The multi-trophic context of plant defense: ecological and evolutionary implications of variation in milkweeds

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Emily K. Mohl

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Dedication

I dedicate this thesis to Don Alstad, who helped me set off on this journey, and to all of those who have walked with me in the exploration of milkweed aphids and their natural enemies. These people include elementary school students and their teachers, undergraduates, colleagues, volunteers, passersby, statisticians, professors, and especially my family, who cannot walk with me through a field without stopping to turn over the leaves of the milkweed.

Abstract

Plants exhibit great diversity in defense-related traits, such as toxins and spines. Most explanations for this variation assume that such traits are costly because they require resources to produce and maintain; however, controlled experiments frequently fail to document costs of plant defenses. One explanation for this pattern is that ecological context matters. I develop the hypothesis that certain plant traits impose ecological costs in a multi-trophic context because they disrupt predation on herbivores. Because milkweed plants are toxic and harbor specialist herbivores that sequester the toxins to defend themselves against generalist predators, I expect plants to incur ecological costs of defense in this system. I investigate the impact of variation among milkweed species on the ecology and evolution of predators that consume herbivores, testing for costs of defense in a multi-trophic context. I show that plants can strongly impact the preference and performance of some predators and generate patterns consistent with ecological costs of defense. However, I also demonstrate that other traits and processes, such as plant tolerance of herbivory and predator tolerance of plant-derived defense, may have the potential to mitigate such ecological costs. Consequently, ecological costs of defense are unlikely to be fixed but are instead subject to coevolutionary dynamics. As introductions, extinctions, and range shifts change the pool of potentially interacting species, a more predictive understanding of the way individual traits affect, and are affected by, the community context in which they occur will improve our ability to prioritize and manage human impacts on these systems.

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Introduction

Defense against enemies comes at a cost. Whether studying the economies of countries or the biology of plants, accounting for the total costs of a strategy is challenging. Some plants defend themselves with traits like thorns or toxins, but many plants appear relatively undefended. Plant defense theory explains this observation by positing that resources allocated for defense against herbivores cannot be used for other plant functions. However, we frequently fail to observe these costs in simple experiments because context matters (Koricheva 2002). The costs of defense depend on the network of species with which a plant interacts. Competing plants may increase the opportunity costs of defense. Similarly, defenses that harm pollinators or predators are costly.

Accounting for costs of defense has practical consequences. For example, the invasion of the Asian soybean aphid prompted American researchers to breed defended soybean varieties. They also screened Asian insects that specialize on consuming soybean aphids and released one species in an attempt to control the aphids without pesticides. While we can observe whether the soybean plant defenses interfere with predator consumption of aphids in the short term, we can only begin to estimate the long term impact of these actions if we understand the effects of plant defense across multiple trophic levels more generally. Classic trophic theory predicts that predators in a food chain will indirectly benefit plants by reducing herbivores (Hairston et al. 1960). If we are to truly understand variation in plant defense and the costs and benefits that help to explain it, we must investigate interactions across at least three trophic levels (Price et al. 1980).

In my dissertation, I present research investigating the costs of plant defenses in a multi-trophic context, focusing on defenses that prevent natural enemies from consuming herbivores. I expect plants to ecological costs of defenses when herbivores are specialists that perform well on a plant in spite of its defenses but predators are generalists that cannot tolerate the defenses. Because milkweed plants are known for their toxicity and commonly harbor specialist herbivores and generalist predators, I focus most of my research on three trophic levels in the milkweed system (Fig. 1). Milkweeds have a suite of traits that are likely to reduce herbivory, including trichomes and toxic cardenolides. Herbivores select for increased trichome densities in *Arabidopsis* (Mauricio and Rausher 1997), and trichomes are negatively correlated with herbivore abundance (but not damage) in milkweeds (Agrawal 2005a). Furthermore, specialist herbivores may be adapted to traits like trichomes that negatively impact predator foraging (Bottrell et al. 1998). Cardenolides are likely to prevent most generalist herbivores from consuming milkweed, and there is evidence that they negatively affect even some specialist herbivores (Zalucki et al. 2001, Agrawal 2004, Agrawal 2005a, Rasmann et al. 2009). A number of specialist herbivores have, like monarch butterflies, evolved ways to overcome and even sequester cardenolides, protecting themselves against their own predators. One such specialist sequestering herbivory is the bright yellow aphid, *Aphis nerii*. Despite its ability to grow on many different species of milkweeds and sequester toxic cardenolides, *A. nerii* is attacked and consumed by a number of generalist aphid predators. If plant defenses that vary across milkweeds impose ecological costs because they reduce predation by generalist predators attacking a specialist herbivore, I expect to be able to detect them in this system.

In the first chapter of my dissertation, I develop and analyze a model of optimal defense that incorporates ecological costs of defenses. I show that when defenses negatively affect plant growth, herbivory, and predation rates, and when these relationships have the appropriate curvature, ecological costs of defense can generate variation in optimal defense. Specifically, when predation is high, it is typically better for plants to be less defended than when predation is low. I also investigate the effects of varying the susceptibility of herbivores and predators to plant defenses. Based on the idea that a “jack of all trades is a master of none,” generalist and specialist consumers are expected to respond differently to plant defenses. Using the model, I investigate the implications of communities with different compositions of specialist and generalist herbivores and predators. Although some of the results are straightforward (when herbivores are more susceptible to defenses, plant defense is favored), I show that the shape and the magnitude of the effect of defense on herbivory or predation can interact to predict optimal defense investment in a tri-trophic context. In the following chapters of my dissertation, I begin to test some of the assumptions and predictions of this model.

First, I test the hypothesis that the more defended of two milkweed species also benefits less from predators. Although the overall results are consistent with this hypothesis and predators are more abundant on the less defended plant species, trends in the data suggest the exact mechanisms may have been different than expected. Plants may resist herbivory with traits that reduce herbivore consumption or survivorship, or plants may tolerate herbivory. Plant tolerance, measured as the relationship between damage and fitness, varies across individuals and species (Hochwender et al 2000, Agrawal and Fishbein 2008), although the mechanisms of tolerance are not well understood in many species. Trends in our data suggest that plant resistance may not be sufficient to explain different effects of predators on plants. Plant tolerance of herbivory may also impact these effects.

Then, I investigate the effects of plants on predators. First, I present evidence that the generalist aphid predator*, Aphidoletes aphidimyza* (Diptera), both prefers and performs better on the less-defended of the two milkweed species. Finally, I show that two populations of the generalist aphid parasitoid, *Lysiphlebus testaceipes*, appear to be locally adapted to the plant species common in the region from which they are collected. These results suggest that, like herbivores, generalist predators may also evolve adaptations to plant-derived defenses.

Overall, the data presented here confirm that plants affect predator foraging and fitness, and that predators affect plant fitness. Thus, there is the potential for ecological costs of defense to be important. However, predators and plants both appear to be able to evolve in response to specialist herbivore adaptations. Although I initially expected resistance traits, like toxins or trichomes, to disrupt interactions between plants and the third trophic level, I now hypothesize that plant tolerance of herbivory may facilitate interactions with the third trophic level. While models of optimal defense make predictions assuming a static environment, plant species have a long history of co-evolution with their herbivores and predators. As predators evolve tolerance of plant traits and plants evolve tolerance of specialist herbivores, the predictions of optimal defense models will change.

Figures

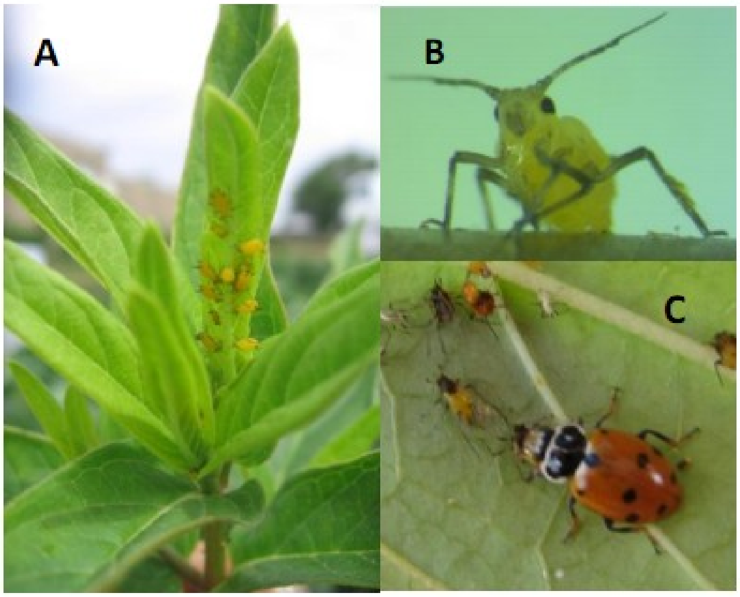

Figure 1. The milkweed system. A) A new aphid colony on a milkweed plant. B) A specialist aphid that sequesters toxins from the host plant. C) Generalist predators consume aphids.

Chapter 1

Plant defense in a tri-trophic context: Optimal defense in models with predation and ecological costs

Summary

Allocation costs are central to most theoretical explanations for variation in defense. However, evidence is accumulating that ecological costs of defense may better explain variation in defense. We hypothesize that plant defenses may be particularly costly when specialist herbivores are able to utilize plant traits, such as toxins or trichomes, to protect themselves from predation. We modify the resource availability model (Coley et al. 1985) that includes allocation and opportunity costs of defense to incorporate predation. In our model, plant defense also reduces predation, generating ecological costs. We show that environmental variation in top-down forces, such as predation, can predict variation in optimal defense, just as bottom-up variation in maximal growth rate does. When defense reduces predation, environments with high maximal predation rates should favor low levels of defense. The magnitude and the shape of the defense-herbivory and the defense-predation curves also affect optimal defense, although the predictions are dependent on other parameters. Our results demonstrate the potential for the tri-trophic context to influence a plant's optimal defense investment and highlight the importance of considering both top-down and bottom-up factors that may influence the evolution of plant defenses.

Introduction

How can we explain variation in plant defense?

Explaining the existence and persistence of variation in the natural world is a central goal of biological theory. We define plant defenses as traits that reduce consumption and/or growth rates of consumers (herbivores or plant pathogens), and these defenses exhibit dramatic quantitative and qualitative diversity within and among individuals and across species. For example, plant traits as diverse as trichomes, latex, leaf nutrient content, leaf toughness, secondary metabolites, and even phenology have all been identified as plant defenses (e.g., Herms and Mattson 1992, Agrawal and Fishbein 2006, Carmona et al. 2011). As plants develop, the concentrations of defensive compounds change, as do herbivore preference and performance (Lawrence et al. 2003, Boege and Marquis 2005, Barton and Koricheva 2010). Defensive compounds are not distributed equally across parts within a plant, with younger leaves typically having greater concentrations than older leaves (Lawrence et al. 2003, McCall and Fordyce 2010). Individual plants display a variety of induced responses to herbivory that frequently involve increasing concentrations of defensive compounds (Karban and Baldwin 1997). Within populations, heritable genetic variation for both constitutive and induced responses exists, and both are subject to selection by herbivores (Zangerl and Berenbaum 1990, Mauricio and Rausher 1997, Karban 2011). Across species, from tropical trees (Fine et al. 2006) to milkweeds (Agrawal and Fishbein 2008), phylogenetically controlled comparisons find variation in composite indices of defense. Finally, herbivore attack on plants is far from uniform (e.g., Coley 1987, Mooney et al. 2010). For example, herbivores consume 10-15% of plant productivity on average in terrestrial environments but in some environments they consume up to 90% (Cyr and Pace 1993). Together, plant defenses are thought to influence critical ecological and evolutionary processes that shape patterns of community structure and biodiversity (Ehrlich and Raven 1964, Fine et al. 2006, Poelman et al. 2008, Futuyma and Agrawal 2009). Consequently, theory that explains and predicts the pattern of variation in plant defense has broad implications.

Stamp (2003) reviewed non-coevolutionary theories that explain variation in plant defense, including the Optimal Defense Hypothesis (Rhoades 1979), the Growth Rate or Resource Availability Hypothesis (Coley et al. 1985), the Carbon:Nutrient Balance Hypothesis (Bryant et al 1983), and the Growth Differentiation Balance Hypothesis (Herms and Mattson 1992). While all of the hypotheses recognize that the costs and benefits of plant defenses depend on environmental variation, they differ in the type of environmental variation to which they most attribute variation in plant defense. Optimal defense theory focused on plant apparency, or the risk of herbivory based on growth habit, as a critical axis of variation determining the benefits of defense (Rhoades 1979). Optimal defense theory, like most other theories, also identifies resource availability as a critical predictor of variation in plant defense. Central to most of these explanations are resource allocation costs. Although coevolutionary theory may also explain variation in plant defense, costs affect the predictions of coevolutionary models (Simms 1992) and can constrain coevolutionary escalation (Thompson 2005).

Classic optimal defense theory posits that plants must allocate resources to produce defensive compounds or structures that could otherwise be applied to growth and reproduction; consequently, investment in defense reduces fitness in the absence of herbivory and is predicted to occur only when the benefits exceed the costs (Rhoades 1979). Beyond allocation costs, a number of authors have noted that plant defenses impose opportunity costs that reduce plant growth potential (Coley et al. 1985, Herms and Mattson 1992). Although optimal defense theory initially posited that plants in resource-poor environments would have the fewest resources to allocate to defense (Rhoades 1979), the growth rate and the growth-differentiation balance hypotheses predict that the opportunity costs of defense will be greatest in resource-rich environments where plants have faster growth rates and experience greater competition (Coley et al. 1985, Herms and Mattson 1992). Still other observations about plant responses to resource limitation generated predictions that some defenses may have no costs; for example, when the availability of certain resources exceeds the plant's growth demand, those excess resources are hypothesized to be diverted to defense without any associated allocation costs (Bryant et al. 1983, Herms and Mattson 1992). Although the assumptions and predictions of the major theories developed to explain variation in plant defense are complicated (Stamp 2003), there is a common emphasis on the allocation costs of plant defenses.

However, there is growing realization that other types of costs may also influence the evolution and distribution of plant defenses, including costs of self-toxicity and ecological costs incurred when traits that have a net defensive benefit in one environmental context impose costs in another (Simms 1992, Strauss et al. 2002). Ecological costs can take on a variety of forms: traits that defend against one herbivore species may increase susceptibility to other herbivores or pathogens; disrupt mutualistic pollinators, predators, or endosymbionts; reduce the ability of a plant to tolerate consumption; or reduce competitive ability. Under allocation costs, resource availability and risk of herbivory are thought to be the primary axes of environmental variation predicting variation in defense (Strauss et al. 2002). However, the specific community of mutualists, competitors, and consumers in any environment can influence the ecological costs of plant defense.

The evidence for costs of defense

Added to the complexity of predictions about the effects of environmental variation on the costs of defense are the complex ways in which defenses, and their costs, are expressed and measured. Variation in plant defense can be attributed to genetic, developmental, and environmental variation, as well as interactions between these factors. For example, within populations of the common milkweed, *Asclepias syriaca*, there is genetic variation in the concentration of toxic cardenolides in plant tissue. At the same time, abiotic environmental factors affect cardenolide concentrations in plants, and herbivory by some but not all insects can induce changes in cardenolide concentrations in *A. syriaca* (Agrawal et al. 2012). Although the ontogeny of cardenolide production appears not to be described, plants can show dramatic variation in defense investment at different developmental stages (Lawrence et al. 2003, Boege and Marquis 2005, Barton and Koricheva 2010).

Methodological issues also generate variation in the ways costs of defense are measured. The most basic measures attempt to calculate the amount of carbon and nutrients used for different plant functions (Simms 1992). However, evolutionary costs have been measured using genetic or phenotypic correlations between plant defense and fitness, usually in the presence and absence of herbivores. These types of studies vary in scale (within populations, among populations, and among species), and in the degree of control exerted over the genetic background and environmental context in which measurements are taken (Simms 1992, Bergelson and Purrington 1996, Strauss et al. 2002, Koricheva 2002). Additionally, both fitness and defense are frequently measured in different ways. Growth is often measured as a proxy of plant fitness, especially for perennial plants, although some studies also measure components of reproduction. Defense can be measured at the level of specific traits, such as secondary metabolite concentration or leaf toughness, or it can be measured using bioassays to detect resistance to a particular type of herbivore or a community of herbivores (Simms 1992, Bergelson and Purrington 1996, Strauss et al. 2002, Koricheva 2002). Because the effect of a plant trait is likely to vary across herbivores, and because tolerance can also influence herbivore abundance, each method has limitations.

Given the variability in the expression and measurement of defense and its costs, it is not surprising that costs of defense have been variable and costs have frequently been difficult to detect (Simms 1992, Bergelson and Purrington 1996, Strauss et al. 2002, Koricheva 2002). Nevertheless, a meta-analysis of 70 studies found an overall negative correlation between defense and fitness, suggesting that defense is costly. However, the magnitude of this effect was not significantly affected by the presence of herbivores, and the effect was not present under controlled conditions (Koricheva 2002). These results are inconsistent with allocation costs of defense, in which resources allocated to defense should benefit plants in the presence of herbivores and reduce plant fitness in their absence. In contrast, fitness costs of plant defense that appear under uncontrolled conditions indicate an important role for ecology. Ecological costs of defense can occur when defense traits reduce a plant's competitive ability, pollinator rate, or predation rate. Despite the importance of allocation costs for much of plant defense theory, evidence points to an important role of ecological costs of plant defense.

Some evidence also suggests that ecological costs may help to explain why some plant defenses are induced rather than constitutive. Ecological costs are plausible explanations for induced plant responses that have opposite effects on different herbivore species, opposite effects on herbivores and pathogens, or more extreme effects on predators than on herbivores (Agrawal 2005b, Karban 2011, Wei et al. 2011). However, evidence from a number of inter-specific studies is consistent with a trade-off between growth and defense (Fine et al. 2006, Van Zandt 2007, Mooney et al. 2010), although such a trade-off is not universally detected. For example, a trade-off between growth and resistance to aphids was detected among *Asclepias* species in the field (Mooney et al. 2010), but not when specific growth and defense traits were measured in a more controlled laboratory environment (Agrawal and Fishbein 2008). Together, these results suggest that allocation costs probably do occur and may be important, but that ecological context is likely to impact the magnitude of allocation costs and to impose other constraints as well.

Ecological Costs: Bottom-up and Top-down explanations for variation in plant defense

Aspects of a plant's ecology that can influence costs of defense include resource-related factors (the community of resource competitors and nutritional endosymbionts), consumption-related factors (the community of herbivores, predators, and parasites), and dispersal-related factors (the community of pollinators and seed dispersers) (Strauss et al. 2002). Coley et al.'s resource availability hypothesis (1985) and the growth-differentiation balance hypothesis (Herms and Mattson 1992) both attribute variation in plant defense to bottom-up factors related to resources. However, they do not incorporate the potential for variation in the probability or risk of herbivory, elements which are fundamental to the plant apparency concept of optimal defense theory (Rhoades 1979, Hamilton et al. 2001). Whereas apparency theory predicts that ephemeral plants can escape herbivory, the "third trophic level" includes consumers of herbivores that may also reduce a plant's risk of herbivory. For more than fifty years, we have known that top-down forces can impact community structure (Fig. 1.1A; Hairston et al. 1960). Predation risk is expected to strongly affect herbivore foraging behavior and consumption (Lima and Dill 1990), and evidence shows that predators can generate trophic cascades through trait mediated pathways (Schmitz et al. 2004, Kaplan and Thaler 2010) as well as consumption mediated pathways. If the third trophic level predictably alters a plant's risk of herbivory and ultimately affects plant fitness, and if plant defense traits alter these impacts, then top-down forces may also explain variation in plant defense.

An individual plant will experience herbivory at a rate that depends upon the rate at which herbivores immigrate to the plant, the rate at which individual herbivores consume the plant, and the amount of time an individual stays on the plant. The rate at which predation reduces herbivory depends similarly upon the rates of predator immigration, the effects of predators on herbivory rates, and the time that predators stay on plants. These processes will be influenced by environmental factors, including the availability of alternate hosts in the environment, the trophic structure of the community, and abiotic factors. At the same time, they will also be influenced by the demography and behavior of the herbivores and predators, characteristics which plant defense traits are likely to impact.

Plant traits have significant effects on the fitness and foraging behavior of insect predators and parasitoids of herbivores (Price et al. 1980). In many cases, studies of tri-trophic interactions have concluded that plant defenses reduce predator fitness, foraging, and/or consumption of herbivores (reviewed by Ode 2006). When plant defense traits generate a cascade of reduced predation, increased herbivory, and ultimately greater plant damage, they can impose top-down ecological costs (Fig. 1.1C). Plant defense is expected to impose top-down ecological costs primarily when plants are attacked by specialist herbivores that are consumed by generalist predators. For example, the fact that many specialist herbivores sequester toxins from host plants and use them for their own defense can generate what Malcolm (1992) called “the lethal plant defense paradox.” In environments where specialist sequestering herbivores are abundant and use plant toxins to protect themselves from generalist predators, plant toxins are likely to impose high ecological costs. A similar effect is possible if herbivorous insects are adapted to trichomes that disrupt the foraging behaviors of insect predators or parasitoids (Bottrell et al. 1998, Dalin et al. 2008, Hare 2002). The nature of the effects of plant defense on predators appears to vary across predator species (Kos et al. 2012), and, at least in agricultural systems, plant defense and predation more commonly appear to be additive than antagonistic (Hare 2002). Nonetheless, sequestration of toxic compounds from prey is a relatively widespread phenomenon in the animal kingdom; consequently, there is potential for top-down ecological costs of defense to have widespread impacts. For example, over 250 insect species sequester compounds from 40 plant families (Opitz and Mueller 2009), and sequestration has also been documented in sedentary marine invertebrates (Hay 1991), amphibians (Daly 1995), and potentially even birds (Dumbacher et al. 2004).

