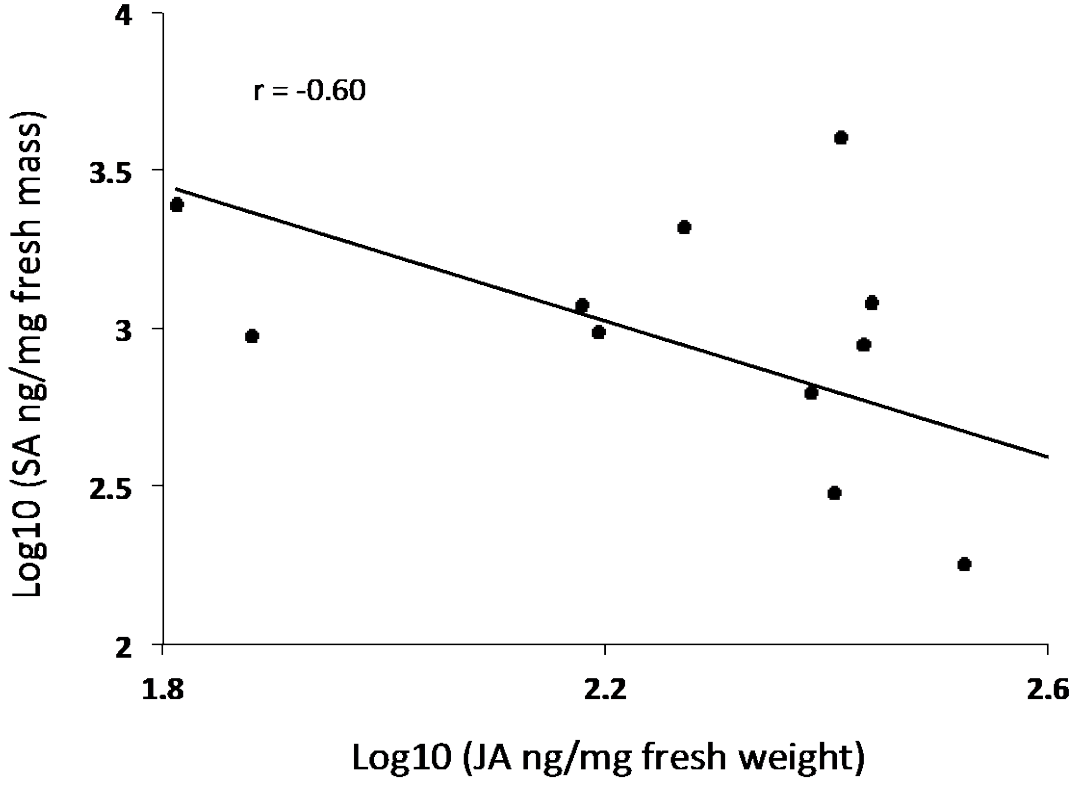

A. tuberosa
Latex amount (mg)
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Monarch
Aphids
No Aphids

Figure 2


r = -0.60
Log10 (SA ng/mg fresh mass)
4
3.5
3
2.5
2
1.8
2.2
2.6
Log10 (JA ng/mg fresh weight)