

Why does a good thing become too much? Interactions between foliar nutrients and toxins determine performance of an insect herbivore

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Summary

1. In terrestrial ecosystems, nitrogen (N) and phosphorus (P) are generally believed to be the most limiting factors for organisms across different trophic levels. However, accumulating evidence suggests that dietary nutrient concentrations higher than optimal can lead to decreases in consumer growth rate.

2. In the current study, we explored mechanisms underlying the negative effects of high nutrient availability on the performance of a specialist herbivore. Specifically, we investigated the responses of the monarch caterpillar *Danaus plexippus* to natural and experimental variation in N and P concentrations of three species of milkweed plants (*Asclepias syriaca*, *Asclepias curassavica* and *Asclepias incarnata*) that also varied in their foliar toxin concentrations.

3. We found that high foliar N concentrations in milkweed were associated with decreases in the growth rate of *D. plexippus* larvae. However, such negative effects of N were only found when larvae were feeding on *A. curassavica*, which also had high foliar concentrations of cardenolide, a widespread chemical defence in the genus *Asclepias*. Foliar N concentration was not correlated with cardenolide concentration. Rather, the per unit toxicity of cardenolide was higher as N increased in excess of demand, resulting in deleterious effects of N. Our results suggest that interactions between nutrient concentrations in excess of demand and high dietary toxin concentrations provide an additional mechanism by which high nutrient availability can reduce the performance of consumers.

Key-words: *Asclepias*, chemical defence, *Danaus plexippus*, herbivore, nitrogen, nutrient–toxin interactions, phosphorus, plant–insect interactions

Introduction

Since von Liebig's time (Von Liebig 1840), ecologists have been studying the nature and consequences of nutrient limitation in organisms. From the classic Law of the Minimum to the Multiple Limitation Hypothesis (Gleeson & Tilman 1992) and the Principle of Ecological Stoichiometry (Sternner & Elser 2002), our increasing understanding of nutritional ecology has shed much light on species interactions and ecosystem processes. In terrestrial ecosystems, nitrogen (N) and phosphorus (P) are generally believed to be the most limiting factors for organisms across different trophic levels (Sternner & Elser 2002; Fagan & Denno 2004; Elser *et al.* 2007). In the context of plant–insect herbivore interactions, foliar N and P concentrations are on average

10 times lower than those in herbivore tissues (Elser *et al.* 2007), therefore increases in foliar N and P concentration can result in higher performance by herbivores (Mattson 1980; Hunter, Watt & Docherty 1991; Speight *et al.* 2008).

However, the principle of ecological stoichiometry predicts there to be an optimal concentration of each nutrient for each organism at which maximal growth rate is attained (Boersma & Elser 2006). As a result, nutrient concentrations higher than optimal can lead to decreases in growth rate. Negative effects of nutrient enrichment on consumer growth rates have been recorded in a number of organisms. For example, high N concentrations can lead to decreases in the fitness of grasshoppers (Joern & Behmer 1998), butterflies (Fischer & Fiedler 2000) and aphids (Zehnder & Hunter 2009), which are often considered limited by N (Mattson 1980). Similarly, high P concentrations can result in negative effects on the growth rates of aquatic

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organisms, which are traditionally thought to be P limited (Boersma & Elser 2006).

Several mechanisms have been proposed to explain such negative relationships between high nutrient availability and organismal performance. First, organisms have an intake target in multivariate resource space (Simpson & Raubenheimer 1993). Therefore, when consuming food whose nutrient composition deviates from the target, they will regulate their total intake to strike a balance between excess and limitation of each nutrient (Raubenheimer & Simpson 1999; Simpson *et al.* 2004). In other words, by preventing the excess uptake of one nutrient, other essential elements may be in deficit. This is especially true in heterotrophs, which obtain their nutrients in mixtures rather than as separate elements. Secondly, excretion and storage of excess nutrients may impose metabolic costs (Boersma & Elser 2006). Thirdly, there can be concomitant changes in other physical/chemical properties of the diet that may potentially decrease growth rate (Plath & Boersma 2001). Specifically, the levels of nutrients and secondary metabolites in food sources are often tightly correlated (Bryant, Chapin & Klein 1983; Herms & Mattson 1992; Vannette & Hunter 2011). As a result, the deleterious effects of high nutrients on consumers may result primarily from higher levels of plant resistance rather than high nutrients themselves (Tao & Hunter 2012).