We expect generalist consumers to be more susceptible to plant defenses than specialists (Fig. 1.2). This prediction is based on the idea that diet breadth is related to a species' tolerance of plant defenses. For example, at low concentrations, plant toxins are expected to negatively affect generalist herbivores, whereas specialist herbivores may only be limited by high concentrations of toxins (Ali and Agrawal 2012). Tradeoffs could limit a generalist consumer's performance across differently defended plants (Futuyma and Moreno 1988). However, even if a consumer's tolerance of one plant defense trait is simply uncorrelated with tolerance of a different defense trait, a specialist population is expected to evolve tolerance more readily because it undergoes more consistent selection than a generalist population (Whitlock 1996).

If generalists are more susceptible to plant defenses than specialists, then the relative abundance of specialist and generalist herbivores and predators in the environment should interact to affect the top-down ecological costs of a defense trait for a plant (van der Meijden 1996, van der Meijden and Klinkhammer 2000). Specifically, we predict that when plant defenses reduce herbivory more than predation, as would be likely in an environment dominated by generalist herbivores, more defended species or genotypes will be favored. In a geographic mosaic of coevolution (Thompson 2005), this situation is likely to occur when a novel plant defense trait evolves or is introduced in a region, or when herbivores are constrained in their evolutionary responses to the plant defense. However, when plant defenses reduce predation more than herbivory, ecological costs of defense have the potential to be great, favoring less-defended species or genotypes. This condition is likely to occur in regions in which herbivores have evolved specialized adaptations to plant defenses but generalist predators have not.

When plant defenses disrupt predation, the potential magnitude of predation benefits is also an important axis of variation that should affect the ecological costs of defense. In environments where predators can generate strong trophic cascades in the absence of plant defenses, whether because they are abundant or effective, plant defenses should have strong ecological costs and less defended plants should be favored. However, rare or ineffective predators will not generate strong ecological costs, even if they are highly susceptible to defense.

The Model

Incorporating tri-trophic interactions into an optimal defense model

One of the values of formal mathematical models is that they facilitate a clear description of the assumptions that generate predictions. To formalize our tri-trophic predictions of ecological costs of defense, we build upon the resource availability model of plant defense (Coley et al. 1985) to include the costs that occur when the defense trait reduces predation on herbivores. In its original formulation, the resource availability model relates a plant's realized physiological growth rate (dC/dt; g/day) to its maximal growth rate (G; g/g/day) in the absence of herbivores, less consumption by herbivores (H; g/day). Investment in defense (D; g/g) both slows the plant's growth rate and reduces herbivory. The model can be expressed as:

$$\frac{dC}{dt} = GC(1 - kD^{a}) - (H - mD^{b})$$

Here C (g) represents the plant's initial biomass, k and a are constants that affect the magnitude and shape of the relationship between defense investment and the growth rate, and m (g/day) and b are constants that relate defense investment to reduction in herbivory. The model must be constrained such that $H\text{-}mD^{b} \geq 0$. To find the optimum growth rate, it is possible to derive this equation with respect to defense and solve for the defense value when the derivative equals 0. When a>b, meaning that initial investment in defense is less costly in terms of growth and more effective in terms of defense against herbivory, the maxima all fall at intermediate values of defense. In this case, optimal defense is a non-linear decreasing function of the maximal growth rate. A critical assumption of this model is that herbivores consume a constant amount rather than a constant proportion of plant biomass (Coley et al. 1985), which we will discuss below.

In order to model the potential for top-down processes to affect optimal investment in defense, we modified some of the assumptions and added additional parameters to the resource availability model (Coley et al. 1985). The original model assumes that investment in defense proportionally reduces the growth rate but additively reduces the rate of herbivory. In formulating the model in this way, the herbivory term drops out of the derivative, meaning that the risk of herbivory does not affect optimal defense. This assumption does not allow for top-down forces to affect optimal defense; nor is it critical to the existence of a tradeoff. We start by reformulating the model so that defense investment proportionally reduces herbivory. We then add a term for predation that reduces herbivore consumption (in units of g/day). Consequently the predation effect could be mediated by consumptive effects of predators, or by nonconsumptive effects that alter herbivore behavior in ways that reduce consumption of the plant (e.g., Schmitz et al. 2004). This yields the following tri-trophic model of optimal defense:

$$\frac{dC}{dt} = GC(1-kD^{a}) - [H(1-mD^{b}) - P(1-nD^{e})],$$

where the terms are the same as above, and *n* and *e* describe the shape and magnitude of the effects of defense on predation. In this model, each of the rate terms (growth, herbivory, and predation) is multiplied by a term that describes the effect of defense investment. The coefficients of the defense term range from 0 to 1 and describe the magnitude of the effect of defense investment on the corresponding rate. For example, we might expect investment in defense to significantly reduce the rate of herbivory of generalists (m->1) but not of specialists (m->0; Fig. 1.2A). The exponents determine the shape of the relationship between defense and the corresponding rate: the relationship is linear when the exponent is one, concave when the exponent greater than one, and convex when the exponent is less than one (Fig. 1.2B). Note that in our model, the shape parameters are described as convex or concave in terms of their affect on the relationship between defense and growth, herbivory, or predation, which is generally negative for each process. The effects of defense on these processes generate the costs and benefits of defense in our model, but the shape parameters are not described directly in terms of costs and benefits as they are in other models (where defense may increase costs and benefits, e.g. Fornoni et al. 2004). The direct effect of defense on growth translates to allocation costs, and the effect of defense on herbivory translate to the benefits of defense. The direct effect of defense on predation represents ecological costs to the plant because defense reduces the rate at which predation slows herbivory. In this model, when either the predation or growth curve is concave and/or the herbivory curve is convex, intermediate levels of defense can be optimal because the benefits of defense will exceed the costs of defense at those levels.

Like the resource availability model, the tri-trophic model, too, must be constrained so that the net herbivory term (in brackets) is not negative, that is, predation can only reduce the rate of herbivore consumption to zero. In our analyses, we constrain k, m, and n between 0 and 1 so that the defense terms never change the sign of the corresponding rates of growth, herbivory, or predation, although we discuss potential implications of relaxing this constraint below. The greater complexity of the tri-trophic model prevents us from analytically deriving an equation for the value of defense (D) that maximizes plant growth rate. Instead, we analyze the model by graphically analyzing the effects of varying specific parameters in order to test hypotheses of interest.

In all of our analyses, we test for the effects of environmental variation on the relationship between plant growth rate and defense. Initially, we set all parameters to 1 except for the herbivory rate, which we set to 2 g/day. These parameters assume linear and equal effects of defense on plant growth, herbivory, and predation. Under these conditions, the net effect of herbivory (accounting for predation) exactly counteracts growth at all levels of defense, resulting in a realized growth rate of zero. Varying any of the rate terms alone (maximal growth rate, herbivory rate, and predation rate) alters the slope of the linear relationship between plant growth and defense in a predictable way. For example, increasing the rate of herbivory increases the slope of the growth-defense function, either by making it less negative or more positive, because defense benefits plants by reducing herbivory in the model (Fig. 1.3). In contrast, increasing the maximal growth or predation rates reduces the slope because defense imposes costs to the plant through these processes (Fig. 1.3).

By changing the shape of the relationship between defense and any of the rate terms, we generate curvature in the model that, under some conditions, allows for an intermediate value of defense to maximize growth rate. Because the shape of the predation- and herbivory-defense curves can interact with those rates to affect the sign of the net herbivory term in ways that complicate inference, we start by modifying the shape of the growth-defense curve. Assuming linear effects of defense on herbivory and predation, when the growth-defense curve is concave ($a>1$), the benefits of defense exceed the allocation costs at low levels of defense, and optimal defense varies in response to each rate variable. As in the resource availability model, individuals with faster maximal growth rates should invest less in defense than those with lower maximal growth rates (Fig. 1.4A). We can also show, however, that optimal defense should increase as the maximal rate of herbivory in the environment increases, or as the maximal rate of predation in the environment decreases (Fig. 1.4B). These analyses demonstrate that in addition to variation in maximal growth rate, variation in the maximal rate of herbivory in an environment, as mediated by predators, can also predict variation in optimal defense. Interestingly, even though the only nonlinear parameter in these models relates effects of plant defense to plant growth, variation in top-down processes generates different predictions from variation in bottom-up processes. Variation in maximal growth rate predicts that the sharpest peaks for optimal defense to occur at low defense levels. As in the resource availability model, deviations from optimal defense will be most costly for fast growing plants with low defense levels (Coley et al. 1985). In contrast, variation in top-down process predicts the sharpest peaks for optimal defense to occur at high defense levels. Under top down processes, deviation from high levels of optimal defense will be most costly for plants in environments with high maximal rates of herbivory and low maximal rates of predation.

Intermediate levels of defense are also optimal when ecological costs are curved such that only large amounts of defense effectively reduce predation ($e>1$), and when the benefits of defense are curved such that small amounts of defense have large benefits in terms of reduced herbivory ($b<1$). We consider these conditions only for the case of variation in the maximal rate of predation since our primary interest is in the potential for ecological costs to maintain variation in optimal defense. Care must be taken to avoid a negative term for net herbivory, so we increase the maximal growth rate to 3 g/g/day and the maximal herbivory rate to 4 g/day, varying the maximal reduction in herbivory due to predation between 0.5 and 2 g/day. When defense has a linear cost in terms of plant growth, but a concave predation-defense curve (Fig. 1.5A) or convex herbivory-defense curve (Fig. 1.5B), variation in maximal predation rate again predicts variation in optimal defense, with greater defenses predicted in environments with lower predation. Under these conditions, however, it is no longer true that top-down processes predict the sharpest peaks at the highest values of optimal defense.

In addition to variation in the maximum predation rate, we also expect ecological costs of defense to depend on the relative susceptibility of herbivores and predators to plant defenses. To investigate this, we can vary the exponents that affect the shape of the relationship between plant defense and the rate of herbivory or predation or we can vary the coefficients that affect the magnitude of this relationship. Changing the coefficients has predictable effects: making the slope of the linear effect of defense on the herbivory rate more negative increases the optimal value of defense; whereas, making the slope of the linear effect of defense on the predation rate more negative reduces the optimal value of defense (Fig. 1.6).

Changing the exponents alters the shape of the relationship between plant defense and the rate of herbivory or predation. We expect the consumption rate of generalists to be strongly affected by low levels of defense with increasing defense levels having little extra effect, corresponding to convex curves with exponents less than 1. In contrast, consumption by specialists should be reduced only at high levels of defense, corresponding to concave curves with exponents greater than 1 (Fig. 1.2B). As the exponent modifying the herbivory term increases, corresponding to a shift from an herbivore community dominated by generalists to one dominated by specialists, optimal defense increases (Fig. 1.7). Because benefits must exceed costs for defense to be favored, this pattern is most probable with concave defense curves for growth and predation. For example, if growth costs of defense are linear, but the herbivore community is dominated by specialists so that the defense curves for herbivory are concave ($b>1$), then no defense is favored because the costs always exceed the benefits of defense. However, when the growth-defense curve is concave, then high levels of defense may be favored, even in communities dominated by specialist herbivores, because the benefits can exceed the costs (Fig. 1.7).

Although the same principles apply, the effects of the shape of the predation curve appear more complex. When growth costs and benefits are linear, no defense is optimal when the predation curve is convex, but optimal defense increases as the predation curve becomes more concave. This makes intuitive sense: when generalist predators are sensitive to lower levels of defenses than herbivores, plants should avoid producing defenses. However, with specialist predators, intermediate levels of defense can benefit plants by reducing herbivory and minimizing the ecological costs. When growth costs are concave, however, plants may maximize benefits of defense at intermediate levels even in a community dominated by generalist predators (predation curve convex; $e<1$; Fig. 1.8). Although ecological costs can be severe at low levels of defense, the growth costs are only realized at high levels, and there is an intermediate region where the linear benefits exceed the costs of defense. Interestingly, there is not a monotonic relationship between optimal defense and the exponent determining the shape of the defense curve for predation (Fig. 1.8). This appears to occur because the defense curves for predation and for growth are combined to determine the overall costs of defense. When these curves are both nonlinear, the shape of the combined curve maximizes defense at an intermediate value to either of the individual curves. For example, when the exponent for the growth curve is 2, increasing the exponent for the predation curve from 1 to 2 first decreases and then increases the optimal value of defense. In a tri-trophic system, the shapes of the growth and predation curves interact to affect optimal defense; consequently both must be understood in order to make predictions about optimal defense.

Discussion

By modifying the resource availability model (Coley et al. 1985) to include a predation term that reduces the rate of herbivory and to make each of the rate terms subject to proportional costs of defense, we have shown that variation in top-down factors can predict variation in optimal defense in the same way that variation in resource availability can. As interest in ecological costs of defense continues to build (e.g. Siemens et al. 2010), this model allows us to investigate the implications of different assumptions about the way ecological costs of plant defense in a tri-trophic context can impact optimal defense. We interpret our analysis of this model in light of the resource availability model (Coley et al. 1985), cost-benefit optimality models (Simms 1992, Mauricio et al. 1997, Fornoni et al. 2004), the evolutionary dilemma model (van der Meijden 1996), and tri-trophic consumer-resource models (Oksanen et al. 1981).

As with the resource availability model, the terms in the tri-trophic defense model describe physiological and ecological processes without explicit reference to fitness. These types of models are typically interpreted in an evolutionary context, assuming that realized growth rate correlates with fitness. Indeed, this model makes explicit the processes that generate the costs and benefits characteristic of other optimality models that do refer to plant fitness (Simms 1992, Mauricio et al. 1997, Fornoni et al. 2004). One critical assumption of the resource availability model, and our tri-trophic model, is that herbivory reduces plant growth by a constant amount rather than an amount proportional to plant growth. This assumption is not explicit in most cost-benefit optimality models, which simply assume a given shape of the cost and benefit functions. However, frequently the benefit function is assumed to saturate at a maximum level determined by herbivore density or pressure in the environment (Simms 1992, Mauricio et al. 1997), which is an analogous assumption. Changing this assumption alters the predictions of cost-benefit optimality models (Siemens et al. 2010). Consumer-resource models, such as the model used to develop the exploitation ecosystem hypothesis (Oksanen et al. 1981), explicitly tie the population dynamics of a consumer to the availability of a resource and to mortality due to predation, thereby avoiding the assumption of constant environmentally-determined herbivory. These models typically predict that in systems with three trophic levels, herbivore density will be determined by predators, which may justify the optimal defense model assumption that herbivory occurs at a rate independent of plant growth. However, we need to investigate further whether community level dynamics can justify the assumption of constant herbivory. Even if herbivores are regulated by predators at the community level, there is evidence that herbivores preferentially attack larger or more vigorous plants (Price 1991, Cornelissen et al. 2008). If herbivores attack or consume individual plants in a non-random manner, then it may not be justifiable to assume that the risk of herbivory is a characteristic solely of the environment and independent of a plant's growth rate. To better understand the implications of plant defense in a tri-trophic context, we need to understand more than effects of plant traits on consumer numerical and functional responses. We need to evaluate the manner in which the community of consumers affects the relationship between plant traits and plant fitness. While this has been achieved in some cases for plant-herbivore interactions (Mauricio and Rausher 1997, Agrawal 2005a, Wise and Rausher 2013), it is still lacking for tri-trophic interactions.

In addition to assuming that the rate of herbivory is reduced by predation, we further assume that plant defense reduces herbivory, predation, and plant growth proportionally in a manner defined by the defense-curve coefficient and shape parameters. The resource availability model also assumes that defense proportionally reduces plant growth, which may be justifiable if faster growing plants experience greater opportunity costs of defense. Whether proportional effects of defense are justifiable for herbivory and predation, as required for top-down processes to generate variation in optimal defense, is less clear. It is possible that as herbivory rates increase, herbivores will be competing more, which will increase the impacts of a unit increase in defense on the rate of herbivory. Similarly, at higher rates of predation, plant defenses may increase the sensitivity of predators to interference. Additivity may be a more parsimonious prediction, but empirical tests of the relationship between consumption and defense at different rates of maximal consumption would indicate which is a more appropriate assumption.

As with other models, we find that curvature in at least one of the defense functions is necessary to optimize defense at an intermediate level (Coley et al. 1985, Simms 1992, van der Meijden 1996, Fornoni et al. 2004). Many previous models assume a specific type of curvature based on biological reasoning. For example, the benefit of increasing defense has been assumed to saturate at the point where herbivores are entirely excluded (Simms 1992, Fornoni et al. 2004), and the costs of increasing defense have been assumed to saturate for specialist herbivores that are attracted to even low levels of defenses in plants (van der Meijden 1996). Our analysis allowed us to examine the effects of different types of curvature. Specifically, we showed that if all other relationships are linear, the defense curve for herbivory must be convex, or the defense curve for growth or predation must be concave, in order for intermediate levels of defense to be favored. Such curvature ensures that the benefits of defense exceed the costs at intermediate defense levels. A convex defense curve for herbivory corresponds most closely with the expectation for generalist herbivores that small amounts of defense are effective at reducing herbivory, and it makes intuitive sense that intermediate levels of defense will be favored in communities dominated by generalist herbivores. A concave growth-defense curve implies that the costs of defense accumulate most rapidly at high levels of defense, which may be a reasonable assumption. A concave predation-defense curve corresponds to an environment with specialist predators that tolerate low levels of defense well. This assumption seems less reliable for predators in general, especially for mobile predators that encounter prey on many different plant species. However, the available data appear to be highly variable. There does not yet appear to be much of a consensus about the empirical shape of either the herbivory- or the growth-defense curves (Bergelson et al. 2001), and further investigation into the shape of all three curves is warranted. Nevertheless, given suitable curvature, our model demonstrates two ways in which environmental variation in top down factors could generate variation in optimal defense: variation in predation pressure or variation in the susceptibility of herbivores and predators.

Predation pressure

When defense reduces the effects of predation on herbivory rates, lower levels of defense will be favored in environments with higher maximal predation rates. As defined in our model, the predation rate directly reduces the herbivory rate. Predation in our model does not require actual consumption of herbivores, but may also include non-consumptive trait-mediated indirect effects that occur when predators cause herbivores to engage in predation-avoidance behaviors that reduce herbivory (e.g. Schmitz et al. 2004). Consequently, environments could vary in predation rates because they vary in the abundance of predators, the consumption rates of predators, or the trait-mediated indirect effects of predators.

The exploitation ecosystem hypothesis posits that as primary productivity increases, trophic structure should change to include increasingly longer food chains, which affects the abundance of predators. As each additional trophic level is added, top down control should shift across alternate trophic levels. For example, in a three-level system, herbivores should be regulated by predators, but they should be regulated by resources in a four-level system (Fretwell 1977, Oksanen et al. 1981). Thus the exploitation ecosystem hypothesis suggests one possible mechanism that could generate variation in predator pressure across environments, and it predicts a specific relationship between top-down and bottom-up factors affecting plants. However, the exploitation ecosystem hypothesis only applies to food chains and does not allow for variation within trophic levels.

Mosaics of coevolution may also explain variation in predation pressure across environments. Interactions with multiple species can limit the potential for tight coevolution between pairs of species, including predators and prey, resulting in geographic mosaics in which regions of reciprocal coevolution between species in “hotspots” is interspersed with regions of non-reciprocal or absent selection in “coldspots” (Thompson 2005) . For example, chemotype matching between wild parsnips and parsnip webworm detoxification enzymes occurs in many populations, but there are mismatches in regions where alternate host plants for parsnip webworms occur (Zangerl and Berenbaum 2003). Similarly, *Drosophila* populations that are resistant to parasitoids but co-occur with other species of non-resistant host species are less resistant than *Drosophila* populations in regions where they are the dominant host, allowing for stronger coevolution of virulence and resistance between host and parasitoids (Kraaijeveld and Godfray 1999). The degree to which predators and herbivores are coevolved could affect predator foraging efficiency as well as the behavioral responses of herbivores to foraging predators. Consequently, aside from studying the coevolution of herbivores and plants, it may also be important to study the coevolution of herbivores and predators in order to better predict optimal defense in plants. If predators are also coevolved with local plant populations, then high predator pressure may be coupled with low predator sensitivity to plant defense. If other relationships are linear, then locally adapted and abundant predators may be represented by the top curve in Fig. 1.5A. A long-term research goal should be to investigate the interactions between geographic selection mosaics and tri-trophic population dynamics to determine their influence on the evolution of plant defense.

The susceptibility of herbivores and predators

Besides variation in overall predation pressure, variation in the relative sensitivity of herbivores and predators to plant defenses is also expected to generate variation in optimal defense. Again, coevolutionary explanations for variation in the local adaptation of herbivores and predators to plant defenses may help to generate predictions about which environments will favor more or less defense. Furthermore, when a plant species is introduced into a novel environment, it is likely to escape coevolved specialist herbivores, and its evolutionary response can impact the outcomes of biological control programs (Muller-Scharer et al. 2004). If introduced plants escape specialist herbivores, especially sequestering specialists, then they are likely to experience a significant change in the relative susceptibility of herbivores and predators. In their native range, plant defenses may have little effect against sequestering specialists but strong effects against generalist predators; however, in the novel range, plant defenses may be effective against generalist herbivores and have little effect on the predator community.