In the current study, we explored mechanisms underlying the negative effects of high nutrient availability on the performance of a specialist herbivore. Specifically, we investigated the responses of the monarch caterpillar *Danaus plexippus* to natural and experimental variation in N and P concentrations of three species of milkweed plants that also varied in their foliar toxin concentrations. In contrast to the mechanisms described above, we found that the deleterious effects of high N concentration on monarch performance were only expressed under high foliar toxin concentrations. Our results suggest that interactions between nutrient concentrations in excess of demand and high dietary toxin concentrations provide an additional mechanism by which high nutrient availability can reduce the performance of consumers.

Materials and methods

We carried out experiments with three species of milkweed: *Asclepias syriaca*, *Asclepias incarnata* and *Asclepias curassavica*. Under control conditions, these milkweed species exhibit a broad range of foliar N (1.1–5.2%) and P concentrations (0.1–0.8%), with one species expressing low N and P concentrations (*A. syriaca*, N: $1.62 \pm 0.28\%$; P: $0.18 \pm 0.02\%$), and two species expressing high N and P concentrations (*A. curassavica*, N: $3.32 \pm 0.26\%$, P: $0.35 \pm 0.08\%$; *A. incarnata*, N: $3.68 \pm 0.26\%$; P: $0.27 \pm 0.02\%$; Fig. S1). In milkweeds, the major defensive chemicals are cardenolides, which can disrupt animal Na^+/K^+ ATPase (Agrawal *et al.* 2012). Our chosen milkweeds vary in foliar cardenolide concentrations from low (*A. incarnata*) through intermediate (*A. syriaca*) to high (*A. curassavica*) (Agrawal & Malcolm 2002; Sternberg *et al.* 2012).

Asclepias syriaca seeds were collected in September 2010 from a natural population at the University of Michigan Biological Station in Pellston, MI, and stored in a refrigerator at 4 °C until use. *Asclepias incarnata* and *A. curassavica* seeds were purchased from Butterfly Inc, San Ramon, California. At the end of March 2012, *A. syriaca* and *A. incarnata* seeds were cold stratified for 6 weeks and then germinated on damp filter paper in petri dishes at 25 °C. *A. curassavica* seeds, which do not need stratification, were germinated simultaneously. After germination, seedlings were planted in 4-inch plant pots containing a 1:1 mixture of potting soil (Sun-Grow Horticulture, Agawam, MA) and sand (Kolorscape). Plants were kept in a controlled growth chamber at 25 °C and an L16:D8 light cycle. When plants were 6 weeks old, 3 by 3 levels of nitrogen and phosphorus fertilizer were applied in a fully factorial design (Fig. S1). Nitrogen was added as ammonium nitrate at total levels of 0, 4, 8 g m⁻², and phosphorus was added as calcium phosphate monobasic at total levels of 0, 0.4, 0.8 g m⁻². Fertilizer was applied once every week for a total of 5 weeks; each plant received one fifth of its total fertilizer allocation each week. We have shown previously that these levels of fertilization generate a broad but realistic range of C:N:P stoichiometry in milkweed plants (Tao & Hunter 2012). For *A. incarnata* and *A. curassavica*, we used 10 replicates per treatment for a total of 90 plants for each species (9 nutrient treatments * 10 plants each = 90 plants). For *A. syriaca*, due to a low germination rate, we used 6 replicates per treatment for a total of 54 plants (9 nutrient treatments * 6 plants each = 54 plants). In the current experiment, our N fertilization resulted in significant increases in foliar N concentrations ($F_{2,187} = 35.98$, $P < 0.001$), while the effects of P fertilization on foliar P concentration were not significant ($F_{2,187} = 0.80$, $P = 0.45$; Fig. S1). As a result, we have used foliar N and P concentrations, rather than treatment levels as independent variables in regression analyses. This provides us with considerable variation in foliar nutrient concentrations (Fig. S1) with which to examine effects on herbivore performance.

Danaus plexippus eggs were obtained from a colony maintained in our laboratory. 250 eggs were collected for the experiment and stored in a refrigerator for 2 days prior to the experiment to synchronize hatching. Each neonate caterpillar was assigned randomly to receive foliage from a single plant. Caterpillars were maintained individually in 163 mL plastic containers. Each day, we retrieved fresh leaves from each plant and fed them *ad libitum* to their associated caterpillars. The experiment lasted for 7 days in total. This period represents 50% of the average larval period of monarchs under our rearing conditions: plants were not large enough to rear all caterpillars through to pupation, so we kept the number of rearing days constant to better compare growth rates among treatments. Effects of foliage quality on monarch growth are known to be most important during early instars (Zalucki *et al.* 2001). After 7 days of feeding, all caterpillars were starved for 24 h to void their gut contents. They were then oven-dried, and their dry mass measured on a microbalance. The average daily growth rate of each caterpillar was calculated by dividing its log transformed mass by 7.

To measure foliar cardenolide concentrations, the major defensive chemicals in the genus *Asclepias*, we followed Zehnder & Hunter (2009). Briefly, 6 leaf discs from the fourth pair of leaves of each plant were taken and ground in methanol using a ball mill and sonicated at 60 °C for 1 h. Another 6 leaf discs were taken and oven-dried to provide estimates of sample dry weights. The supernatant from samples in methanol was evaporated at 45 °C for 70 min until dry. Samples were then resuspended in 150 µL methanol containing 0.15 mg mL digitoxin as an internal standard and analysed using reverse-phase ultra-high-performance liquid chromatography (UPLC, Waters Inc., Milford, MA, USA). Running time for each sample was 9 min. Peaks were detected by absorption at 218 nm using a diode array detector, and absorbance spectra were recorded from 200 to 300 nm. Peaks with

symmetrical absorption maxima between 216 and 222 nm were recorded as cardenolides. Total cardenolide concentration was calculated as the sum of all separated cardenolide peaks, corrected by the concentration of the internal standard and the estimated sample mass. For the same leaves from which discs were taken, the remaining leaf material was oven-dried and ground for subsequent carbon (C), N and P measurements. Foliar C and N concentrations were measured on a CHN analyzer (Costech, Valencia, CA, USA), and foliar P concentrations were quantified by an autoanalyzer using an acid digestion method (Tao & Hunter 2012). Only N and P concentrations were used in subsequent analyses because insect herbivores are generally considered to be N and/or P limited (Sterner & Elser 2002; Fagan & Denno 2004).

STATISTICAL ANALYSIS

To examine the effects of plant traits on *D. plexippus* growth rates, we used general linear models with N, P, cardenolide concentration, species identity and all possible interactions as fixed factors, and growth rate of *D. plexippus* as the dependent variable. Because of expectations that caterpillar responses to nutrient availability would be nonlinear (see introduction), we also included quadratic terms for both N and P and their interactions with other terms. A minimal model was derived using a backward selection procedure (Kleinbaum *et al.* 2007) in which terms were retained in the model if their removal significantly ($P < 0.05$) reduced the explanatory power of the model (Crawley 2007). To test potential effects of N, P and species identity on the concentrations of cardenolide in leaves, we used the three factors and their interactions as independent variables and cardenolide concentration as the dependent variable.

Because of strong interactive effects of plant species and foliar traits on insect performance, we developed species-specific models to explore the effects of foliar N, P, cardenolide concentration and their interactions on the growth rate of *D. plexippus*. Because we found strong interactive effects of foliar N and cardenolide concentrations on monarch growth rates on some plant species (below), we illustrated the nature of these interactions with contour plots using weighted least squares to fit a local polynomial trend surface to approximate the changes in caterpillar growth rate across N and cardenolide gradients in each milkweed species. We provide additional scatterplots (Fig. S3) illustrating the interactions between foliar N and cardenolide concentration on caterpillar growth rate.

All statistical analyses were performed in R 2.13.0 (R Development Core Team 2011), and contour plot was generated by the Lattice package.