In the literature, there are a number of ways to distinguish generalists and specialists. Perhaps most commonly, identifying a species as a specialist or generalist involves a rough comparison of the diet breadth of a given species on a continuum compared to other similar species. For example, an insect might be considered specialized when feeding on plants within a single genus (e.g. Ali and Agrawal 2012). However, local adaptation is related to a species diet breadth (Lajeunesse and Forbes 2002), and generalists and specialists have been defined in terms of their responses to plant defenses. For example, in the evolutionary dilemma model (van der Meijden 1996), generalists respond negatively to plant compounds, but specialists respond positively to the same traits. In our model, the magnitude of the effect of defense and the shape of the defense curve make different predictions about the effects of the herbivore and predator community on optimal defense. Based on the slope of a linear effect of defense, our model shows that optimal defense increases as herbivores are more sensitive to defense and decreases as predators become more sensitive to defense, as would be expected to occur when the community shifts from specialists to generalists. Thus generalist herbivores and specialist predators favor higher levels of defense. However, our model also predicts that, under certain conditions, optimal defense will increase as the shape of the defense curve becomes more concave for both herbivores and predators. Thus under certain conditions, higher defense might be favored by a more specialized community of herbivores or predators that is relatively tolerant of low levels of defense. This result contradicts the idea that a plant introduced into a community dominated by generalist herbivores should always evolve increased defense. Our analysis reveals that we cannot simply make predictions based on generalizations about specialists and generalists; instead, we must describe both the shape and the magnitude of the relationship between defense and herbivory or predation.

Our assumption that a plant's defense reduces consumption of it both by generalist and specialist herbivores deviates from the assumption in van der Meijden's evolutionary dilemma model (1996) that assumes herbivore pressure by specialists increases with defense and that defenses are only effective against generalist herbivores. While it is true that many consumers are attracted to secondary metabolites, especially volatile compounds produced by plants, other consumers may be repelled by the same compounds (Heil 2004). There is also evidence that high levels of defensive compounds do negatively impact even coevolved specialist herbivores (see Ali and Agrawal 2012). The shape of the relationship between consumer "pressure" and defense is likely complex, and while a variety of predictions exist, the shape of the actual relationship does not yet appear to be well established and probably depends on environmental context. In part, the challenge lies in the variety of processes that are integrated into single parameters in most models. For example, consumer pressure integrates consumer preferences, foraging behavior, and consumption rates, and, depending on the time scale of the interaction, reproductive rates and fitness. The degree to which plant defense traits are genetically and phenotypically correlated will also affect the relationship between “plant defense” and consumption. For example, if the production of volatile compounds that can attract insects from a distance is linked to the production of secondary chemicals, then consumer foraging may increase while other aspects of fitness may decline with defense (e.g., Wei et al. 2011). It is possible to modify the tri-trophic model to incorporate a positive net effect of defense on the rate of herbivory and/or the rate of predation by allowing the coefficients (m and n) to be negative. For example, if defense has no effect on the rate of herbivory ($m=0$), then it is possible to show that a positive effect of defense on predation rates ($n<1$) can also result in optimal intermediate levels of defense (Fig. 1.9).

Evidence

Despite mixed evidence for allocation costs of defense and the shape of defense curves, interspecific data appear to be largely consistent with some of the predictions and interpretations of the resource availability hypothesis. Ideas associated with the resource availability hypothesis but that are not inherent in the model may help to explain the fact that the predictions seem to be born out even though some of the assumptions have not been widely confirmed. The evolutionary explanation for the resource availability hypothesis argues that species evolved in resource-rich and resource-poor environments have different suites of traits, with those in resource-rich environments evolving higher maximum growth rates, more rapid turnover of leaf tissue, lower constitutive defenses, and a greater ability to tolerate herbivory (Coley et al. 1985). Most of the tests compare the defenses of fast-growing and slow-growing species, and in general, slower-growing species have longer leaf lifespans, higher constitutive levels of defense, and lower rates of herbivory (Endara and Coley 2011). While these data do demonstrate that variation in defense correlates with other growth-related traits, they don’t preclude the role of herbivory and top down forces from influencing the evolution of defense. For example, if predators tend to be more abundant in resource rich environments, this might favor the joint evolution of low defense and fast growth, even if fast growing species do not incur greater opportunity costs of defense or herbivores do not consume a fixed amount rather than percent of plant tissue. When possible, tests of specific model assumptions provide important evidence in addition to tests that confirm predicted patterns.

Ecological tradeoffs mediated by consumers can generate divergent selection in different environments. For example, pollinators can cause divergent selection on flower color (Schemske and Bradshaw 1999) and predators can cause divergent selection on coat color in mice (Hoekstra 2006). We know that herbivores can select for increases in plant defense traits (Berenbaum et al 1986, Mauricio and Rausher 1997, Agrawal 2005a), and that specialist and generalist herbivores can exert opposite selection pressures on some plant defenses (Lankau 2007). This evidence shows that heterogeneity in the herbivore community may generate ecological costs of defense from the top down. However, it is not yet clear whether top-down forces are consistent enough to generate divergent selection or whether the effects of plant defense traits on the third trophic level translate into effects on plant fitness (Hare 2002).

Although there is growing evidence that indirect interactions can have important evolutionary implications (Biere and Tack 2013, Walsh 2013), we do not yet have much evidence about the degree to which the third trophic level exerts selection on plant defense traits. In order for the third trophic level to exert selection on plant defense traits, the effect of natural enemies must vary across individual plants in response to those traits (van der Meijden and Klinkhammer 2000). The scale and manner in which plant traits affect predators critically affects the potential for predators to impose selection on those traits. For example, plant defense traits that cause an important predator to spend less time foraging or to forage less effectively are likely to be under selection by the third trophic level. However, plant defense traits that affect the fecundity of a mobile predator at some point in the future are less likely to experience selection by the third trophic level because the predator’s mobility decouples the plant defense trait from any consequence for plant fitness. In one study, the plant chemical isopimpinellin was associated with reduced parasitism of parsnip webworm in Europe, and the chemical xanthotoxin was associated with reduced survivorship. Interestingly, isopimpinellin was the only chemical that showed lower proportional representation in European populations than American populations, where parasitism is absent (Ode et al. 2004). Although other explanations remain to be ruled out, these results are consistent with the hypothesis that selection by the third trophic level will be stronger on traits affecting predator foraging, which directly impacts herbivory on an individual plant, than those affecting predator fitness. Even if a plant trait affects predator foraging, predators susceptible to the defense trait must have a density or trait-mediated indirect impact on plant growth. There is some evidence that predator effects on herbivores do not translate directly into benefits for different plant species (Mooney et al. 2010). Consequently, it is still an open question whether predators exert selection on plant traits.

Conclusion

Many hypotheses have been proposed to explain the dramatic variation in plant defenses observed in the natural world. Resource availability and allocation are central to most of these explanations. We have shown that simple modifications to the resource availability model allow variation in top-down forces to predict variation in optimal defense as well. Our results depend upon the assumptions that 1) plant defenses proportionally reduce the rates of plant growth, herbivory, and predation, 2) there is appropriate curvature in the relationship between defense and at least one of the rate terms (growth, herbivory, or predation), and 3) herbivory and predation rates are constant rates determined by the environment rather than a proportion of the respective growth or herbivory rates. As ecological costs of defense are increasingly used to explain observations and data about the distribution of defense in the natural world (Koricheva 2002), our model incorporates specific ecological and allocation costs of defense. We posit that sequestering specialist herbivores are so damaging to plants precisely because they use plant defenses to protect themselves from predation and disrupt trophic cascades, and our model shows that ecological costs of can change optimal defense. When defense reduces predation, environments with high maximal predation rates should favor low levels of defense. The magnitude and the shape of the defense-herbivory and the defense-predation curves also affect optimal defense, although the predictions are dependent on other parameters. While predation-derived ecological costs clearly cannot explain all variation in optimal defense, investigating the assumptions and predictions of this model will illuminate whether and under what conditions top-down processes are particularly important.

Acknowledgements

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Figures

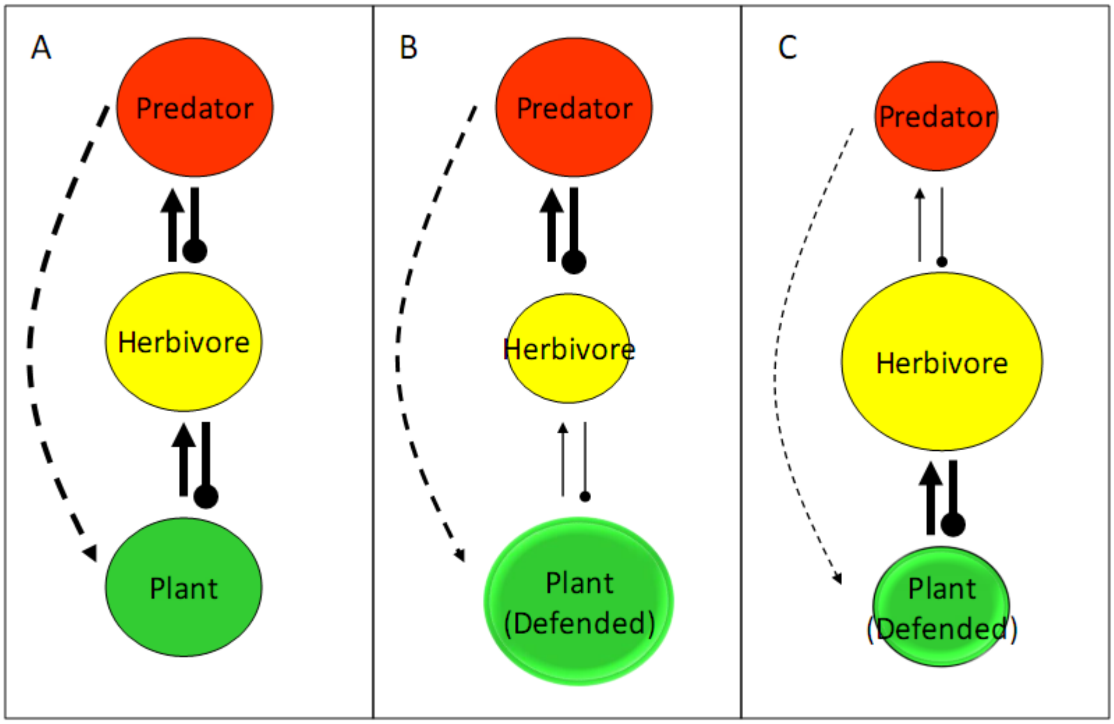

Figure 1.1. Plant defense in a tri-trophic system. A) In a community with three trophic levels, theory predicts that food-limited predators will regulate herbivores resulting in greater plant biomass, a trophic cascade (Hairston et al. 1960, Oksanen et al. 1981). B) Plant defenses that benefit plants will reduce herbivory without disrupting predation; however, the magnitude of the trophic cascade will be less because herbivores suppress defended plants less. C) The lethal plant defense paradox (Malcolm 1992): If herbivores are well-adapted to plant defenses that protect them from predation, then plant defenses may impose ecological costs. Herbivores will not be suppressed by defenses or by predators, and the magnitude of the trophic cascade will be reduced.

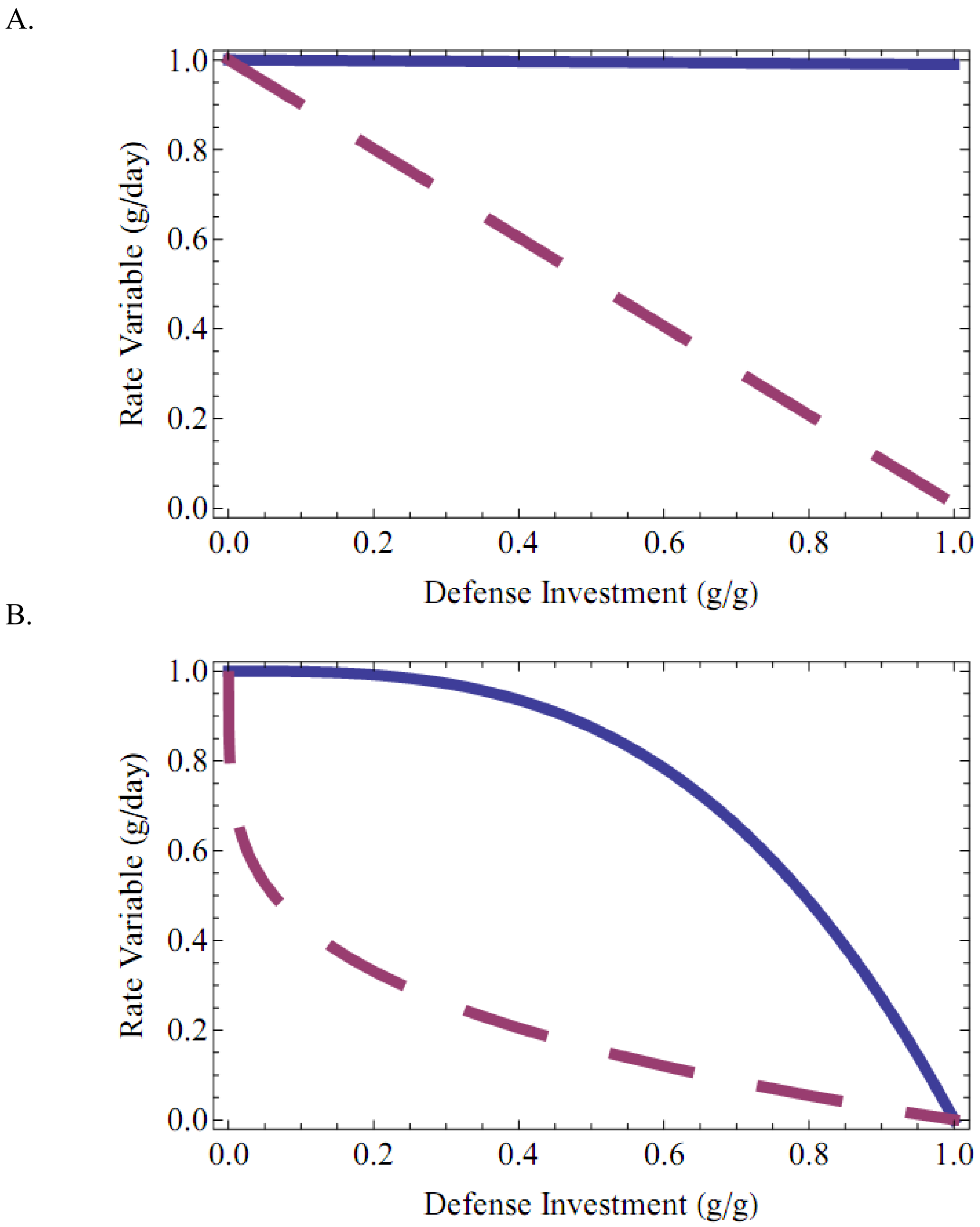

Figure 1.2. The sensitivity of consumers to defense. Consumer sensitivity is described by both the coefficient of the defense term (A) and the exponent, or shape parameter (B). A) The rate term for a specialist consumer (herbivory or predation) would be expected to be relatively independent of defense (m/n=.01; solid line); whereas the rate term for a generalist consumer would be highly sensitive to defense (m/n=.99; dashed line). B) At the same time, consumption by a specialist is expected to be reduced only at high levels of defense (Ali and Agrawal 2012), described with a concave defense curve (b/e>1; solid curve); whereas, generalist consumption is expected to be reduced at low levels of defense, described by a convex curve (b/e<1; dashed curve).

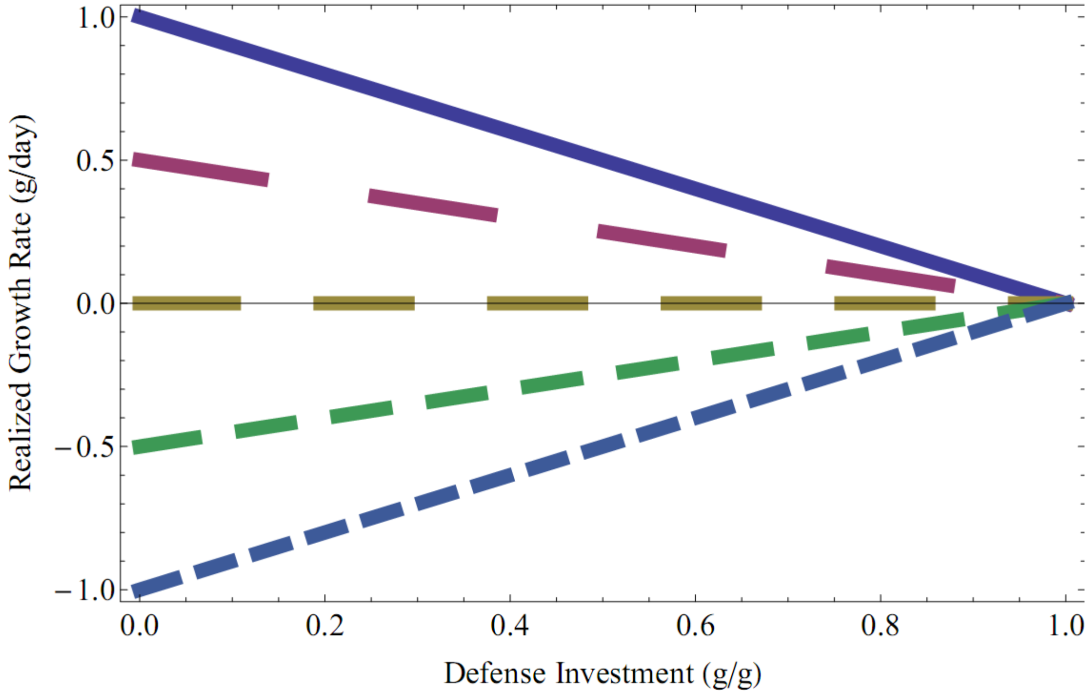

Figure 1.3. Variation in rate terms. By varying the rate terms (maximal growth rate, herbivory rate, and predation rate), we can observe the way each term mediates the effects of defense on realized growth rate. Increasing the rate of herbivory increases the slope of the relationship between defense and the realized growth rate (lines from top to bottom represent herbivory rates of 1, 1.5, 2, 2.5 and 3 g/day). Increasing the maximal growth rate or predation rate decreases the slope of the relationship between defense and the realized growth rate (lines from top to bottom represent maximal growth or predation rates of 2,1.5,1, 0.5, and 0 g/g/day or g/day). All other parameters, when held constant, are as follows: $p = g = a = b = e = g = n = k = m = c=1; h=2$.

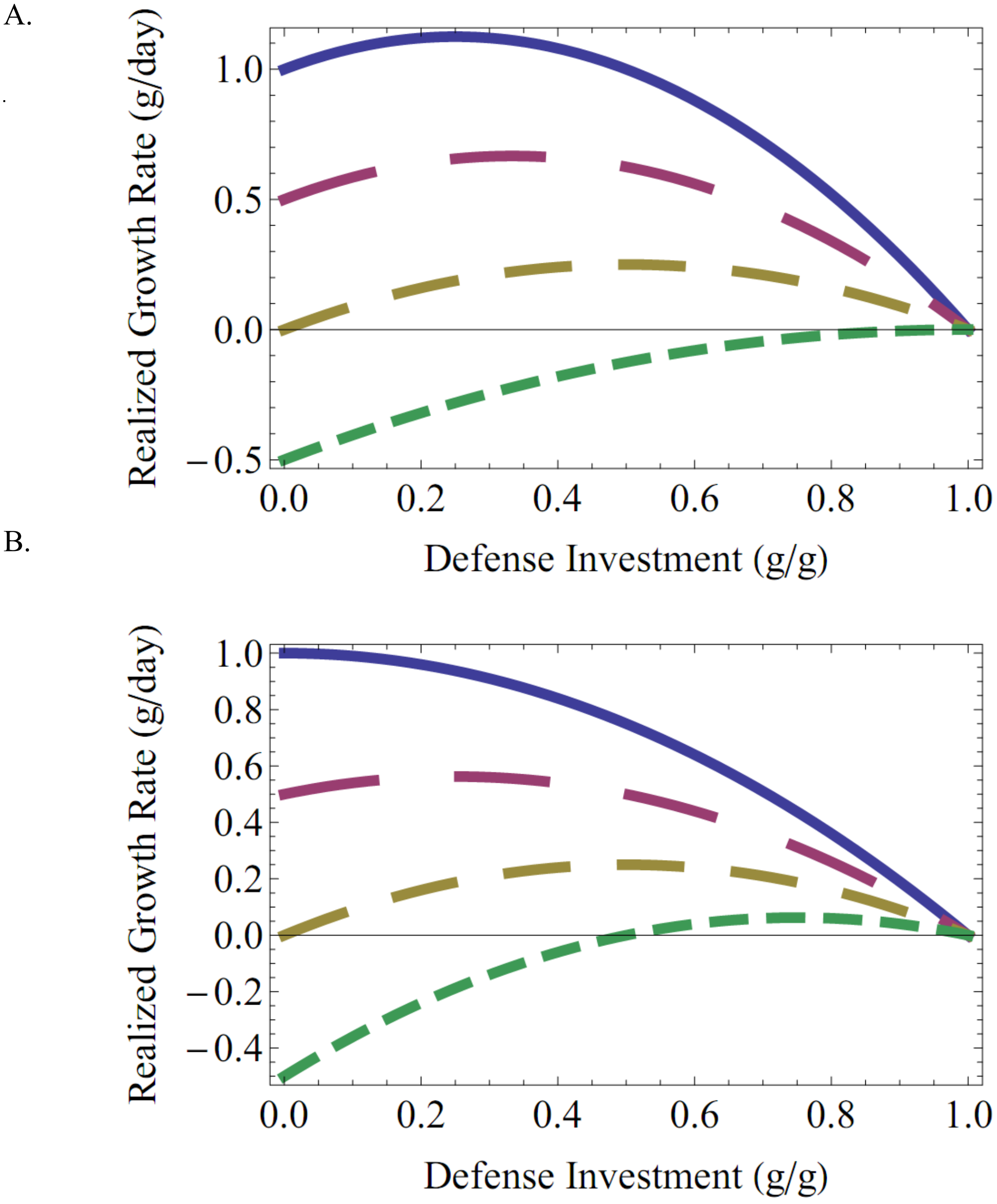

Figure 1.4. Bottom-up and top-down processes. Both bottom-up and top-down processes can generate variation in optimal defense. A) Maximal growth rate decreases from top to bottom (2, 1.5, 1, 0.5 g/day), favoring increased values of defense. B) Variation in herbivory and predation rates also change optimal defense. Varying different parameters produces an identical set of curves. As the rate of herbivory increases from top to bottom (1, 1.5, 2, 2.5 g/day), optimal defense increases. As predation rates decrease from top to bottom (2, 1.5, 1, 0.5 g/day), optimal defense increases. Except when varied, parameters in both panels are as follows: a = h = 2; b = e = g = n = k = m =c= 1.