Results

There were significant linear and quadratic effects of foliar N concentration on the growth rate of *D. plexippus*. Namely, the growth rate was greatest at intermediate foliar N concentrations, initially increasing and then decreasing as foliar N concentration increased (Table 1; linear term, $F_{1,185} = 55.53$, $P < 0.001$, slope = 0.242; quadratic term, $F_{1,185} = 4.83$, $P = 0.03$, slope = -0.031). This combination of slopes indicates that, across the three milkweed species, the highest caterpillar growth rates were achieved when foliar nitrogen concentration was around 3.9%. Caterpillars that fed on *A. incarnata* and *A. curassavica* had higher growth rates (3.14 ± 0.02 and 3.13 ± 0.01 , respectively) than did caterpillars feeding on *A. syriaca* (2.84 ± 0.02 , units are $\log \mu\text{g day}^{-1}$ for all species) (species effect

Table 1. Results of a general linear model examining the effects of foliar nitrogen (N) concentration, milkweed species, foliar cardenolide concentration and their interactions on the growth rate of *Danaus plexippus* larvae feeding on *Asclepias syriaca*, *Asclepias incarnata* and *Asclepias curassavica*

	<i>F</i>	<i>P</i>
N	$F_{1,185} = 55.53$	<0.001
Species	$F_{2,185} = 34.43$	<0.001
Cardenolide	$F_{1,185} = 4.42$	0.04
N ²	$F_{1,185} = 4.83$	0.03
N × Species	$F_{2,185} = 4.63$	0.01

$F_{2,185} = 34.43$, $P < 0.001$). Across all three milkweed species, caterpillar growth rates declined with increasing foliar cardenolide concentration ($F_{1,185} = 4.42$, $P = 0.04$; slope = -0.02 ± 0.008). In addition, the effects of foliar N concentration on the growth rate of *D. plexippus* varied among milkweed species ($F_{2,185} = 4.63$, $P = 0.01$, see full details below). Together, these five variables explained 43.58% of variations in the growth rate of *D. plexippus*. There were neither independent nor interactive effects of foliar P concentration on the growth rate of *D. plexippus* larvae, and its effects on larval performance are not considered further.

Asclepias curassavica and *A. incarnata* had similar foliar N concentrations ($3.32 \pm 0.26\%$ and $3.68 \pm 0.26\%$, respectively), while *A. syriaca* had significantly lower foliar N concentrations than the other species ($1.62 \pm 0.28\%$) ($F_{2,193} = 100.05$, $P < 0.001$). On average, foliar N concentration in *A. syriaca* was 51% and 56% lower than that in *A. curassavica* and *A. incarnata*, respectively. Cardenolide concentrations were highest in *A. curassavica* at $2.79 \pm 0.16 \text{ mg g}^{-1}$, followed by *A. syriaca* ($0.57 \pm 0.05 \text{ mg g}^{-1}$) and *A. incarnata* ($0.05 \pm 0.01 \text{ mg g}^{-1}$) ($F_{2,190} = 290.67$, $P < 0.001$). In other words, the foliar cardenolide concentration of *A. curassavica* was 4.9 and 56 times higher than the foliar cardenolide concentrations of *A. syriaca* and *A. incarnata*, respectively. There were no relationships between foliar N, nor its interaction with milkweed identity, and the concentration of cardenolides in milkweeds (Table S1; N: $F_{1,181} = 0.14$, $P = 0.71$; N by species interaction: $F_{2,181} = 0.01$, $P = 0.99$), illustrating that N levels did not affect cardenolide production in any of the milkweed species that we studied. However, there was a significant positive relationship between foliar P concentration and cardenolide concentration ($F_{1,181} = 14.22$, $P < 0.001$, slope = 5.28 ± 0.99 ; Table S1, Fig. S2).

We also developed species-specific general linear models using foliar N, P, cardenolide concentration and all possible interactions as independent variables (Table 2). The results from species-specific models corroborate those from the full model that the effects of foliar N concentration on caterpillar growth rate differed among species. In *A. curassavica*, increases in foliar N concentration were associated with decreases in the growth rate of *D. plexippus*

Table 2. Results of general linear models examining the effects of foliar nitrogen (N) concentration, phosphorus (P) and cardenolide concentration and their interactions on the growth rate of *Danaus plexippus* larvae on individual species of *Asclepias*

	<i>Asclepias syriaca</i>	<i>Asclepias curassavica</i>	<i>Asclepias incarnata</i>
N			
F	$F_{1,41} = 5.45$	$F_{1,59} = 9.42$	$F_{1,69} = 0.35$
P	0.02	0.003	0.55
Slope	0.34 (0.17)	-0.16 (0.1)	0.08 (0.07)
P			
F	$F_{1,41} = 3.56$	$F_{1,59} = 0.12$	$F_{1,69} = 0.17$
P	0.07	0.73	0.69
Slope	4.01 (1.96)	-2.99 (1.66)	0.58 (0.91)
Cardenolide			
F	$F_{1,41} = 2.78$	$F_{1,59} = 2.67$	$F_{1,69} = 0.21$
P	0.10	0.11	0.65
Slope	-0.13 (0.48)	-0.16 (0.13)	-1.87 (3.81)
N × P			
F	$F_{1,41} = 1.16$	$F_{1,59} = 0.03$	$F_{1,69} = 0.13$
P	0.27	0.85	0.72
Slope	-1.62 (0.80)	0.76 (0.39)	-0.24 (0.23)
N × Cardenolide			
F	$F_{1,41} = 0.22$	$F_{1,59} = 5.83$	$F_{1,69} = 0.29$
P	0.65	0.02	0.59
Slope	-0.25 (0.24)	-0.007 (0.002)	-0.12 (0.88)
P × Cardenolide			
F	$F_{1,41} = 1.37$	$F_{1,59} = 0.21$	$F_{1,69} = 6.18$
P	0.24	0.65	0.02
Slope	-5.14 (2.52)	0.03 (0.03)	11.76 (15.49)
N × P × Cardenolide			
F	$F_{1,41} = 3.01$	$F_{1,59} = 3.49$	$F_{1,69} = 0.03$
P	0.09	0.07	0.87
slope	1.78 (1.03)	-0.02 (0.12)	-0.54 (3.37)

($F_{1,59} = 9.42$, $P = 0.003$, slope = -0.16 ± 0.1 , Fig. 1a). In *A. incarnata*, there was no significant relationship between the growth rate of *D. plexippus* and variation in foliar N concentrations ($F_{1,69} = 0.35$, $P = 0.55$, Fig. 1b). In contrast, increasing N concentrations were associated with an increase in larval growth rate in *A. syriaca* ($F_{1,41} = 5.45$, $P = 0.02$, slope = 0.34 ± 0.17 , Fig. 1c).

We have illustrated the interactive effects of foliar N concentration and cardenolide on larval growth rates by

contour plots (Fig. 2, S3). In *A. curassavica*, at low cardenolide levels, changes in *D. plexippus* growth rates were relatively minor across gradients in foliar N concentration. However, at high cardenolide concentrations, *D. plexippus* growth rates declined steeply with increasing foliar N concentration (Fig. 2a, S3a) (N × cardenolide interaction $F_{1,59} = 5.83$, $P = 0.02$, Table 2). In other words, the deleterious effects of excess N on larval growth were expressed increasingly at high cardenolide levels. The complimentary view is that reductions in larval growth rate caused by increasing cardenolide concentrations are greatest at high N levels, suggesting that the per unit toxicity of cardenolide increases as N increases in excess of the optimum. In contrast, the cardenolide concentrations are too low in *A. incarnata* ($0\text{--}0.32 \text{ mg g}^{-1}$) to facilitate any negative effects of high N concentration on larval growth (N × cardenolide interaction $F_{1,69} = 0.29$, $P = 0.59$, Fig. 2b). Finally, foliar N concentrations in *A. syriaca* are always below those that interact significantly with foliar cardenolide (N × cardenolide interaction $F_{1,41} = 0.22$, $P = 0.65$, Fig. 2c).