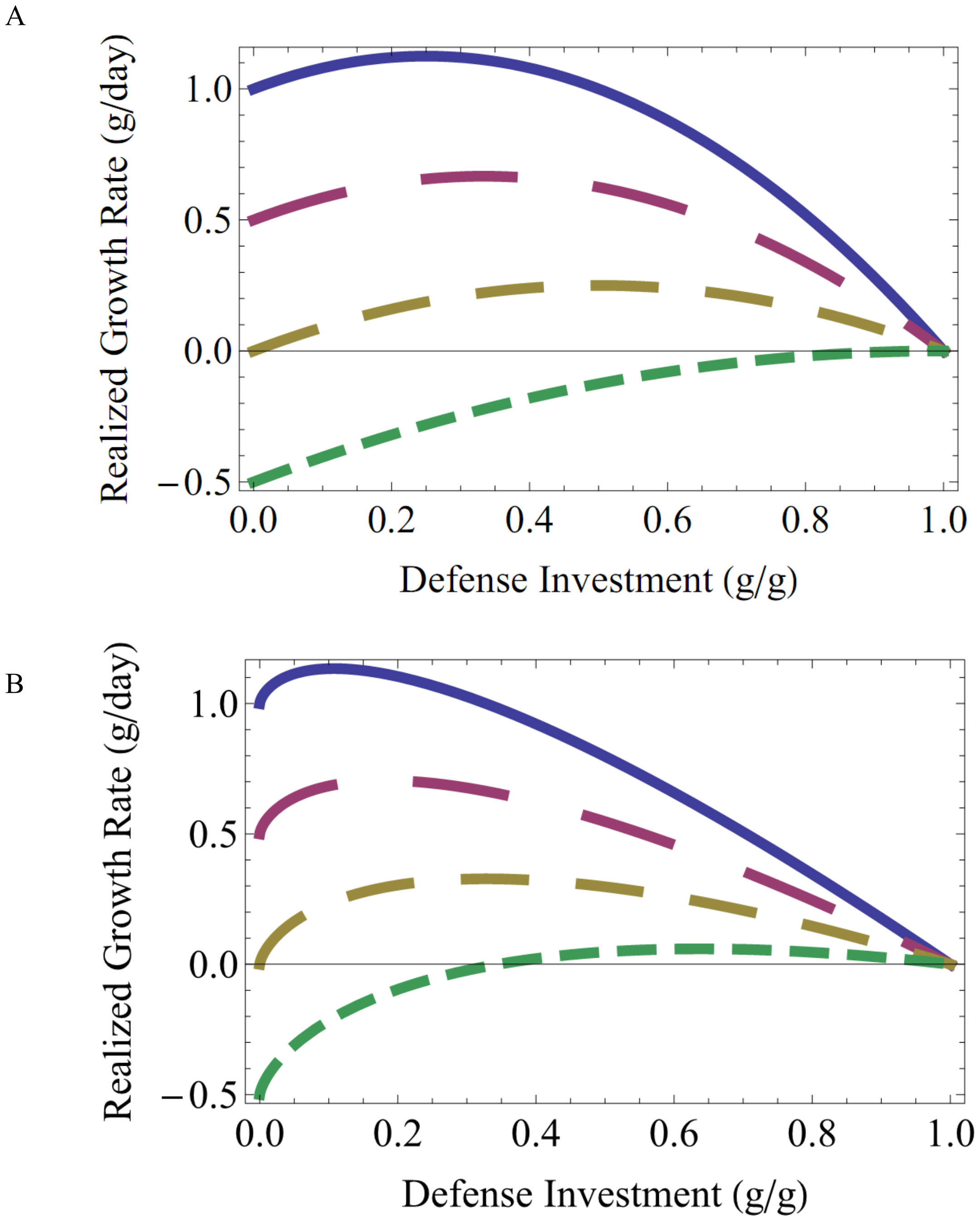

Figure 1.5. Nonlinear consumption curves. Nonlinear consumption curves can contribute to variation in optimal defense. A) When the predation-defense curve is concave (e=2), increasing the maximal predation rate reduces optimal defense. B) Likewise, when the herbivory-defense curve is convex (b=0.8), increasing the maximal predation rate reduces optimal defense. Predation rate declines from top to bottom in both figures (2, 1.5, 1, 0.5 g/day). Unless otherwise noted, parameters are as follows: a = n = k = m=b=e = 1; g = 3; h = 4.

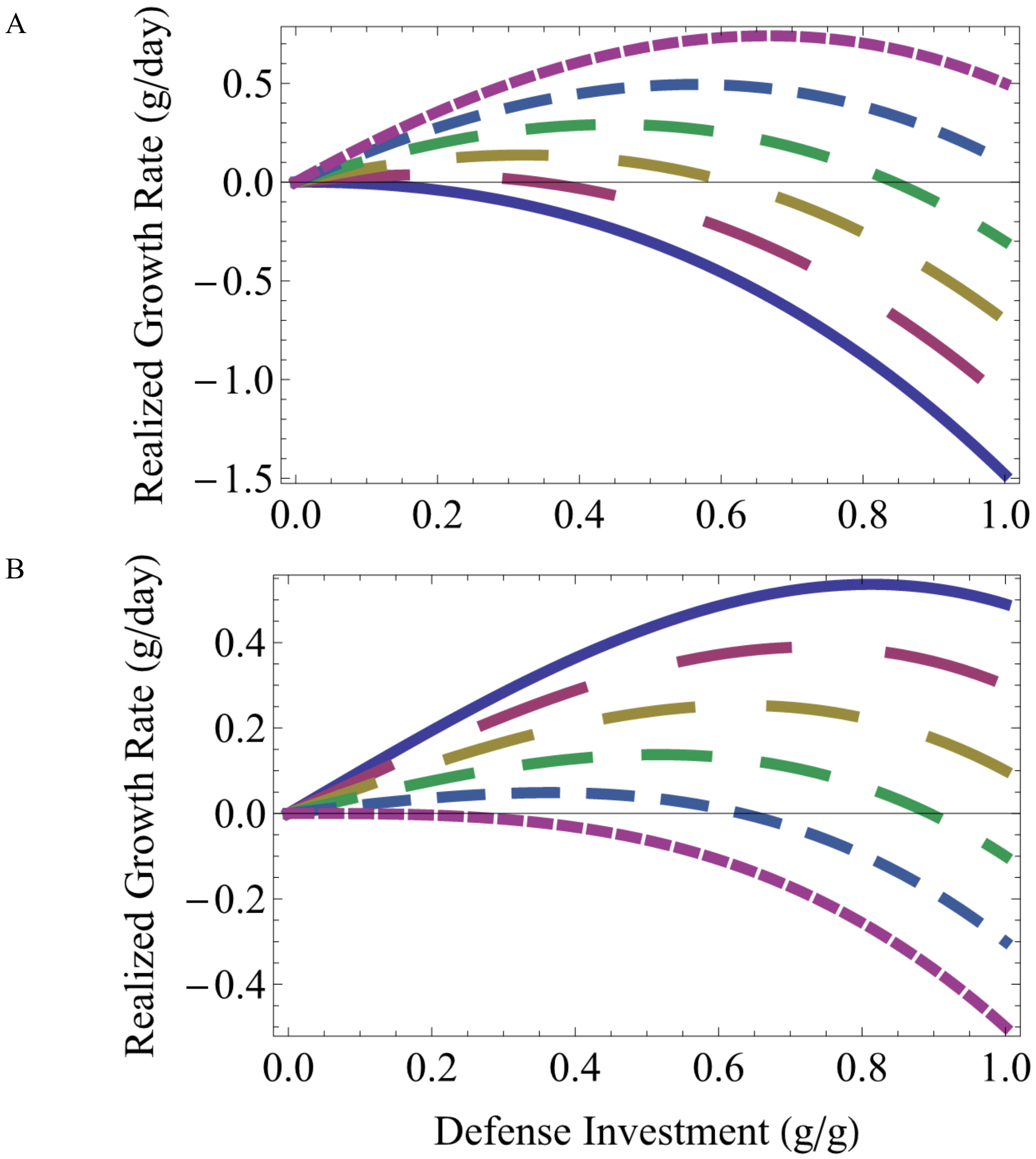

Figure 1.6. Variation in the magnitude of sensitivity to defense. The strength of the linear effect of defense on the rate of herbivory and the rate of predation alters optimal defense. A) Increasing the magnitude of the effect of defense on herbivory (m increases from .01 to 1 from solid to dashed) increases optimal defense. B) Increasing the magnitude of the effect of defense on predation (n increases from .01 to 1 from solid to dashed lines) decreases optimal defense. Fixed parameters in both plots: p =b=g= 1; a = 3; h = 2; k = .5. In A: e = 2; n =1. In (B): e = 1; m = .5.

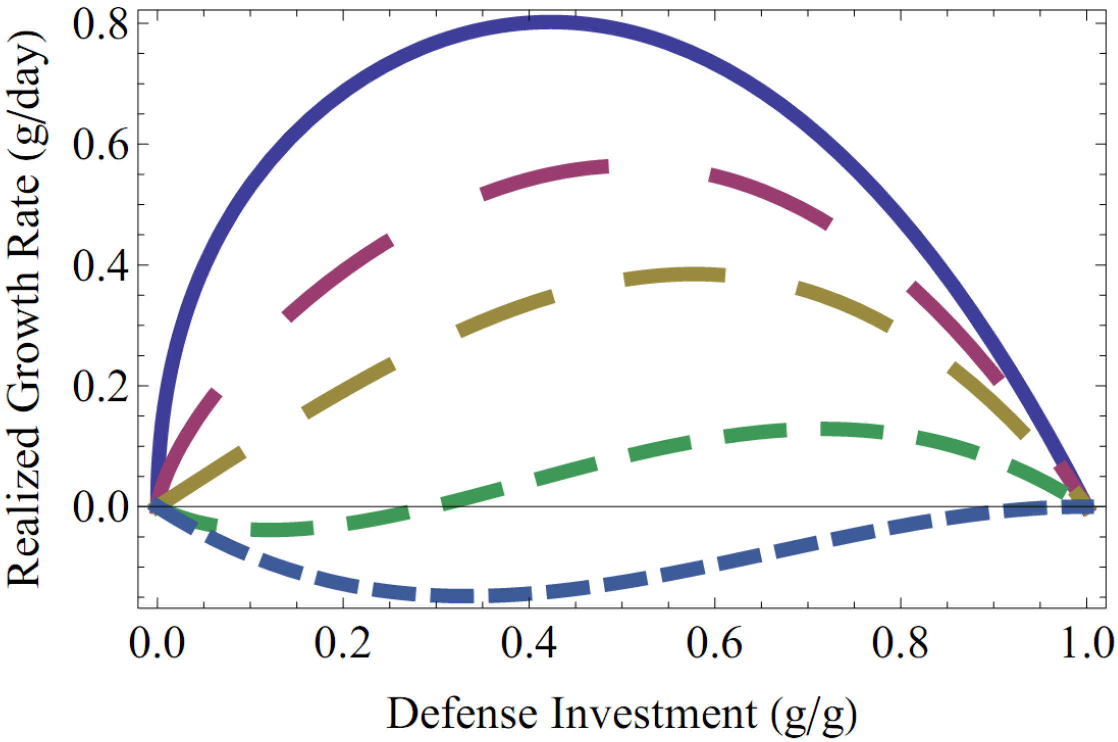

Figure 1.7. The shape of the defense-herbivory curve. As the herbivory-defense curve shifts from convex to concave (b changes from 0.5 to 2 from solid to dashed), higher levels of defense are favored. Parameters: g=p=m=k=n=e=c=1; h=2; a=3. Note that if a=1, then there is not an internal optimal level of defense for b≥1; growth is negative for any amount of defense.

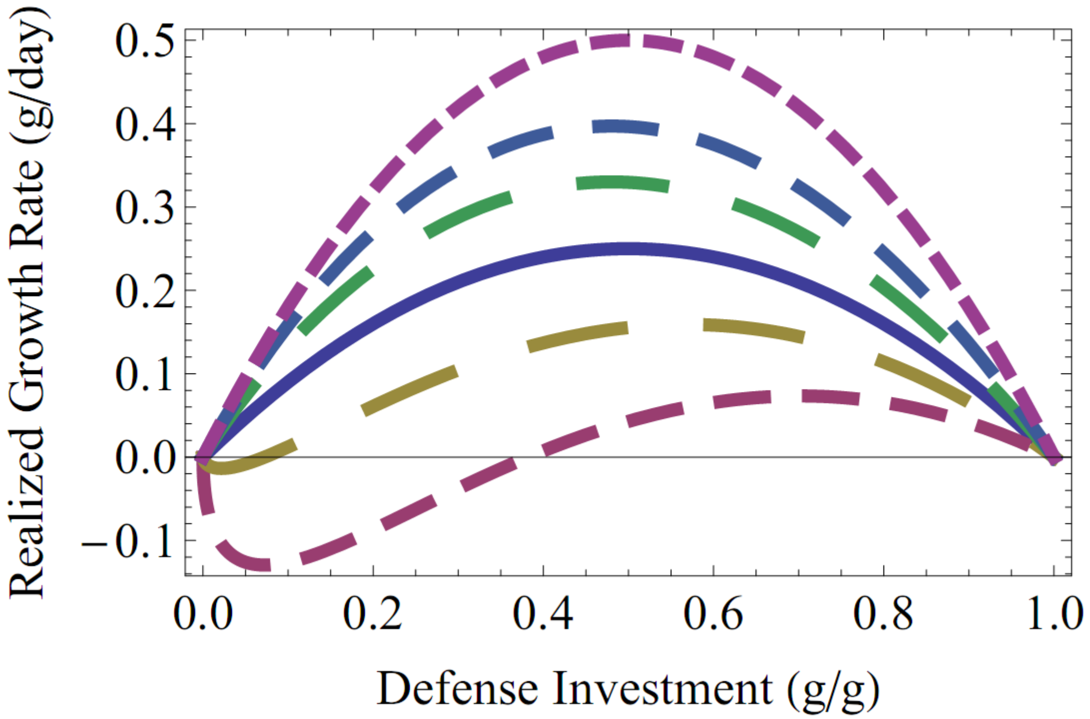

Figure 1.8. Nonlinear effects of defense on multiple rate parameters. There is a non-monotonic relationship between the predation-defense shape parameter and optimal defense when growth costs are also nonlinear. As the predation-defense curve shifts from linear to convex (e changes from 1 to 0.25 from the solid curve down), optimal defense increases. However, as the predation-defense curve shifts from linear to more concave (e changes from 1 to 2 from the solid curve and up), optimal defense first decreases and then increases. Parameters: g=p=m=k=n=b=c=1; h=a=2. Note that if a=1, then there is not an internal optimum for e≤1; growth is negative for any amount of defense.

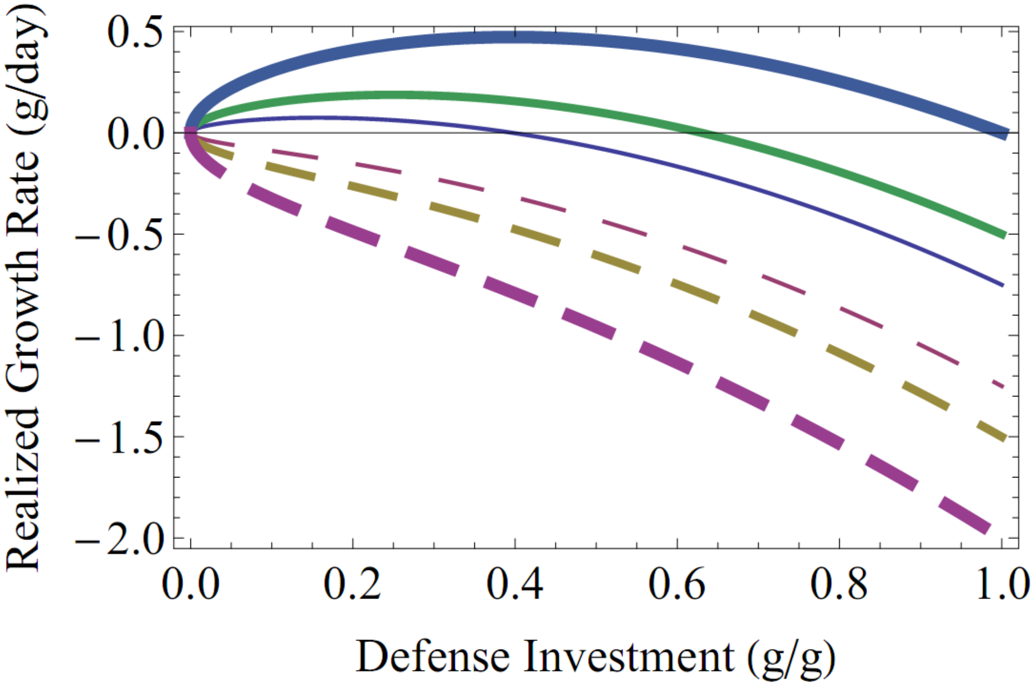

Figure 1.9. Alternative effects of defense. When defense has no effect on herbivores, then no defense is optimal when defense reduces predation (dashed lines; 0<n<1). However, intermediate levels of defense can be optimal if defense has a net effect of attracting predators (solid lines; -1<n<0). The thickest lines represent n=1. Parameters: g=k=p=b=1; h=a=2, m=0, e=0.5.

Chapter 2

Interspecific differences in milkweeds alter predator density and the strength of trophic cascades

Summary

Plant defense traits may influence the strength of trophic cascades in a variety of ways. Plant resistance to, or tolerance of, herbivores reduces the potential magnitude of a trophic cascade. Plant traits can also affect predator foraging or consumption rates in ways that either increase or decrease the strength of trophic cascades. In this study, we manipulated predator access to aphid populations on two species of milkweed, the slower growing and putatively more-defended *Asclepias syriaca* and the faster growing, putatively less-defended *Asclepias incarnata*. We observed trophic cascades that persisted across years for both species, but the strength of the cascade was greater on *A. incarnata*. The milkweed species did not show clear differences in resistance. More predators were observed per aphid on *A. incarnata*, and cage treatments generated patterns consistent with predator aggregation on *A. incarnata,* but not *A. syriaca*. However, predator effects on aphids did not consistently differ across species; consequently, plant tolerance to herbivory may be the primary driver of the difference in trophic cascade strength observed. We also observed that the timing of predator exclusion affects growth and survival differently, and we hypothesize that resource allocation patterns could explain the differences we observed in growth and tolerance between milkweed species.

Introduction

Since ideas about top-down control of ecosystems began to be formalized (Oksanen et al. 1981, Fretwell 1977, Hairston et al. 1960) and debated, authors have recognized that plant resistance has the potential to disrupt trophic cascades (Murdoch 1966, Polis and Strong 1996, Leibold 1989, Chase et al. 2000). In fact, one hypothesis explaining variation in the strength of trophic cascades across ecosystems is that terrestrial producers are often more defended, particularly with structural defenses, than algae, thereby attenuating cascades on land (Strong 1992, Shurin et al. 2006, Polis 1999).

A number of meta-analyses have identified the plant-herbivore link as a critical influence on community structure, consistent with a potentially important role for plant resistance (Halaj and Wise 2001, Schmitz et al. 2000, Brett and Goldman 1996, Shurin et al. 2002, Borer et al. 2006). Here, we compare trophic cascade strength between plant species to investigate the influence of resistance traits on trophic cascades. By focusing on "species cascades" (Polis 1999) rather than community-level cascades, we investigate the effects of species defense traits on processes mediating cascade strength in the absence of species turnover.

Plant resistance or tolerance can reduce herbivore control of plant biomass, thereby reducing the potential for predators to generate trophic cascades (Bell 2002, Mooney et al. 2010, Cronin et al. 2010). Plant resistance traits that reduce survival, growth, or reproduction of herbivores include secondary chemicals, latex, spines, and trichomes. The traits that underlie variation in plant tolerance to herbivory likely include phenology, resource allocation, and plant architecture (Stowe et al. 2000, Tiffin 2000), but tolerance is often operationally measured as the relative effect of damage on fitness (Simms and Triplett 1994). Plant defense traits may also affect predator foraging, consumption, and survival with implications for cascade strength. Thus plant defenses may reduce trophic cascade strength through two pathways: by reducing herbivore control of plant biomass or by reducing predation.

Plant resistance may reduce predation through density- or trait-mediated effects. There is evidence that parasitoids exhibit temporal density dependence in response to aphid density over some parts of the range (Helms et al. 2004). If predators also respond in a density dependent manner, then resistant plants with low aphid densities may experience reduced predation. At the same time, plant resistance traits may affect predation by altering prey quality or affecting predator foraging (Price et al. 1980, Malcolm 1992, Ode 2006). Plant resistance traits, such as toxins or trichomes, may be particularly likely to reduce predation when specialist herbivores are well-adapted to defenses that negatively affect generalist predators. Plants traits, such as extrafloral nectaries, food bodies, domatia, and herbivore-induced volatile compounds, may also increase predation on herbivores (Heil 2008, Kessler and Heil 2011). These traits, called indirect defenses, are expected to increase the strength of trophic cascades.

In this experiment, we used two milkweed species that vary in growth and resistance to explore how plant traits affect the strength of trophic cascades. We exposed the plants to specialist aphid herbivores and either allowed or excluded predators and parasitoids. We hypothesized that trophic cascades would be weaker on the putatively more-defended species, either because resistance reduced herbivory directly, because resistance reduced predation on specialist herbivores, or because the more-defended species is also more tolerant of herbivory. Although tolerance and resistance are frequently expected to tradeoff (van der Meijden et al. 1988), previous evidence indicates that the more-defended species in our study is also the more tolerant of damage (Agrawal and Fishbein 2008). Our experimental design allowed tests of the first two predictions and inferences about tolerance.

Methods

Natural History

Milkweeds (*Asclepias* spp.) are perennial plants in the Apocynaceae known for a suite of putative defense traits, including physical traits like trichomes and latex (Agrawal 2004, Agrawal 2005a), secondary chemicals in the form of cardenolides that disrupt the NA+/K+ ATPases in animal cells (Malcolm 1995, Malcolm and Zalucki 1996, Agrawal et al. 2012), and tolerance to clipping (Hochwender et al. 2000, Agrawal and Fishbein 2008). *Asclepias incarnata* and *A. syriaca* are native to North America, and have been characterized as belonging to different plant defense syndromes (Agrawal and Fishbein 2006). *A. syriaca* appears to be a slower-growing, more-defended species, exhibiting high levels of latex and trichomes (Agrawal and Fishbein 2008, Agrawal and Fishbein 2006) and inducible cardenolides (Malcolm and Zalucki 1996, Mooney et al. 2008). In contrast, *A. incarnata* exhibits low physical resistance (Agrawal and Fishbein 2006b) and cardenolide concentrations that are equal to or less than *A. syriaca* (Agrawal and Fishbein 2008, Agrawal and Fishbein 2006, Agrawal 2004). At the same time, *A. incarnata* has a faster growth rate, a taller and more branching growth habit (E.K. Mohl unpublished data), and less tolerance of damage (Agrawal and Fishbein 2008), than does *A. syriaca*, which branches less above ground but forms below-ground ramets.

Most herbivores found on milkweed plants are specialists (Agrawal and Malcolm 2002) that either avoid or sequester cardenolides (Agrawal and Fishbein 2006). Nonetheless, many generalist and specialist herbivores appear to be negatively affected by plant resistance traits, including cardenolides (Agrawal et al. 2012). We focus in this study on the aposematic aphid, *Aphis nerii*, which specializes on plants in the Apocynaceae from which it sequesters cardenolides. Several lines of evidence suggest that concentrations of cardenolides in *Aphis nerii* increase with concentrations in its host plant (Malcolm 1990, Malcolm 1992). Furthermore, the cardenolides in *Aphis nerii* are thought to affect the preference and performance of arthropod predators, including spiders (Malcolm 1986, Malcolm 1989), lady beetles, hover flies, and lacewings (Pasteels 1978, Malcolm 1992), and host plant species affects parasitism rates and parasitoid mortality on *Aphis nerii* (Helms et al. 2004, Desneux et al. 2009). Nonetheless, we observed a variety of aphid predators, parasitoids, and ants interacting with *Aphis nerii* on both plant species (Appendix A). Like most aphid species, *Aphis nerii* exhibit a wing polyphenism in which adults develop into either wingless or winged forms in response to environmental cues, including high aphid densities (Zehnder and Hunter 2007a, Hall and Ehler 1980).

A recent study found that predators generate trophic cascades across 16 milkweed species. Cascade strength was not significantly correlated with resistance to, or predator effects on, *Aphis nerii*, but instead appears to be related to high response to soil fertility and low tolerance of herbivory (Mooney et al. 2010).

Outline of Experimental Design

We used a randomized complete block factorial design to test for effects of milkweed species (*A. incarnata* and *A. syriaca*) and predator exclusion on aphid abundance, predator abundance, and plant growth. Plants were seeded with aphids and either caged in the “No Predators” treatment, or exposed to predators in one of two

"Predators" treatments. Uncaged plants were open to the environment (uncaged) while sham cage treatments controlled for the microclimate effects of caging while allowing predator access. Each plant species-predator exclusion combination was randomly applied to one of six plants along both the north and south side of each of 12 plots of soybeans, for a total of 144 milkweed plants distributed within a 0.8 ha field at the University of Minnesota experimental station at St. Paul, MN, USA. Plant growth, aphid abundance, and the identity and abundance of aphid predators were recorded weekly for 10 weeks throughout the growing season.

The soybean plots consisted of either of two varieties of soybeans: one that was bred for resistance to the soybean aphid, *Aphis glycines*, and a near-isoline susceptible variety (Chacón et al. 2012). While some preliminary analyses found significant higher-order interactions between soybean variety, predator exclusion, and milkweed species treatments; these results did not show strong or consistent patterns. We therefore exclude soybean variety from our analysis here.

Experimental Protocols

Rearing and Planting: Aphids used in the experiment all descended from a single *Aphis nerii* female selected on April 24, 2009 from a colony grown on *Asclepias syriaca*, and colonies were reared for at least a month on either *A. syriaca* or *A. incarnata* before being used in the experiment. Seeds of both species were cold-stratified and planted in 14 cm pots in the greenhouse on April 16, 2009. Seedlings were thinned to one plant per pot on May 8 and allowed to grow in a greenhouse until the experiment began. On June 12, we added 2 adult aphids to the apex of each plant in the greenhouse and allowed the aphids to reproduce. Aphids were reared on the same milkweed species as the experimental plant to which they were transferred. From June 15-19, we monitored aphid populations daily; once a plant had ≥ 20 aphids, we transplanted it to its randomly assigned location in the field and immediately applied the appropriate cage treatment.

To exclude predators, we used wire tomato cages (100x36x36 cm; Burpee, Warminster, PA, USA) enclosed in white no-see-um mesh sleeves (Quest Outfitters, Sarasota, FL, USA) that were buried in several inches of soil at the bottom of the cages and rolled closed and clipped at the top. This design allowed us to exclude predators and to open the cages for weekly counts. Plants were staked and watered as needed, and we extended the cage length as necessary to accommodate plant growth. Sham cages were identical to predator exclusion cages, but with slits cut into the sides and top of the mesh sleeve. Temperatures in caged and sham caged treatments were slightly elevated, and predator and aphid counts were different in the sham and uncaged "Predators" treatments; however, we believe microclimate effects of caging were minor compared to predator exclusion effects (Appendix B).

Counts: Each week, we randomly chose a plot to begin the counts and proceeded sequentially. For each plant, we measured the height, counted the number of leaves, counted winged (alate) and unwinged (apterous) aphids, and recorded any aphid predators found on the plant. We identified predators by sight in the field, attempting to disturb them as little as possible during our counts. We observed a variety of generalist aphid predators, including coccinellid larvae and adults, lacewings, predatory flies, predatory bugs, and spiders (Appendix A). We also report counts of parasitized aphids, or mummies, separately from aggregate predator counts (Appendix A).

During weekly counts, we opened the cages, and recorded and removed any non-aphid herbivores from all treatments and any predators found in exclusion cages. Any insects inside the cages but not on the experimental plants were noted but not included in the counts. Winged aphids that accumulated on the mesh inside the cages as the season progressed frequently escaped during counts. Cumulative calculations demonstrate that there were fewer than 4% winged aphids on average on plants of either species in predator exclusion cages.

Cage Switching: After five weeks, we switched half of the caged "No Predators" and the uncaged "Predators" treatments. Cage switching created two new levels of cage treatments: "Predators Early", and "Predators Late", for a total of five levels of cage treatment. Cage switching allowed us to determine the effects of the timing of predator exclusion treatments, to measure responses of predators to different aphid densities, and to separate the effects of excluding aphid predators and enclosing winged aphids. As expected, aphid populations declined when predators had access to previously caged plants and the declines in aphid density were largely attributable to predation (Appendix C).

Survivorship to second year: After August 31, 2009, we removed the cages and concluded the experiment for the first season. However, we allowed the plants to overwinter and measured their survival and growth during the following year. No treatments were applied during the second season, but we measured growth and survival of the plants on June 16.

Statistical Analysis

Trends in leaf, aphid, and predator counts over time were nonlinear and errors were not normally distributed, violating assumptions of standard repeated measures analyses. To capture the effects of plant species and cage treatments over the course of the season, we calculated cumulative measures of leaf number, aphid abundance, and predator abundance based on 10 weekly counts using the following formula:

$$\sum_{i=1}^{n} \frac{(x_i + x_{i-1})}{2} \times t_i,$$

where n=number of sampled dates, x_i=count on date *i*, and t_i=the number of days since the previous count (Hanafi et al. 1989). These calculations synthesize the abundance over time into a single value representative of the area under the curve (Ruppel 1983, Chacón et al. 2012), and hereafter we refer to them as cumulative leaf days (CLD), cumulative aphid days (CAD), and cumulative predator days (CPD). We show cumulative plots of leaves, aphids and predators over time in Figure 2.1. Because repeated measures of biomass on experimental plants are not feasible, leaf number was chosen as the most reliable, repeatable measure of plant size (Appendix E).

Unless otherwise described, we used linear mixed effects models with plot as a random factor and species and cage treatments as factorial fixed factors. When it improved the fit, we included the initial plant size (leaf number at week 1) as a covariate. Because variances were often non-constant across classes, we used models that allowed the variance to vary by species, species crossed with cage, or fitted values when it improved model fit; in such cases we chose the model that minimized the Aikike Information Criterion (AIC score). We used model simplification (Crawley 2007) to combine levels of cage treatments when it did not significantly reduce model fit; specifically, we tested for significant effects of (1) cage switching, with models using only early-season or late-season predator exclusion status, (2) the timing of predator exclusion, with models that combined early and late "Predators" treatments, and (3) the type of predator access, with models that combined sham and uncaged "Predators" treatments. Responses were square root transformed to improve the fit to normality. We report Wald Chi Square test statistics based on type II tests calculated using the car package in R (Fox and Weisberg 2011). Linear contrasts between means of interest were analyzed using the multiple comparisons package in R; p-values were adjusted for multiple comparisons using the single-step method (Hothorn et al. 2008).

The effects of plant species and predator exclusion treatments on the probability of plant survival to mid-June of the second growing season were assessed using a binomial generalized linear model. To account for the different growth patterns of the two milkweed species, we estimated treatment effects on per-aphid predator density and per-leaf aphid density by modeling the effects of species and cage treatments on the relationship between cumulative consumer and cumulative resource abundance at the end of the season. We excluded the "No Predator" treatment from the predator density analysis and switching treatments from both analyses. We used linear contrasts between species to test for differences in resistance to aphids in the "No Predators" treatment.

Cage switching appeared to affect both predator and aphid trajectories (Fig. 2.1, Appendix D). Aphid abundances were very different in the uncaged "Predators" and "Predators Late" treatments during week 6, so we used Wilcoxon Rank-Sum tests to compare predator abundance in these two treatments on each species as a measure of predator aggregation to patches of high aphid density. To tease apart the effects of predation and changing plant quality on aphids; we modeled the effect of species and cage treatment on the ratio of early:late season cumulative aphid days and contrasted the No Predators and Predators Late treatments (Appendix D).

Finally, to facilitate comparison of the relative magnitude of predator effects on aphids and plant growth across species, we calculated the effect size of predator exclusion on week 10 cumulative leaf days and cumulative aphid days as the ln(Predators treatment/No Predators treatment) within each plot. We used standard linear models to analyze the effects of plant species and response measure (CLD or CAD) on the absolute value of the predator exclusion effect size. All statistical analyses were conducted in version 3.0.1 (R Core Team 2013).

Results

Trophic Cascades

Predators generated trophic cascades that affected plant growth in the first season and survival to the second season for both milkweed species. Predator exclusion reduced plant growth for *A. syriaca* by 25% and for *A. incarnata* by more than 60% (Fig. 2.1A). The effects of predator exclusion on cumulative leaf days at week 10 occurred primarily within the first half of the season (Likelihood $\text{Ratio}_{df=8}=15.41$, $P=0.052$), and the uncaged and sham "Predators" treatments were not significantly different (Likelihood $\text{Ratio}_{df=4}=4.29$, $P=0.37$) and therefore combined. Cumulative leaf days at week 10 were significantly higher for *A. incarnata* than for *A. syriaca* ($\chi^2_{d.f.=1}=48.77$, $P \ll 0.0001$) and for treatments exposed to predators in the first half of the season ($\chi^2_{d.f.=1}=48.13$, $P \ll 0.0001$). The strength of the trophic cascade, as measured by the effect of predator exclusion on cumulative leaf days, was greater for *A. incarnata* than *A. syriaca* (interaction effect: $\chi^2_{d.f.=1}=46.12$, $P\ll0.0001$). Linear contrasts confirm that predator exclusion reduced growth, as measured by cumulative leaf days, for both *A. incarnata* ($z=9.07$, $P_{adj} \ll 0.0001$) and *A. syriaca* ($z=3.47$, $P_{adj}=0.001$).

More than 90% of the plants exposed to predators during the second half of the growing season in 2009 survived until June of 2010, but predator exclusion in late 2009 reduced survivorship to 70% for *A. syriaca* and less than 15% for *A. incarnata* (Fig. 2.1B). The effects of predator exclusion on survivorship occurred primarily within the last half of the season ($\text{Deviance}_{df=4}=9.31$, $P=0.054$), and the uncaged and sham

"Predators" treatments were not significantly different (Deviance $_{df=2}$=3.36, P=0.19) and therefore combined. Overall, late season predator exclusion reduced plant survival ($\chi^2_{d.f.=1}$=51.55, P<<0.0001) and *A. incarnata* experienced higher mortality than *A. syriaca* ($\chi^2_{d.f.=1}$=11.96, P=0.0005). Late season predator exclusion affected the survival of *A. incarnata* more than *A. syriaca* (Interaction: $\chi^2_{d.f.=1}$=6.18, P=0.013).

Aphid and Predator Abundances

Cage treatment significantly affected the cumulative abundance of predators (CPD; $\chi^2_{d.f.=4}$=264.21, P<<0.0001; Fig. 2.2A,B) and aphids (CAD; $\chi^2_{d.f.=4}$=164.06, P<<0.0001; Fig. 2.2C,D) after 10 weeks. The sham and uncaged "Predators" treatments accumulated significantly different numbers of predators (Likelihood Ratio$_{d.f.=4}$=16.54, P=0.0024) and aphids (Likelihood Ratio$_{d.f.=4}$=31.86, P<0.0001), so both levels were retained for the analysis. Nevertheless, linear contrasts confirmed that there were more predators in both the sham and uncaged "Predators" treatments than in the "No Predators" treatment for both species (P_{adj}< 0.001 for all contrasts). Aphids were more abundant in "No Predators" treatments than in either "Predators" treatment on both species (P_{adj}< 0.001 for all contrasts).

A. incarnata accumulated more predators ($\chi^2_{d.f.=1}$=74.42, P<<0.0001; Fig. 2.2A,B), and more aphids ($\chi^2_{d.f.=1}$=18.54, P< 0.0001; Fig. 2.2C,D) than *A. syriaca* did over the 10 weeks. Except for parasitoid mummies (Appendix B: Fig. 2SB1), each class of predator was observed more frequently on *A. incarnata* than on *A. syriaca* (Appendix A: Table 2SA1). Additionally, there was an interaction between plant species and cage treatment for both cumulative predator days ($\chi^2_{d.f.=4}$=64.42, P<<0.0001) and cumulative aphid days ($\chi^2_{d.f.=4}$=12.73, P=0.013) at 10 weeks. Predator access in the non-switching treatments increased cumulative predator days and reduced cumulative aphid days more on *A. incarnata* than it did on *A. syriaca*.

Responses Standardized by Resource Availability

The slope of the relationship between cumulative predator days and cumulative aphid days at week 10 is steeper on *A. incarnata* than it is on *A. syriaca* in the

"Predators" treatments (SpeciesxCAD $\chi^2_{d.f.=1}$=8.71, P=0.0032, Fig. 2.3A), and the intercept is also higher ($\chi^2_{d.f.=1}$=143.15, P<<0.0001). Sham cages tended to increase the intercept ($\chi^2_{d.f.=1}$=4.16, P=0.041) of the relationship between CPD and CAD for both species of plants.

As expected, predator exclusion increased the slope of the relationship between cumulative aphid days and cumulative leaf days at week 10 (CagexCLD $\chi^2_{d.f.=2}$=7.37, P=0.025; Fig. 2.3B). The sham and uncaged "Predators" treatments were significantly different (Likelihood Ratio$_{df=6}$= 48.47; P <0.0001), so they were not combined. Aphids accumulated at a faster per-leaf rate on *A. syriaca* than they did on *A. incarnata* (SpeciesxCLD $\chi^2_{d.f.=1}$=9.06, P= 0.0026); however, between-species contrasts of the slope and intercept of the relationship in the "No Predators" treatments reveal no significant difference in across species (intercept z=0.94, P=0.39; slope z=-0.86, P=0.44). Given the differences in predator abundance and density across milkweed species (above), we might expect an interaction between species and cage treatment to affect the slope of the relationship between aphids and leaf number, and indeed the trend in the data appears consistent with the prediction that predators reduced aphid density more on *A. incarnata* than on *A. syriaca*. However, the three way interaction was not significant ($\chi^2_{d.f.=2}$=0.39, P=0.82). Predator exclusion effects are qualitatively similar when aphid density is based upon an estimate of biomass (Appendix E); however, an analysis at week 5 suggests aphids initially accumulate at a faster per-leaf rate on *A. incarnata* than *A. syriaca* (Appendix F).

Cage Switching

The timing of predator exclusion, but not cage switching, affected plant growth and survival (above); however, both cage switching and the timing of predator exclusion affected aphid and predator abundance (Appendix D). In the "Predators Late" treatment, we observed a sharp increase in predators during the two weeks immediately after cages were removed from the plants, consistent with predator aggregation to high aphid densities. Furthermore, it appears that predators rapidly suppressed aphid populations in these treatments and then disappeared from the plants (Fig. 2.2A-D). However, the effects were not consistent across species. Predator abundance at week 6 was significantly greater in "Predators Late" treatments than "Predators" (uncaged) treatments on *A. incarnata* ($W = 106.50$, $P = 0.045$), but not on *A. syriaca* ($W = 78.00$, $P= 0.42$). Cumulative aphid days during weeks 6-10 were affected by an interaction between species and cage treatment ($\chi^2_{d.f.=4}=12.15$, $P=0.016$). Linear contrasts show reduced aphid growth on the "Predators Late" plants compared "Predators" (uncaged) plants for *A. incarnata* during weeks 6-10 ($z=-4.57$, $P_{adj}<0.0001$; Appendix D: Fig. 2SD1). Although a similar trend was observed for *A. syriaca*, the effect was not significant ($z=-1.97$; $P_{adj}=0.111$). Plants in the "Predators Late" treatment had fewer than 5% winged aphids at the time of cage switching, and the effects of removing cages on aphid populations exceed the effects of declining plant quality on aphids in the "No Predators" treatment (Appendix D).

Predator Effect Size

Predators have a positive effect on plants that increases through time (Fig. 2.1E,F); however, predator effects on aphids are negative, increasing in strength early in the season but declining later with plant quality in predator exclusion cages (Fig. 2.1C,D). At week 10, predator effects on aphids (cumulative aphid days) did not significantly differ across species whether effect sizes were based on sham cage treatments ($t=1.37$, $P_{adj}=0.46$) or uncaged treatments ($t=-1.72$, $P_{adj}=0.27$). However, predator effects on plant growth (cumulative leaf days) tended to be greater for *A. incarnata* than for *A. syriaca* (sham cage treatments: $t=3.53$, $P_{adj}=0.003$, uncaged treatments: $t=2.24$, $P_{adj}=0.099$). In sham cages, predator effect sizes did not differ for aphids (CAD) and plant growth (CLD) ($F_{1,44}=0.10$, $P=0.75$). In uncaged replicates, predators affected aphids more than plant growth ($F_{1,44}=29.11$, $P<0.0001$), but this difference varied by species (Interaction: $F_{1,44}=7.84$, $P=0.0076$) and was much greater for *A. syriaca* than for *A. incarnata*.