Discussion

High foliar N concentrations in milkweed were associated with decreases in the growth rate of *D. plexippus* larvae. However, such negative effects of N were found only when larvae were feeding on *A. curassavica*, which also had high foliar concentrations of cardenolide, a widespread chemical defence in the genus *Asclepias*. The per unit toxicity of cardenolide was higher when N occurred in excess of optimum concentrations for larval growth, providing an additional mechanism by which high nutrient availability can cause declines in consumer fitness (Boersma & Elser 2006). When feeding on *A. incarnata*, which had similar high foliar N concentrations, excess N did not have significant negative effects on larval growth because cardenolide concentrations are on average 56 times lower than those of *A. curassavica*. In addition to illustrating a new mechanism for the negative effects of excess nutrients on consumers, our work emphasizes that future studies of species

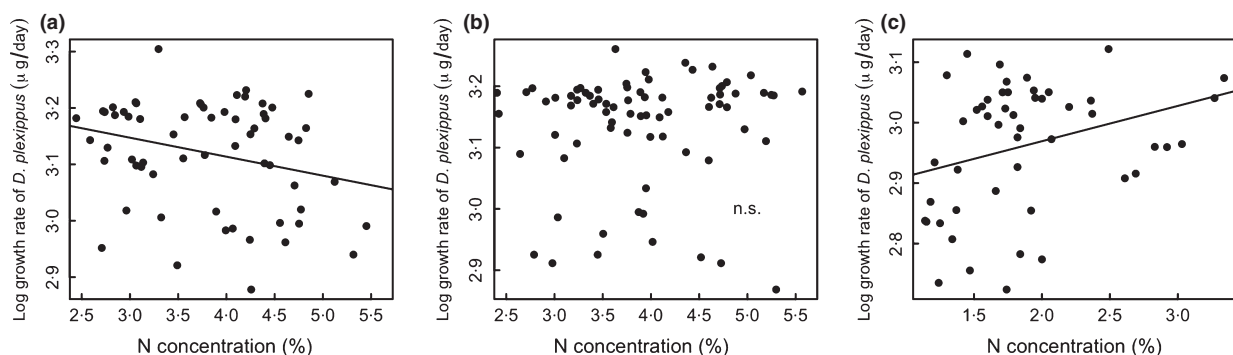


Fig. 1. Relationship between foliar nitrogen (N) concentration and the growth rate of *Danaus plexippus* larvae feeding on *Asclepias curassavica* (a), *Asclepias incarnata* (b) and *Asclepias syriaca* (c). Regression lines illustrate significant relationships.

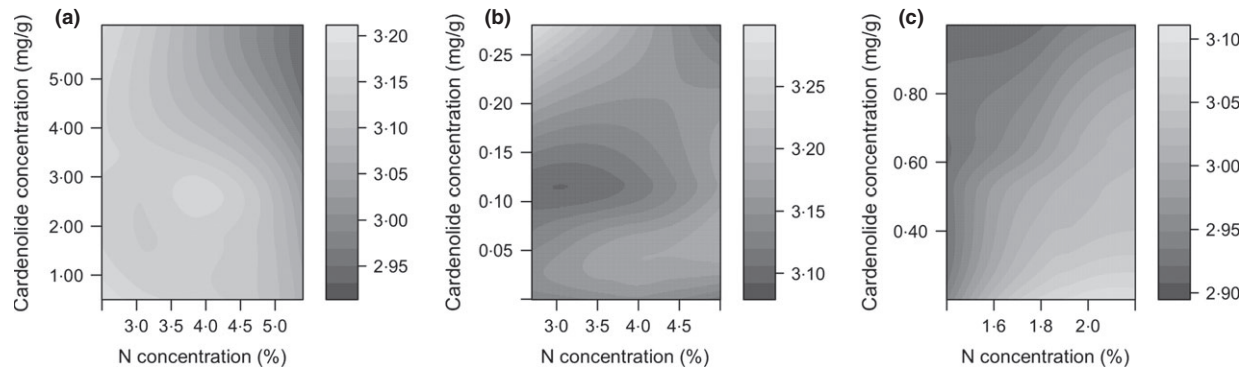


Fig. 2. The growth rate of *Danaus plexippus* larvae across variation in foliar nitrogen (N) and cardenolide concentrations. In the contour plots, the x-axis represents N concentration (%), the y-axis represents cardenolide concentration (mg g^{-1}) and colour represents the larval growth rate of *D. plexippus* ($\log \mu\text{g day}^{-1}$). Responses are illustrated for larvae on *Asclepias curassavica* (a), *Asclepias incarnata* (b) and *Asclepias syriaca* (c).

interactions should focus increasingly on interactions between nutrients and defence chemicals (Raubenheimer & Simpson 2009).

During preingestion periods, nutrients in excess of demand can inhibit food consumption, resulting in lower organismal growth rates, as has been shown for some grasshoppers (Raubenheimer & Simpson 1999). In *D. plexippus*, high nitrogen concentration has resulted in reduced consumption of *A. syriaca* (Lavoie & Oberhauser 2004). However, we do not think reduced consumption is the mechanism underlying the negative effects of N on caterpillar growth in our study. First, the N fertilization levels in Lavoie & Oberhauser (2004) were about 10 times higher than those in our study. Secondly, if the high foliar N levels in our study acted to reduce consumption, we should have seen similar declines in caterpillar growth rates on *A. incarnata* as we saw on *A. curassavica* as they share similar foliar N concentrations. However, caterpillar growth rates were unaffected by high foliar N concentrations in *A. incarnata*. A second mechanism to explain reductions in consumer growth under high nutrient concentrations proposes that postingestive costs of excreting excess nutrient may translate into reduced fitness (Boersma & Elser 2006). Although there is indirect evidence for this mechanism operating in aphids (Zehnder & Hunter 2009), it is difficult to measure costs of excretion directly. As before, our current experiment suggests that this is an unlikely explanation for our data. If operating, we would expect similar excretion costs in caterpillars fed on *A. incarnata* and *A. curassavica*, which had similarly high foliar N concentrations. A third proposed mechanism, which perhaps is relatively common, is that nutrient enrichment results in correlated changes in foliar defences that themselves influence consumer performance (Plath & Boersma 2001; Tao & Hunter 2012). Although fertilization by N:P:K (potassium) fertilizers has been shown to reduce total concentrations of cardenolide across several milkweed species (Agrawal *et al.* 2012), associations between foliar nutrient concentrations and foliar cardeno-

lide concentrations are less well-established. In our experiment, N concentrations were not correlated with cardenolide production in any of the three species that we studied ($P = 0.73, 0.89, 0.93$ for *A. curassavica*, *A. incarnata* and *A. syriaca* respectively). Rather, we observed increases in foliar cardenolides associated with high foliar P concentrations. Because foliar N and foliar cardenolide concentrations are unrelated in our study, we can discount the possibility that negative effects of high foliar N on monarchs result from concomitant increases in foliar cardenolides.

In general, relationships between foliar nutrients and chemical defences are not as simple as once thought (Bryant, Chapin & Klein 1983). Rather, the effects of nutrient availability on chemical defence are now recognized to vary with plant functional group and life history and cannot easily be predicted from current theories of plant defence (Koricheva *et al.* 1998; Hamilton *et al.* 2001; Vannette & Hunter 2011). Studies that associate variation in foliar defence with foliar nutrient availability should include a broad mixture of intra- and interspecific studies, to measure the importance of phylogenetic signal and phenotypic plasticity in such responses (Agrawal & Fishbein 2006). The fact that cardenolide production is correlated with foliar P concentration in milkweeds suggests that synthetic pathways of cardenolides may be tightly controlled by P-containing intermediates and/or cell energetic needs. As anthropogenic N deposition increases, understanding the mechanisms by which nutrient availability influences foliar chemical defence will become increasingly important (Throop & Lerdau 2004; Zehnder & Hunter 2008).