Discussion

Even though milkweeds display a variety of resistance traits and aphids sequester toxins from their host plants, both plants and aphids in our study responded to predator exclusion. Our results demonstrate that predator access to plants reduces the negative impact of aphids on the growth and survival of milkweed plants, generating trophic cascades for both milkweed species. Consistent with our hypothesis, trophic cascades were stronger on *A. incarnata* than they were on *A. syriaca*, which is the putatively better-defended of the two species (Agrawal and Fishbein 2008, Desneux et al. 2009). We did not find strong evidence that the milkweed species differed in their resistance to aphids. Although we did find differences in predator abundance, density, and aggregation to aphids across milkweed species, these differences did not translate into significant differences in aphid suppression, limiting our ability to conclude that differences in predation drive differences in cascade strength. Consequently, we hypothesize that differences in milkweed tolerance herbivory may be the primary driver of the differences in cascade strength we observed. Specifically, we hypothesize that the fitness of *A. incarnata* is more affected by changes in aphid density than is the fitness of .

Resistance

We did not find strong support for the prediction of lower aphid densities in the absence of predators on the putatively more-defended *A. syriaca*. Although we observed higher aphid abundances on *A. incarnata* than on *A. syriaca* in each of the cage treatments, leaf number appears to account for much this difference. In the absence of predators, we find no significant effect of species on the per-leaf rate of aphid accumulation; thus we have no evidence that resistance affects cascade strength for our two species. We interpret our results with caution because the two milkweed species have different growth forms: *A. syriaca* has larger leaves which may reduce competition among aphids. Nonlinear temporal dynamics further complicate comparisons: we observed higher per-leaf aphid densities in the early season on *A. incarnata* (Appendix F), where aphids tended to accumulate earlier than they did on *A. syriaca* (Appendix D). However, over the whole season, per-leaf densities were higher on *A. syriaca*, which could occur if resistance declines over time in *A. syriaca*, as has been shown for induced resistance in herbs more generally (Barton and Koricheva 2010). Finally, inferring resistance based on the relationship between aphid abundance and plant growth may confound tolerance and resistance since aphid populations growing at the same per-capita rate on two different plants would grow at a faster per-leaf rate on the less tolerant plant. Nonetheless, as a specialist aphid, *Aphis nerii,* may be well adapted to moderate levels of defenses in both milkweed species. Although one study found that the maximum growth rate of *A. nerii* was negatively correlated with cardenolide concentrations among milkweed species, it also reported a positive correlation with trichome density (Agrawal 2004). A different study inferred that cardenolide sequestration was likely to be a general mechanism that limited the potential for tradeoffs in the performance of *A. nerii* across different host plants (Groeters 1993).

Predation

While we saw no evidence of differential resistance to *Aphis nerii* by our two milkweed species, *A. syriaca* is still expected to have stronger resistance against other herbivores as well as insect predators and parasitoids (Malcolm 1989, Desneux et al. 2009, Agrawal et al. 2012). Indeed, *A. syriaca* experienced reduced predator densities in our study. *A. syriaca* supported fewer predators across diverse feeding guilds and accumulated predators at a lower per-aphid rate than did *A. incarnata*. Furthermore, significant aggregation to, and suppression of, high aphid densities after cage switching was only found on *A. incarnata*. These patterns could be attributed to differences in per-plant aphid abundances across species, or they might be caused by differences in induced indirect defenses like volatile compounds that affect predator foraging. They are also consistent with theory predicting plant resistance to interfere with indirect defenses provided by predators (Price et al. 1980, Malcolm 1992, Ode 2006). Because predators accumulated at a faster per-aphid rate on *A. incarnata* than on *A. syriaca*, even over similar ranges of cumulative aphid days, we hypothesize that predators are responding to a trait-mediated effect across species. Results from controlled studies of the preference and performance of the most abundant predator, *Aphidoletes aphidimyza*, are consistent with this hypothesis (Chapter 3). Other studies in the milkweed system found that high-cardenolide plants support fewer aphid predators (Malcolm 1992) and reduce the virulence of monarch butterfly pathogens (de Roode et al. 2008); however, a phylogenetically controlled study across 16 milkweed species did not find a significant relationship between resistance to and predation on *Aphis nerii* (Mooney et al. 2010). These differences may occur because cardenolides do not confer resistance to *Aphis nerii*, because phylogenetic constraint of relevant defenses reduced the ability to detect a relationship, or because of differences in response measured. It appears that plant resistance more clearly affects natural enemy fitness or abundance, and less clearly affects the impacts natural enemies have on herbivores.

Despite the fact that *A. syriaca* supported significantly lower densities of predators, milkweed species did not significantly altered the effect of predator exclusion on the relationship between cumulative aphid days and cumulative leaf days or the effect size of predators on cumulative aphid days at week 10. We note that there was a non-significant trend toward a greater effect of predators on aphid density on *A. incarnata*, as would be expected for the putatively less-defended plant with greater predator densities. However, our measure of per-aphid predator density does not account for different consumption rates by different predator species, which would more accurately quantify predation. Furthermore, complex interactions between plant species and aphid behavior or temperature growth curves could counteract the effects of predator density. Finally, cumulative aphid days reflect the combined effects of plant quality and predators throughout the season, and this may mask effects that occur on shorter time scales. Ultimately, direct measurements of the effects of plant traits on the numerical and functional responses of specific predators may be necessary in order to clarify the relationship between plant traits, predator density, and cascade strength.

Tolerance

Because there was no clear difference in resistance, and because predator effects on aphids did not significantly vary between milkweed species, we infer that greater tolerance of aphid herbivory best explains the weaker trophic cascades on this species than on *A. incarnata.* Although we do not have direct measures of tolerance to aphid herbivory from our study, a different study reported that *A. syriaca* better tolerated clipping damage than *A. incarnata* did. In fact, after removing above-ground biomass, *A. syriaca* regained 7 times the fraction of biomass that *A. incarnata* did (Agrawal and Fishbein 2008). If tolerance of aphid herbivory explains variation in cascade strength across milkweeds (see also Mooney et al. 2010), this result corresponds with the finding that tolerance best explains variation in community-level herbivore control (Cronin et al. 2010).

Tolerance to herbivory has both an environmental and a genetic component. The effect of resource availability on tolerance depends both upon the resources limiting plant growth and on the type of herbivory (Wise and Abrahamson 2005, Wise and Abrahamson 2007). Thus, we might expect different patterns of tolerance to aphid herbivory if different resources limit each milkweed species. *A. incarnata* is typically found in wetland habitats (Agrawal et al. 2008, Ivey et al. 1999, Woodson 1954), so it may have been water-limited in our experimental field, contributing to its low tolerance. Genetic variation in tolerance can be related to differences in traits that are constitutively expressed, such as phenology or resource allocation prior to damage, or to those that are induced in response to herbivory, such as shifts in the aforementioned traits (Fornoni 2011). Evidence from our cage switching experiments suggests that both milkweed species respond to aphid herbivory by altering their growth patterns, but that *A. syriaca* allocates relatively more resources to storage.

Predator exclusion early in the season influenced growth patterns for the whole season, but predator exclusion late in the season affected survival, particularly for *A. incarnata*. This suggests that milkweed plants exposed to intense aphid herbivory early in the season invest more in underground storage rather than leaf production, facilitating their survival to the second season. Herbivore-induced sequestration of resources, like carbon and nitrogen, has been observed across different types of plants, including *A. syriaca* exposed to both leaf and root chewing herbivores (Tao and Hunter 2013). Induced resource sequestration against aphids is under-studied, but it is expected against specialist herbivores, especially when early season herbivory predicts intense damage later (Orians et al. 2011). We believe induced resource sequestration may facilitate milkweed fitness in the face of herbivory by the sequestering specialist *Aphis nerii,* which has the potential for rapid population growth.

Our results also suggest that constitutive allocation patterns may impact tolerance to aphid herbivory. *A. syriaca,* the more tolerant species, has a large storage rhizome that affects its ability to tolerate clipping damage at low nutrient levels (Hochwender et al. 2000), which *A. incarnata* lacks (Agrawal et al. 2008). The resource availability hypothesis posits that adaptation to resource gradients causes growth rate and tolerance to covary positively across species but trade off with resistance to herbivory. Part of the argument is that slow-growing species in resource-poor environments invest more in leaves with long lifespans and cannot tolerate loss of these tissues, favoring resistance (Coley et al. 1985). This prediction was confirmed using tropical forest tree seedlings, where slow-growing species suffered higher mortality as a result of defoliation than did fast-growing species, regardless of the environment in which they were planted (Fine et al. 2006). However, we observed *A. syriaca* to be both the slower growing and the more tolerant of the two species we studied, and we hypothesize that constitutive allocation to storage explains both of these traits. In contrast, we hypothesize that *A. incarnata* rapidly mobilizes resources in response to changes in herbivory levels, causing a strong growth response to predator exclusion and reduced survival after late season damage. This pattern may not be limited to the two species studied here. Across multiple milkweed species, evidence supported an evolutionary tradeoff between response to fertilization and tolerance to aphid herbivory (Mooney et al. 2010), and a separate study demonstrated that tolerance to damage and root-shoot ratios were positively correlated but phylogenetically constrained (Agrawal and Fishbein 2008).

Conclusion

We have demonstrated trophic cascades occurred on both milkweed species that persisted across years; however, the magnitude of the effect was much greater on *A. incarnata*, which both tolerated aphids less well and supported a higher density of predators than *A. syriaca* did. Because predator effects on aphids did not reliably differ between the species, we infer that the difference in cascade strength is primarily due to the difference in tolerance to herbivory between these species. These results tie together and build upon previous research that has demonstrated 1) a macroevolutionary decline in resistance traits and an increase in tolerance to clipping in milkweeds (Agrawal and Fishbein 2008), 2) an evolutionary association between trophic cascade strength, response to fertilization, and tolerance of aphid herbivory (Mooney et al. 2010), and 3) the mechanisms for tolerance in *A. syriaca* may involve both induced resource sequestration (Tao and Hunter 2013) and allocation to roots (Hochwender et al. 2000). Together, we have an emerging picture that tolerance is particularly important when milkweeds face consumption by specialist herbivores. Plant resistance traits that are effective against generalist herbivores may be particularly costly in the presence of sequestering specialists if they interfere with predators (Price et al. 1980, Malcolm 1992, Ode 2006). Tolerance to specialist herbivory may help to resolve the lethal plant defense paradox. In the milkweed family, storage and induced resource sequestration contribute to tolerance. We suspect that the degree of resource allocation to storage corresponds with variation across species in both their growth responses and their tolerance of herbivory, which contributes to the difference in cascade strength.

If specific traits predictably alter the relative importance of direct and indirect effects, this will facilitate our ability to predict the outcomes of perturbations to ecosystems. Meta-analyses have regularly observed stronger direct effects of predators on herbivores compared to indirect effects on plants (Halaj and Wise 2001, Schmitz et al. 2000, Brett and Goldman 1996, Shurin et al. 2002, Borer et al. 2006). One hypothesis is that this attenuation is more pronounced for specialist grazers and sap suckers than for generalist grazers (Schmitz 2010, p. 37). In our study with a specialist sap-sucking herbivore, we observed attenuation of predator effects only on *A. syriaca* in the uncaged treatment. We attribute this attenuation primarily to *A. syriaca*'s tolerance of aphid herbivory, which raises the question of whether plants in general are more tolerant of specialists and sap suckers than other classes of herbivores. Furthermore, if resource storage is a general mechanism of tolerance, particularly in terrestrial plants where storage and reallocation between different structural tissues is possible, then tolerance could be important in damping the strength of community-wide trophic cascades in terrestrial ecosystems. We also observed that plant species directly affected predator abundance, density, and aggregation patterns in our study; however, this variation did not always translate into an effect on aphid density. The particular traits underlying the attenuation of indirect plant effects on aphids mediated through predators are worthy of further investigation.

Because both resistance and tolerance affect trophic cascades, understanding the conditions favoring each will improve our ability to predict the nature of trophic control in ecosystems. The growth rate hypothesis for plant defense (Coley et al. 1985) predicts that resistance will be favored in slow-growing plants in resource-limited environments because they do not tolerate herbivory. However, in our study, the fast-growing species was less tolerant of aphid herbivory. Although tolerance and resistance are often expected to tradeoff (van der Meijden et al. 1988), we might expect to see a positive association between resistance and tolerance if each is effective against a different suite of herbivores, which may be particularly likely when specialist herbivores derive benefit from resistance traits. The potential for complex interactions between plant traits and predator effects on herbivores precludes simple predictions about the net effects of bottom-up and top-down processes. Ultimately, a better understanding of the relationship between tolerance, resistance, and resource availability may be critical for understanding and predicting the nature of trophic cascades.

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Figures

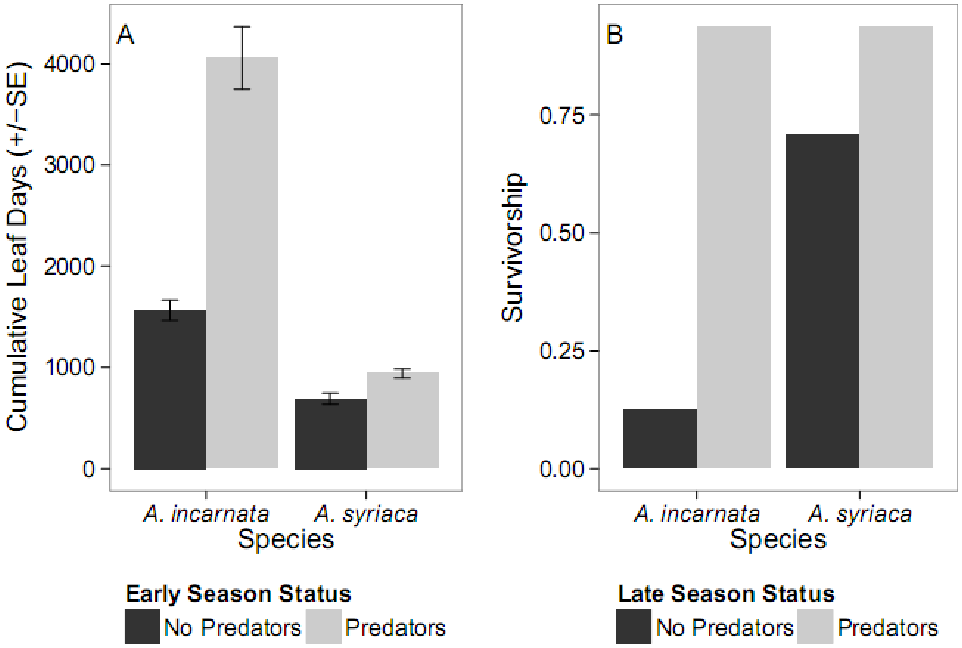

Figure 2.1. Effects of predator exclusion. A. Plant growth, measured as cumulative leaf days over 10 weeks, was significantly affected by an interaction between species and early season predator exclusion treatments. B. The fraction of plants that survived until the second growing season (June of 2010) was significantly affected by an interaction between species and late season predator exclusion treatments (n=24 for each species when predators were excluded in late 2009, and n=48 for each species exposed to predators in late 2009). For both plots, data from the two "Predators" treatments (sham and uncaged) were not significantly different and were therefore combined.

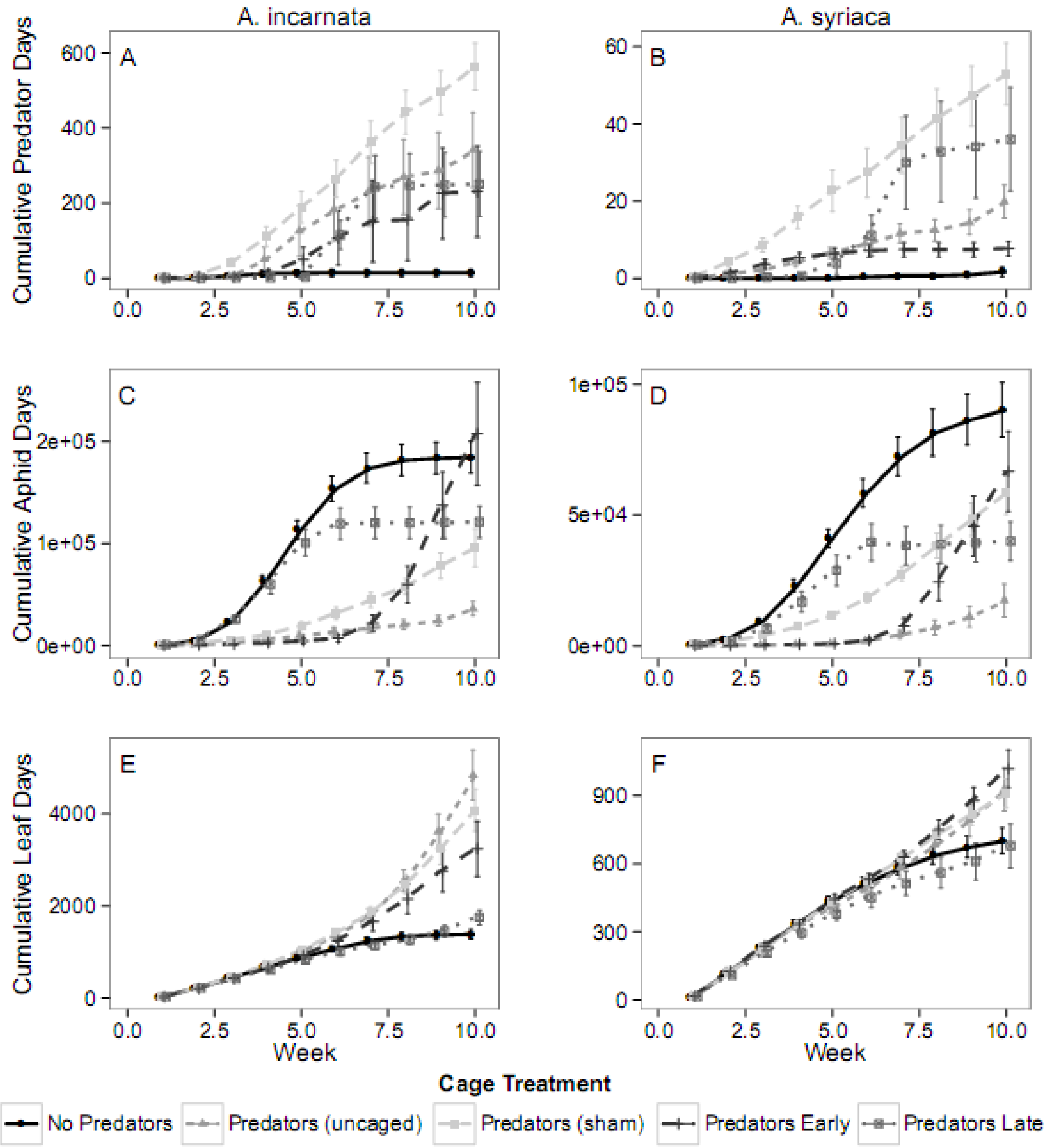

Figure 2.2. Cumulative plots. Shown are cumulative plots of predators (A and B), aphids (C and D), and leaf number (E and F) over time by species and cage treatment. The slope on a cumulative plot corresponds to the abundance at a given time point; the peak abundance occurs at the steepest point on the curve. A positive slope on a cumulative plot indicates a positive count between time points; a slope of zero indicates extinction. Note that the scales are different for each plot.

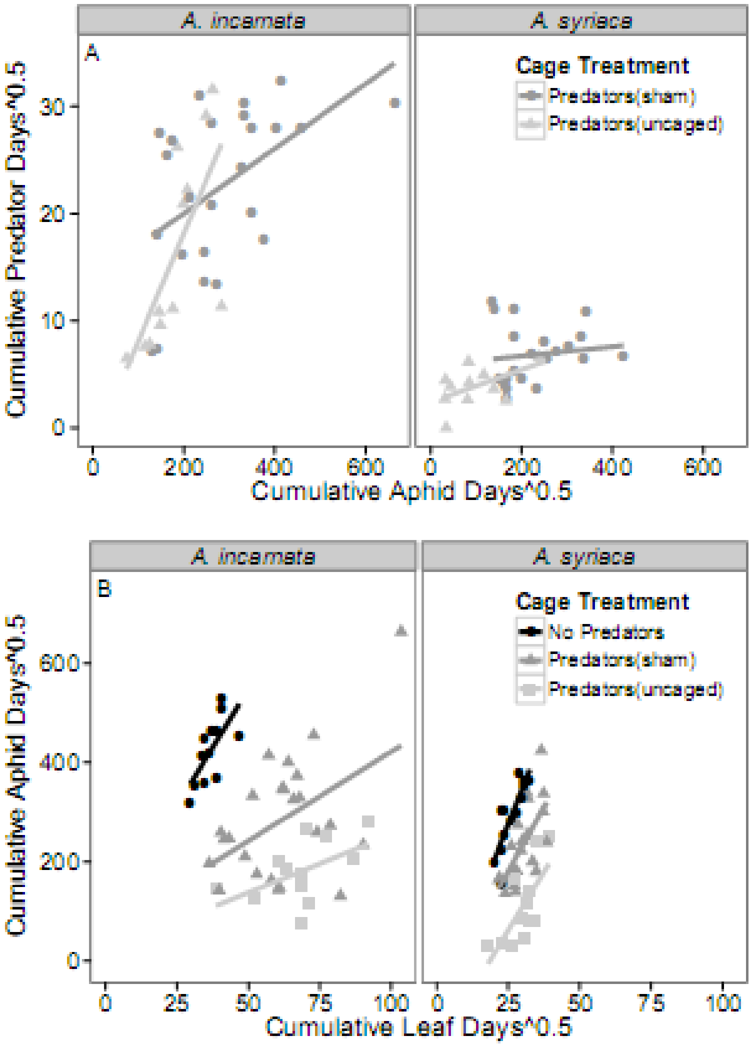

Figure 2.3. Responses standardized by resource availability. Plant species and cage treatment affect the relationship between consumers and resources. Each point in each plot represents the cumulative abundance over 10 weeks of a consumer and a resource on one plant. The slope of the relationship standardizes the abundance of consumers by the availability of resources. Cage switching treatments are excluded. A. Cumulative predator days plotted as a function of cumulative aphid days (n=36 *A. incarnata* and n=35 for *A. syriaca*). B. Cumulative aphid days plotted as a function of cumulative leaf days (n=48 plants of each species).