In our study, the strong interaction between foliar N and cardenolide concentrations suggests that the negative effects of high N concentration on monarch growth arise from increasing toxicity of cardenolides in *A. curassavica*. Although there is accumulating evidence that dietary nutrients can affect the impact of secondary metabolites on herbivore performance (Raubenheimer & Simpson 2009), the majority of such evidence to date has involved low nutrient

levels. For example, during preingestion periods, inhibitory effects of chemical defences on feeding may be greater at low nutrient levels, because the stimulatory effects of nutrients are not enough to counteract inhibitory effects of defences (Glendinning & Slansky 1994). The result is a higher expression of deterrence per unit concentration of chemical defence when the nutrient is limiting (Simpson & Raubenheimer 2001; Cruz-Rivera & Hay 2003). Alternatively, during postingestion periods, there can be direct chemical interactions between nutrients and secondary metabolites, such that effective concentrations of nutrients vary with levels of defence chemicals. For example, responses of brushtail possums to N variability in *Eucalyptus* leaves depend on the foliar concentration of condensed tannins (DeGabriel *et al.* 2009). When foliar N levels are high, possums suffer less per unit increase in condensed tannin concentrations, because after precipitation by condensed tannins, there remains enough protein for the herbivore. In our study, foliar N concentrations were lowest in *A. syriaca*. We found no interactive effect of foliar N and cardenolide on caterpillar growth on *A. syriaca*, presumably because cardenolide concentrations are also relatively low. In contrast, monarch growth rate appeared more strongly affected by foliar cardenolide concentration on *A. incarnata* under low P concentrations (Table 2). While this might suggest that cardenolide toxicity is higher under low P concentrations, we observed no such effect on either *A. syriaca* or *A. curassavica*, suggesting some alternative mechanism. Future studies should include adding cardenolides experimentally to low N and P milkweed species to explore in more detail this end of the N/P/cardenolide spectrum.

We are only aware of one other published example where foliar chemical defences appear more toxic under high dietary nutrients. In their work with African grasshoppers, Simpson & Raubenheimer (2001) found that excess protein led to higher toxicity of condensed tannins during postingestion periods. In our system, we also speculate that the interaction between cardenolide and excess N occurs during postingestion periods because cardenolides in milkweed act as feeding stimulants rather than as deterrents (Zalucki, Brower & Malcolm 1990), making preingestion effects of high cardenolide on monarchs unlikely. To test this hypothesis, future studies should include direct measures of consumption, excretion and growth efficiency of herbivores.

Summary & conclusions

Although many classic studies have explored the independent effects of foliar nutrients and secondary chemicals on consumers, studies of their interactions remain scarce. Here, we show that high foliar N concentrations increase the per unit toxicity of a potent plant defence, reducing the growth rate of a specialist insect herbivore. With increased nutrient loading in both aquatic and terrestrial ecosystems, such interactions between chemical defence and plant

nutrients may become increasingly prevalent. Exploring the nature and mechanisms underlying such interactions will be important in future studies of plant defence and coevolution between plants and herbivores.

Acknowledgements

This work was supported by an Angeline B. Whittier fellowship and a Barbour fellowship from the University of Michigan to L.T., and NSF DEB-0814340 to M.D.H. Hannah DeRose-Wilson and Mary Kate Cartmill provided great help with plant and insect maintenance. Michael Grant from the Biological Station of UM performed the nutrient analyses. We thank the editor Marc Johnson and four anonymous reviewers for their insightful suggestions. The authors declare no conflict of interests.

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Received 7 February 2013; accepted 7 August 2013

Handling Editor: Marc Johnson

Supporting Information

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

Fig. S1. Effects of N (a, b) and P (c, d) fertilization on foliar N (a, c) and P (b, d) concentrations of *A. syriaca*, *A. curassavica* and *A. incarnata*.

Fig.S2. Relationship between foliar P concentration (%) on foliar cardenolide concentration (mg g^{-1}) in *A. curassavica* (a), *A. incarnata* (b) and *A. syriaca* (c).

Fig.S3. Scatterplot showing the growth rate of *D. plexippus* larvae across variation in foliar nitrogen (N) and cardenolide concentrations.

Table S1. Results from a general linear model examining the effects of milkweed species, foliar nitrogen (N) concentration, foliar phosphorus (P) concentration and their interactions on foliar cardenolide concentrations in *A. syriaca*, *A. incarnata* and *A. curassavica*.