Supplementary Material

Appendix A. Predator and Parasitoid Observations

We attempted to observe predators without disturbing them; consequently, in some cases we grouped multiple species together into easily recognizable guilds for predator counts (Table 2SA1). Every class of predator occurred more frequently on *A. incarnata* than *A. syriaca*. Predatory fly larvae were the most abundant predators overall, and were nearly 45 times as abundant on *A. incarnata* as on *A. syriaca*. This group is primarily responsible for the differences in overall predator abundance across milkweed species. Nearly all individuals in this category were predatory midge larvae, *Aphidoletes aphidimyza*, although a few syrphid larvae were also observed. Ladybeetle larvae were the second most abundant group of predators overall and the most abundant on *A. syriaca.* Although they were an order of magnitude less abundant than fly larvae, ladybeetle larvae grow much larger and potentially consume more aphids than fly larvae. Adult ladybeetles, which we observed consuming many aphids, were intermediate in abundance, while other predators, such as *Orius* adults and spiders, were among the least abundant groups of predators, although we did not record nymphs for these groups.

Parasitized aphids, or mummies, are not included in predator counts and are analyzed separately. In order to avoid collecting mummies from experimental plants, we collected mummies from "sentinel plants." Like experimental plants, we started sentinel plants from seed in the greenhouse and seeded them with *A. nerii* at the same time, although they were 1 month younger than the experimental plants. We planted a single sentinel plant of each species along the east and west margins of each experimental plot. Mummies from sentinel plants were collected into gelcaps (size 0; Solaray, Park City, Utah) and reared in a growth chamber (16d:8n; 25°C) for emergence and subsequent identification.

Mummies were virtually absent on our experimental plants during the first half of the season but became more abundant at the very end of the season (Fig. 2SA1). There was no effect of milkweed species on the total mummies observed over the course of the season on a given plant in the uncaged or sham treatments (Wilcoxon Rank Sum test: W=627.5, P=0.9797). We collected 779 mummies between July 10 and Sept. 30 on sentinel plants. Of these, 201 were chewed, sunken, or unidentifiable, leaving 578 potentially viable mummies. Forty-nine of these emerged as primary parasitoids, all of the species *Lysiphlebus testaceipes*. Hyperparasitoids from various families emerged from another 218 mummies, leaving 311 whole mummies that we collected that never emerged.

Table 2SA1. Cumulative counts of predators.

	Predatory Larvae			Adult Ladybeetles				Other adult predators		
	Fly Larvae	Ladybeetle Larvae	Lacewing Larvae	*Harmonia axyridis*	*Coccinella septempuctata*	*Hippodamia convergens*	*Hippodamia variegata*	*Orius* sp.	Spiders	Lacewings
A. incarnata	1951	310	50	95	25	20	16	25	12	6
A. syriaca	44	60	13	14	4	1	3	13	7	2
Totals	1995	370	63	109	29	21	19	38	19	8

Note: We included counts from only the "Predators" (sham and uncaged) treatments over the 10 week experimental period, which summed to 358 observation periods on *A. incarnata* and 349 observation periods on *A. syriaca.*

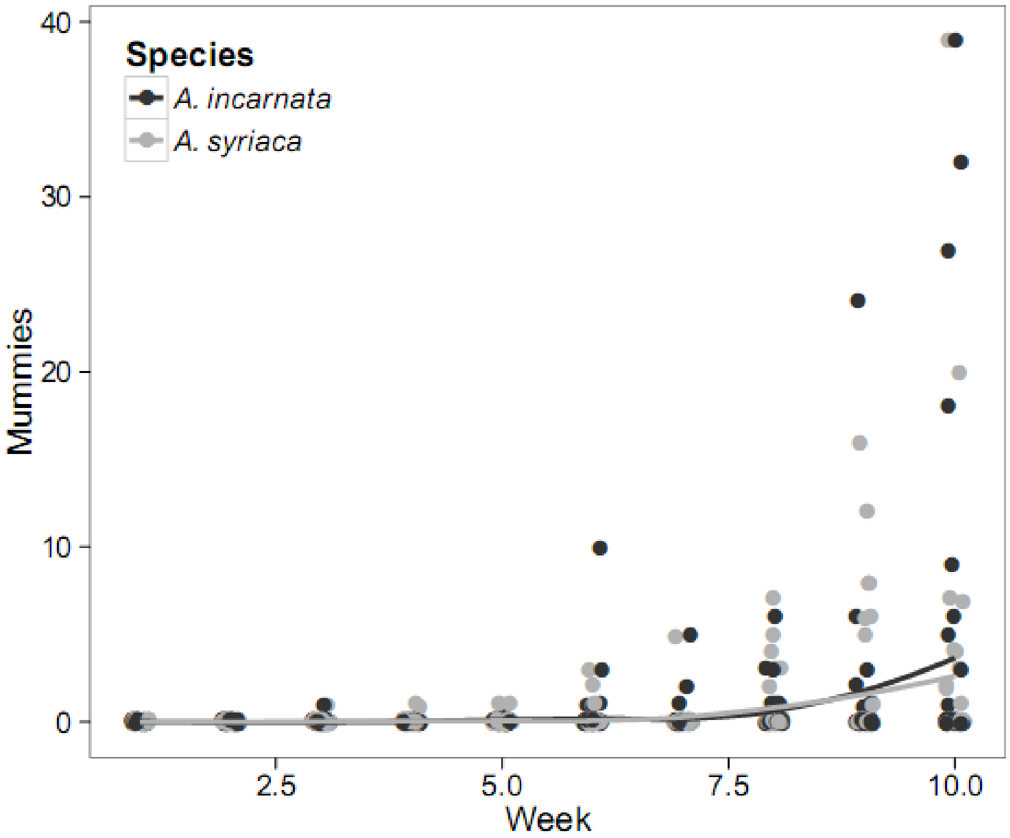

Figure 2SA1. Mummies on each milkweed species over time. Each point represents the number of mummies observed on a single plant on that date; a loess smooth is shown for each species.

Appendix B. Sham cage methods and temperature measurements

Predator exclusion cages have the potential to impact aphid populations and plant growth in a variety of ways separate from the effect of removing predators. Cages have the potential to alter the abiotic environment experienced by both aphids and plants, with typical effects related to shading (lower light and temperatures) and reduced airflow (Luck et al. 1999). We constructed sham cages that were identical to the predator exclusion cages except that we cut two horizontal slits (~23 x 5cm) on each of the four sides of the cage and an additional two slits along the mesh covering the top of the cage. The total area removed was approximately 5.6% of the total surface area of the cage. In our experiment, sham cages were included to control for these effects while allowing predator access to plants. To test for temperature differences between cage treatments, we used the Monarch Watch Temperature Monitoring Kit (www.MWLOG.org) to collect hourly temperature records on pairs of neighboring plants undergoing different cage treatments on 10 different dates during the summer. We calculated the average temperature over a 24 hour period and analyzed the data using a linear mixed effects model with cage treatment as a fixed effect and date of measurement as a random effect.

Temperatures ranged from 9-36°C during the observation period, with an average temperature of 22.1°C. Visual analysis of the data suggested that temperatures in sham and caged treatments were slightly higher than they were in uncaged treatments during the warmest parts of the day (Fig. 2SB1). There was a significant effect of cage treatment on average temperature ($\chi^2_{d.f.=2}$=93.141 , P< 2.2e-16), and combining sham and predator exclusion cages into one variable reduced the fit of the model (Likelihood Ratio=5.015679; P=0.0251), suggesting that sham cages affected temperature differently than predator exclusion treatments. Surprisingly, the model estimated sham cages to have the overall highest temperatures (22.54°C), followed by cage treatments (22.26°C), and then uncaged treatments (21.68°C). There was some measurement error as the instruments recorded temperature measurements under the same conditions within 0.3°C of each other.

We note that there was no difference between sham and uncaged predator access treatments with respect to plant growth, suggesting that any direct effect of caging on plant growth was minor compared to the effects of caging on aphid populations. However, our data suggest that sham cages may have affected aphid populations in two ways, either by increasing temperatures slightly or by altering predator foraging. Overall, more aphids accumulated in sham than uncaged "Predators" treatments on both milkweed species (*A. incarnata*: z=3.410, Padj=0.00936, *A. syriaca*: z=5.498, Padj< 0.001), but more predators accumulated in sham compared to uncaged treatments only on *A. syriaca* (*A. incarnata*: z=2.126, P=0.31803; *A. syriaca*: z=3.707, Padj=0.00300). Temperature is known to affect population growth rates of aphids; however, we do not have data about the effect of temperature on the demographic parameters for *Aphis nerii* specifically. Our data show that although sham cages increased the intercept of the relationship between predators and aphids, predator exclusion had greater effects on aphids in uncaged treatments (see Results sections). This suggests that either sham cages interfered with predator effects on aphids or that the temperature in cages increased aphid growth rates, inflating our measures of predator effects on aphids in the uncaged treatments. However, this effect did not disrupt trophic cascades. Overall, the data are consistent with a positive effect of sham cages on aphid abundance, but we cannot determine whether this is a result of increased temperature or reduced effects of predators, or a potential interaction between them.

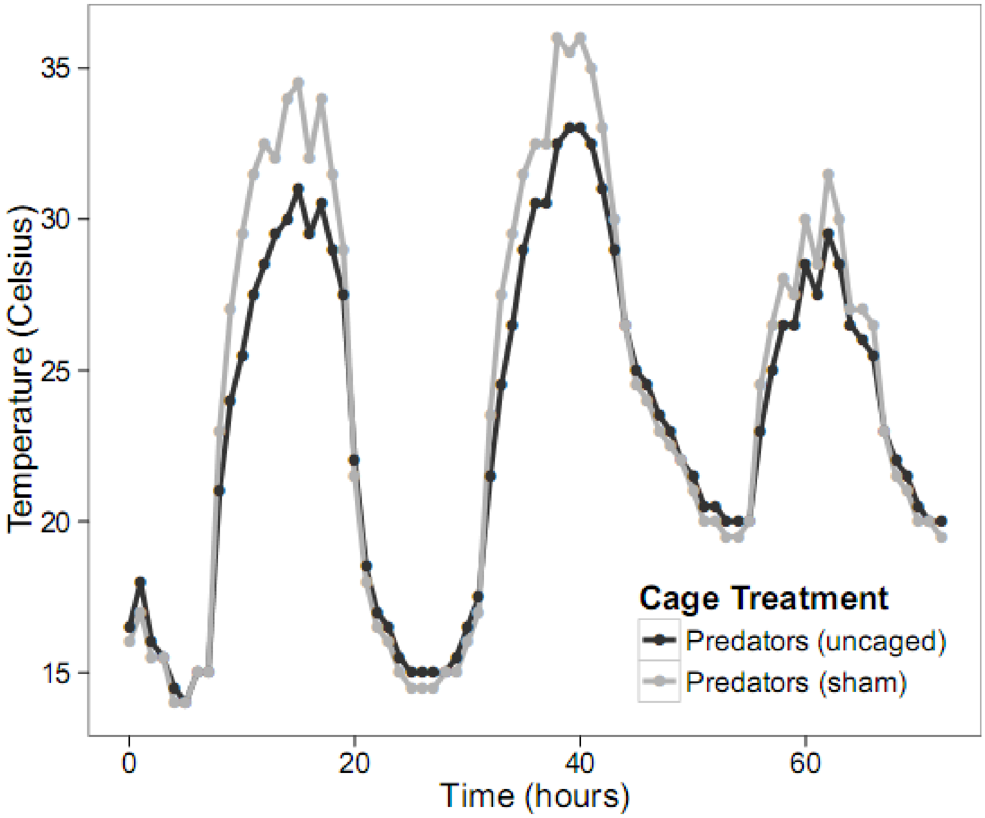

Figure 2SB1. The effect of cage treatment on temperature. This is a representative plot of temperatures in a sham and an uncaged treatment over three days. The x-axis shows hours past 12AM on August 11, 2009. Similar measurements were collected on 10 randomly chosen pairs of plants during the season; only the first 24 hours of data for each plant were used for the analyses.

Appendix C. Effects of predator exclusion cages on winged aphids

Predator exclusion cages also have the potential to prevent the emigration of winged aphids, another factor that could inflate the effect sizes of predators on both sham and uncaged treatments. Plants in the "No Predators" treatment might be expected to harbor many winged aphids, not only because they cannot emigrate, but also because high densities of *Aphis nerii*, particularly over multiple generations, are associated with higher rates of winged aphid development (Hall and Ehler 1980, Zehnder and Hunter 2007a). In addition to crowding, other factors such as plant quality and exposure to predators may affect the rate of winged aphid development (Muller et al. 2001). In order to test whether predator exclusion affected the proportion of winged aphids observed on either species, we calculated cumulative winged-aphid days (CWD) and fit a linear mixed effects model with cumulative aphid days (CAD), species, and cage treatment as completely crossed predictors. For simplicity, we excluded cage switching treatments from this analysis. Cage treatment and species interacted to affect the rate of winged aphid formation ($\chi^2_{d.f.=2}$=10.3554 , P=0.005641, Fig. 2SC1). We performed linear contrasts within each milkweed species to test whether there were differences between the "No Predators" and each of the "Predators" treatments with respect to the slope and intercept of the relationship between CWD and CAD. On *A. incarnata*, we found that predator exclusion cages increased the rate at which winged aphids accumulated compared to uncaged plants (z=3.197; Padj=0.00722), but not plants in sham cages (z=0.155; Padj=0.99998). No other contrasts were significant. This suggests that winged aphids either emigrated more quickly or developed at a lower rate on *A. incarnata* in the uncaged treatment.

Although enclosing winged aphids undoubtedly affected the aphid populations in predator exclusion cages, we believe the major effects of exclusion cages were a result of preventing predation for several reasons. First, there were less than 4% winged aphids on average on plants in predator exclusion cages, so it is unlikely that they drove the differences we observed. One possible explanation for these low abundances is that migrant aphids left the plant and congregated near the tops of the cages where they were released during weekly counts. Second, there was a dramatic reduction in aphid population size as a result of cage switching treatments which cannot be entirely explained by aphid emigration or aphid effects on plant quality. Third, predator exclusion affected aphid population sizes more rapidly than we expect winged aphids to develop. Effect sizes comparing total aphid numbers in caged and uncaged plants differed from zero within the first week, and effect sizes for both sham and uncaged treatments differed from zero by the second week of the season (Table 2SC1). Since *Aphis nerii* takes 5 days to reach maturity on the milkweed species used in this study (Zehnder and Hunter 2007a) and winged aphids take even longer (Groeters 1989), winged aphids would have had to develop almost immediately to explain such rapid effects of predator exclusion cages. During week 1, the median number of winged aphids in any treatment was less than 1; however, winged aphids were more abundant by week 2, when they may have had some impact on effect sizes. Finally, our data indicate that effects of predators on plants are not a linear function of their effects on aphids; predator effect sizes for plants in sham and uncaged treatments were very similar, even though the effect sizes for aphids were not (see Results section). Thus, even if aphid densities were inflated above natural levels in our experiment, we expect that the pattern of cascade strength we observed was a plant response to high aphid densities that would occur under natural aphid outbreak conditions as well.

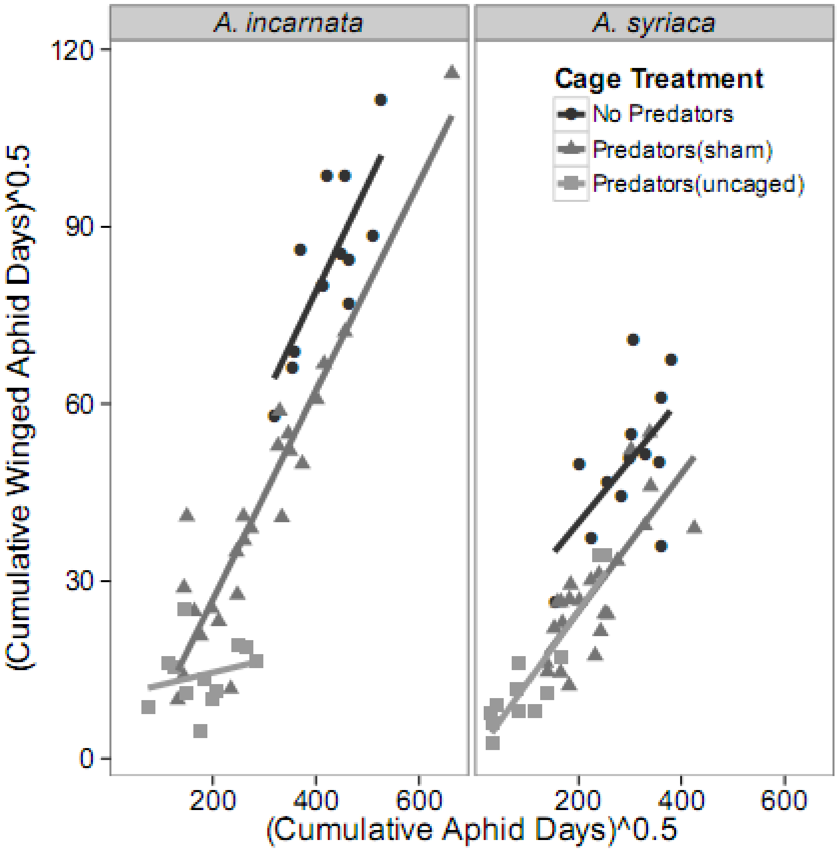

Figure 2SC1. Density of winged aphids. The relationship between cumulative winged aphid days and cumulative aphid days for *A. incarnata* and *A. syriaca* in each non-switching cage treatment.

Table 2SC1. Effect size of predator exclusion on total aphids.

Aphids+Predators Treatment	Time	*A. incarnata*	*A. syriaca*
Sham	**Week 1**	-0.1069 (0.1882)	0.0420 (0.1690)
Uncaged	**Week 1**	-1.3016 (0.3033)	-2.3138 (0.2556)
Sham	**Week 2**	-1.8508 (0.4303)	-0.54493 (0.2492)
Uncaged	**Week 2**	-3.6360 (0.4696)	-3.2722 (0.6005)

1. Effect sizes were calculated as the ln(value with predators/value in predator exclusion treatment) within each block for the total number of aphids at each time point. Effect sizes were calculated separately based on sham and uncaged predator access treatments. Shown are means (standard errors).

2. Effect sizes with confidence intervals that do not overlap zero are shown in grey, indicating a significant effect of predator exclusion.

3. Significant differences in effect size between species occur during week 1 based on uncaged values, when predator exclusion has a greater effect on *A. syriaca* (Welch two-sample t-test: T = 2.5522, df = 42.099, P = 0.01442) and during week 2 based on sham cages when predator exclusion has a greater effect on *A. incarnata* (Welch two-sample t-test: T=-2.6264, df = 33.961, P = 0.01285)

Appendix D. Cage switching effects on aphids

Cage switching treatments give additional insight into the processes and temporal dynamics affecting the outcome of trophic interactions. Because aphids are affected both by plant quality and predators, the effects of cage switching on aphid populations can highlight key processes driving the ultimate patterns we observe. Half of the "Predators (uncaged)" and "No Predators" treatments were switched at week 5: removing cages resulted in dramatic increases in predator abundance and decreases in aphid abundance, and adding cages effectively excluded predators, except for on some *A. incarnata* plants in the "Predators Early" treatment during the second half of the season (Fig. 2.2C). Apparently, predatory fly eggs were laid on some uncaged plants prior to cage switching, and large numbers of predatory fly larvae were subsequently found on some "Predators Early" plants during the second half of the season. Both cage switching and the time of predator exclusion affected the cumulative abundance of both aphids and predators (Table 2SD1).

Predator exclusion early in the season resulted in a decline of plant growth late in the season, particularly for *A. incarnata* (Fig. 2.2E). Aphid abundance was expected to respond to both plant quality and predation. To get at these effects, we calculated the "temporal" effect size as the ratio of cumulative aphid days during the first five weeks to cumulative aphid days during the last five weeks (Table 2SD2). The ln transformation of this ratio generates positive values if cumulative aphid days early in the season exceed cumulative aphid days late in the season, and negative values otherwise. By modeling the effects of plant species and cage treatment on the temporal effect size, we were able to determine whether temporal effects varied across species, across caging treatments, and as a result of interactions between these factors. We specifically used linear contrasts within and across species to determine whether aphid population growth was suppressed more in the "Predators Late" treatment than in the "No Predators" treatment, where plant quality alone affected populations.

Overall, temporal effect size was affected both by cage treatment ($\chi^2_{\text{d.f.}=4}$=278.08, P<<0.0001) and species ($\chi^2_{\text{d.f.}=1}$=9.73, P=0.0018), but not an interaction between them

($\chi^2_{d.f.=4}$=1.61, P=0.81). Temporal effect sizes in most predator exclusion treatments were negative, indicating more cumulative aphid days later in the season. Such a pattern would be consistent with either exponential aphid population growth or increased plant quality through time, or both. The obvious exception is the "Predators Late" treatments, where positive effect sizes indicate more aphids early in the season, consistent with predator suppression of aphids. A comparison of the magnitude of the temporal effect sizes for the "Predators Early" and "Predators Late" treatments within each species indicates that plant quality matters: when plants are exposed to predators early in the season, aphid population growth in the late season is much greater than it is for the same duration early in the season. In the "No Predators" treatments, the temporal effect size was positive for *A. incarnata* and negative, but small compared to other treatments, for *A. syriaca*. This further suggests that aphids cause a decline in plant quality over time that feeds back to reduce aphid population growth late in the season, particularly on *A. incarnata*. Overall, the temporal effect sizes for *A. incarnata* were more positive than for *A. syriaca*. This suggests that *A. incarnata* plants are higher quality earlier in the season while *A. syriaca* plants are higher quality later in the season. Our abundance data (See Results Section, Fig. 2.2) indicates that an alternative explanation, that aphids experience a higher exponential growth rate or carrying capacity on *A. syriaca*, is implausible.

To specifically test whether predators suppress aphids beyond the reduction in aphid population growth due to reduced plant quality, we conducted linear contrasts between the "No Predators" and the "Predators Late" treatments within each species. The temporal effect size was greater in the "Predators Late" treatment than in the "No Predators" treatment for both *A. incarnata* (z=5.90, P_{adj}<0.0001) and for *A. syriaca* (z=3.19, P_{adj}=0.0037). Although there was not an interaction between species and predator exclusion treatment for temporal effect size, we did find that cumulative aphid days during weeks 6-10 were affected by an interaction between species and cage treatment ($\chi^2_{d.f.=4}$=12.15, P=0.016; see Results Section). Aphid populations in the "Predators Late" treatment grew less than those in the "Predators (uncaged)" treatments on *A. incarnata* (Fig. 2SD1). This is likely a result of predator aggregation to plants with high aphid densities; however, seasonal differences in plant quality may also contribute to this result.

Table 2SD1. The significance of switching.

Response	Factor Levels Tested	Likelihood Ratio	df	P-value
CAD Week 10	Predators Early vs. All	60.75	8	<0.0001
	Predators Late vs. All	54.19	8	<0.0001
	Switch vs. All	18.39	4	0.001
CPD Week 10	Predators Early vs. All	61.06	8	<0.0001
	Predators Late vs. All	43.25	8	<0.0001
	Switch vs. All	15.46	4	0.0038

We tested whether switching significantly affected Cumulative Aphid Days (CAD) and Cumulative Predator Days (CPD) by comparing models including all 5 levels of predator exclusion treatments (All) against models that included only predator exclusion treatments applied during the first five weeks (Predators Early) or the last five weeks (Predators Late). Given that switching mattered, we tested whether the timing of predator exclusion significantly affected the response variables by comparing models that combined Predators Early and Predators Late into a single “Switch” variable to models with all five levels of predator exclusion. All p-values are significant, indicating both the significance of cage switching and the timing of predator exclusion for CAD and CPD at Week 10.

Table 2SD2. Temporal effect sizes by species and cage treatment.

Cage	Species	N	Temporal Effect Size: ln(EarlyCAD/LateCAD)	Standard Error	Confidence Interval
No Predators	*A. incarnata*	12	0.5656	0.1483	0.3263
	A. syriaca	12	-0.0707	0.2168	0.4772
Predators (sham)	*A. incarnata*	24	-1.1172	0.5568	1.1519
	A. syriaca	23	-1.1665	0.27	0.5599
Predators (uncaged)	*A. incarnata*	12	-1.6883	0.6301	1.3870
	A. syriaca	12	-2.3744	0.6258	1.3773
Predators Early	*A. incarnata*	12	-4.5982	0.6223	1.3697
	A. syriaca	11	-4.4660	0.5284	1.1774
Predators Late	*A. incarnata*	12	1.6099	0.1105	0.2433
	A. syriaca	12	1.0127	0.2813	0.6192

A positive Temporal Effect Size indicates aphids accumulated more during the first than the second half of the growing season; a negative value indicates more aphid population growth during the late part of the growing season. Note that two plants were excluded from this analysis because the temporal effect size could not be calculated due to zero aphid population growth during the second half of the growing season.

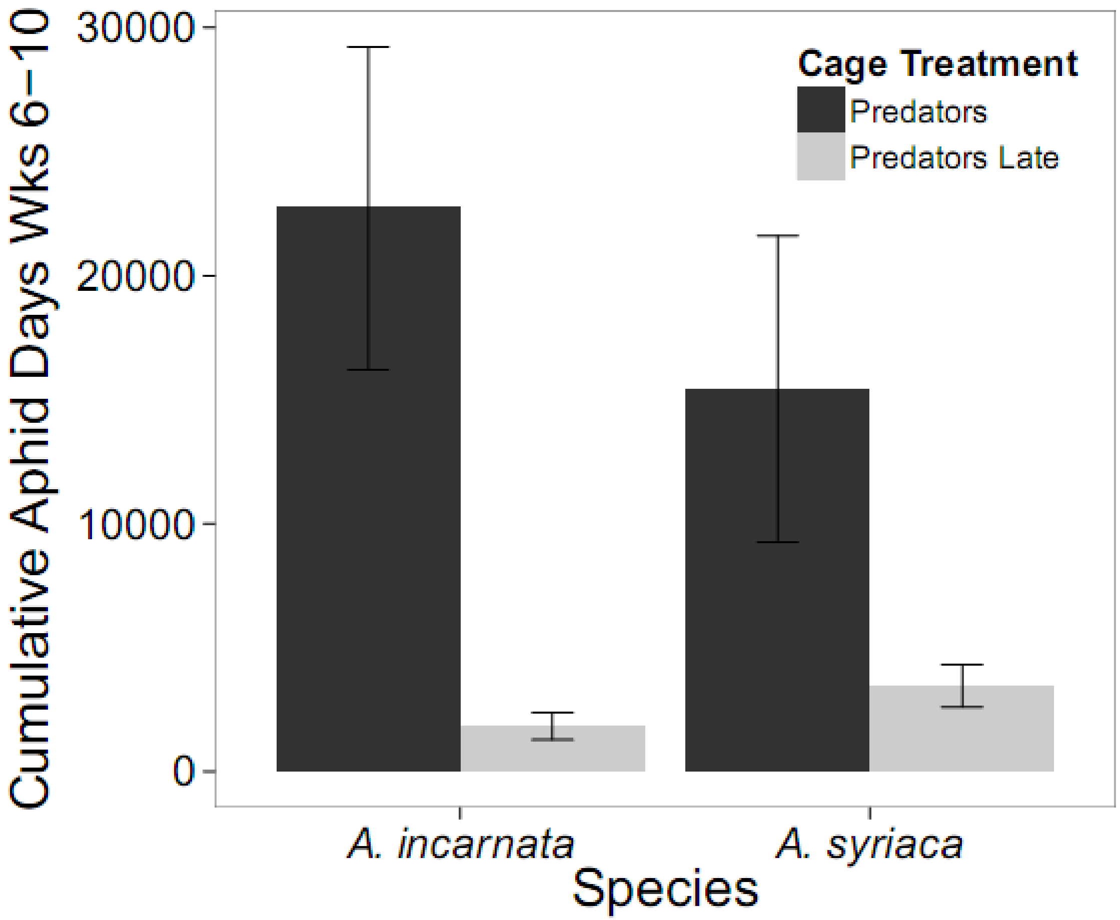

Figure 2SD1. The effect of early season predator exclusion on late season aphid population growth. Immediately after cages were removed from the "Predators Late" treatment, aphids were more abundant on plants than they were on the "Predators" treatment, where predators had suppressed aphids during the first 5 weeks. The difference in cumulative aphid days during weeks 6-10 between treatments is significant for *A. incarnata*, but not for *A. syriaca*, and could be a result of predator aggregation or plant quality effects, or both.

Appendix E. Biomass estimates as a measure of plant growth

To evaluate whether using biomass rather than leaf number as a measure of plant growth would alter our results, we grew fifteen plants of each species in a nearby agricultural field during the summer of 2010. We harvested a subset of these plants in July, August, and September, and then dried and massed the plants. In September, we also harvested a eight *A. syriaca* plants that were contained in predator exclusion cages during the growing season. We fit a linear model using biomass as a response and leaf number at time of harvest as a predictor, and the resulting equations are:

Biomass *A. syriaca*=20.57+1.4(Leaf number) (R^2=0.3091)

Biomass *A. incarnata*=64.6+.66(Leaf number) (R^2=0.8395)

We used these equations to estimate the biomass of the 2009 experimental plants at week 10, based on leaf number at week 10. Leaf counts were missing for two *A. incarnata* plants, preventing an estimate of biomass, so these plants were excluded from the analysis. We then fit a linear mixed-effects model of cumulative aphid days as a function of estimated biomass crossed with cage and species. Both the response and predictor were square-root transformed and we allowed variance to vary with the interaction between species and cage. The results are qualitatively similar to those produced by the analysis of cumulative aphid days as a function of leaf number (Fig. 2SE1, Fig. 2.3B). The slope of the relationship between cumulative aphid days and biomass is steeper for *A. syriaca* (Biomass x Species: $\chi^2_{d.f.=1}$=5.07, P=0.02434) and for plants in cages (Biomass x Cage: $\chi^2_{d.f.=2}$=6.4942, P=0.03892), but is not affected by an interaction between them (Biomass x Species x Cage: $\chi^2_{d.f.=2}$=0.3383, P=0.84439).

Because we were not able to find a combination of measured variables that explained a large fraction of the variation in biomass for *A. syriaca*, and because we anticipate that the relationship between measured variables and biomass changes over the course of the season and in response to different levels of herbivory, we retain leaf number as our measure of plant growth for our analyses.

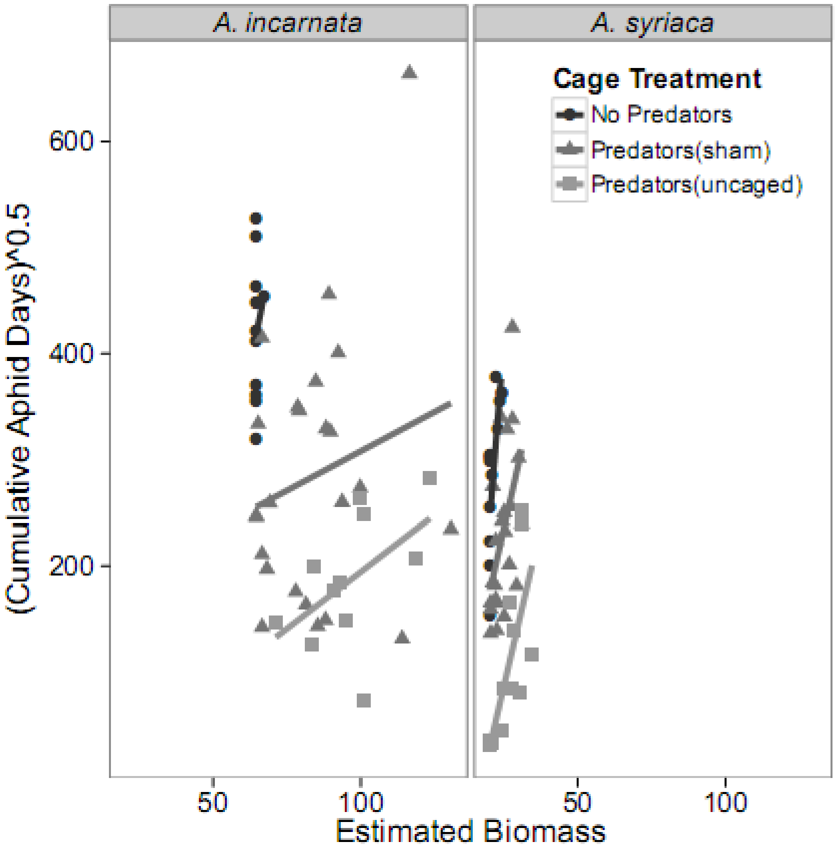

Figure 2SE1. The relationship between cumulative aphid days and estimated plant biomass. The slope of the relationship depends on both plant species and cage treatment. Only data from the three non-switching cage treatments is used.

Appendix F. Mid-season analysis of aphid density

Because aphid population growth depends both on plant quality and predator consumption, we suspected that our analysis of cumulative aphid density at week 10 might mask important temporal dynamics. In particular, visual inspection of plant growth curves (Fig. 2.1 E,F) shows that large differences in plant growth between treatments occurred primarily in the last half of the season, and these likely impacted aphid populations. We thus repeated our analysis of aphid population growth standardized by plant growth on cumulative data from just the first 5 weeks. At week five, none of the cage treatments had been switched; consequently, all plants are included in this analysis under the cage treatment applied at the start of the season. The sham and uncaged "Predators" treatments were significantly different (Likelihood $\text{Ratio}_{df=4}$ =66.19, P<0.0001), so they were not combined.

Predator exclusion increased the slope of the relationship between cumulative aphid days and cumulative leaf days at week 5 (CagexCLD $\chi^2_{d.f.=2}$=10.06, P=0.0065; Fig. 2SF1). In contrast to the pattern observed at the end of the season, at week 5, aphids accumulated at a faster per-leaf rate on *A. incarnata* than they did on *A. syriaca* (SpeciesxCLD $\chi^2_{d.f.=1}$=4.84, P=0.028; Fig. 2SF1). However, the intercept was higher for *A. syriaca* than for *A. incarnata* (Species $\chi^2_{d.f.=1}$=10.74, P=0.001). Between-species contrasts of the slope and intercept of the relationship in the "No Predators" treatments reveal no significant difference between species (intercept z=-0.57, P=0.63; slope z=0.98, P=0.37); consequently, there is no clear evidence for between-species differences in resistance. At the same time, leaf number appears to only affect aphid abundance early in the season on *A. incarnata* in cages, as this was the only slope to deviate significantly from zero (z=4.97, Padj<0.0001). Nevertheless, there is no significant evidence for a three way interaction between species, cage, and slope at week 5 ($\chi^2_{d.f.=2}$=3.32, P=0.19).

Together, these results suggest that early in the season, aphid populations may not be limited by plant size, as measured by cumulative leaf days, except for populations on *A. incarnata* in the absence of predators. At low leaf numbers, our data suggest that there were more aphids per leaf on *A. syriaca* than on *A. incarnata*, which may result from our method of initially seeding all plants with an equal number of aphids, regardless of leaf number. The fact that the slope of the relationship between cumulative aphid days and cumulative leaf days was steeper for *A. incarnata* than for *A. syriaca* during the first five weeks is consistent with our other observations that aphid population growth tended to be higher on *A. incarnata* early in the season and on *A. syriaca* later in the season (Appendix D).

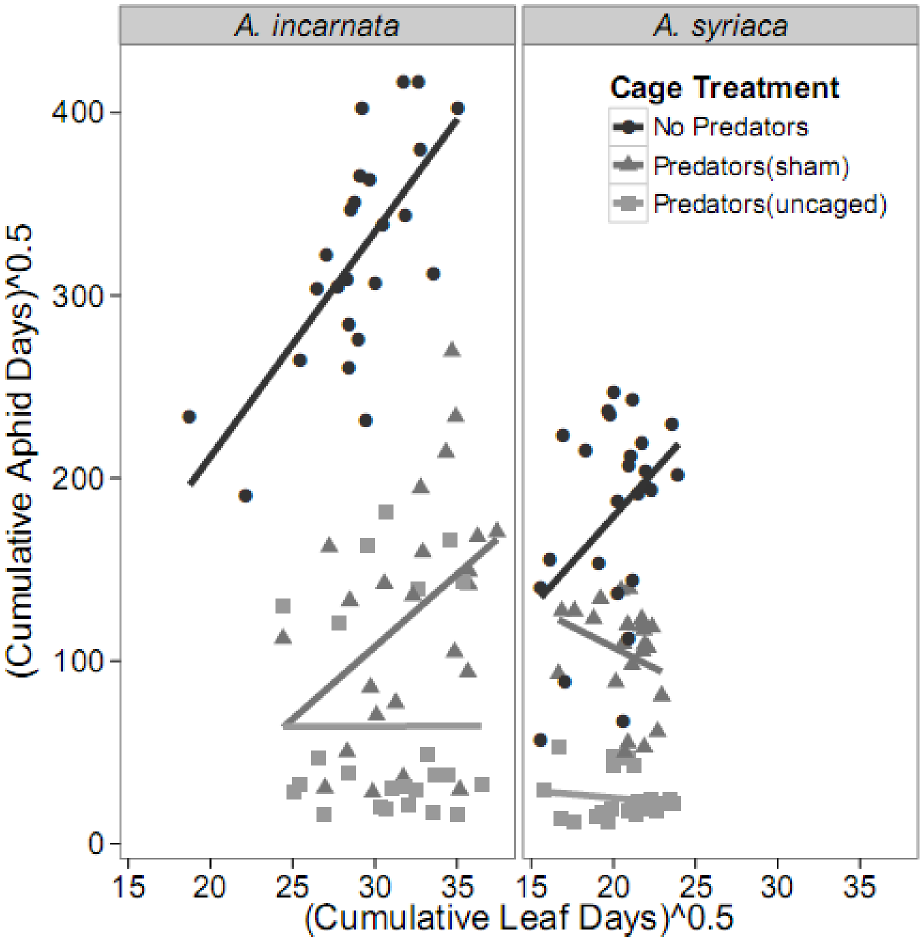

Figure 2SF1. The relationship between cumulative aphid days and cumulative leaf days at week 5. Each point represents the cumulative abundance over five weeks of aphids and leaves on a single plant. The slope of the relationship standardizes the abundance of aphids by plant size.

Appendix G. A table of predator effect sizes

Table 2SG1. Effect sizes of predators on aphids and plant growth across species.

"Predators" Treatment	Response	*A. incarnata*	*A. syriaca*
Sham	Cumulative Aphid Days	-0.68 (0.31)	-0.51 (0.17)
Sham	Cumulative Leaf Days	1.17 (0.18)	0.25 (0.14)
Uncaged	Cumulative Aphid Days	-1.82 (0.22)	-2.40 (0.39)
Uncaged	Cumulative Leaf Days	1.19 (0.13)	0.23 (0.15)

Predator effect sizes were calculated as the ln(value with predators/value in predator exclusion treatment) within each block for both CAD and CLD at week 10. Effect sizes were calculated separately based on sham and uncaged predator access treatments. Shown are means (standard errors).

Chapter 3

Host plant species more strongly affects larval performance than adult oviposition in the aphid predator *Aphidoletes aphidimyza*

Summary

Although preference-performance relationships in insects are typically studied in a bi-trophic context, it is well-known that host plants can affect both the preference and performance of predatory insects. Here, we present evidence from field and laboratory studies that two species of milkweeds, the putatively less-defended *Asclepias incarnata* and the putatively more-defended *A. syriaca*, differentially affect adult oviposition and larval performance in *Aphidoletes aphidimyza*, a predatory midge, independent of aphid species and density. In the laboratory, we observed weak evidence for increased oviposition on the putatively less-defended *A. incarnata*, and a potentially suppressive effect of *A. syriaca*, the putatively more-defended milkweed species, on oviposition. Larval and adult emergence rates provided strong evidence for reduced performance on *A. syriaca*. Comparison of data from the laboratory and the field suggests that the effect of plant species on the predatory fly larvae performance is even greater in the field; nonetheless, predatory flies do not reject *A. syriaca* entirely. We discuss potential explanations for, and implications of, these patterns.

Introduction

Because insect larvae are frequently immobile, adult oviposition behavior can determine the food sources available to their larvae, with strong implications for fitness. The preference-performance hypothesis predicts that adult oviposition preference for, and larval performance on, various food sources will be positively correlated as a result of natural selection (Jaenike 1978, Thompson 1988). If preference and performance are influenced by separate loci, then performance may evolve to match preference, resulting in a wider diet breadth, or preference may evolve to match performance, narrowing diet breadth (Futuyma and Moreno 1988, Rausher 1993). Furthermore, a positive genetic association between preference and performance may facilitate speciation and specialization, particularly when mating occurs in the preferred habitat (Diehl and Bush

1989). The effects of plants on the preference and performance of herbivorous insects have been extensively studied (Gripenberg et al. 2010); however, plants also significantly influence insect predator foraging and fitness (Price et al. 1980) which can impact the expression and evolution of diet breadth. This is important because the diet breadth and foraging behavior of a predator often mediate its effectiveness as a biological control agent in patchy environments (Holt and Kotler 1987; Harmon and Andow 2004, A. Chailleux et al, submitted).

Experimental results frequently support the preference-performance hypothesis, but there are exceptions (Thompson 1988, Gripenberg et al 2010). Mismatches in preference and performance are typically attributed to variables not measured in laboratory settings. For example, preference measures in the laboratory setting generally do not account for the frequency, constancy, or availability of a food source in the field or the evolutionary history of the interaction between two species (Jaenike 1978, Thompson 1988). Additionally, performance measures in the laboratory typically do not account for variation in predation risk among hosts, but predation has been strongly implicated in oviposition or diet preference for some species (e.g., Staley and Yeargan 2005, Singer 2008). The enemy free space hypothesis posits that preference, and ultimately specialization, can serve as a predator-avoidance strategy (Price et al 1980, Bernays and Graham 1988). Furthermore, adult insects in many cases must optimize foraging both for resources that promote their own fitness as well as oviposition sites that increase offspring fitness, and a mismatch between these needs may disrupt a correlation between adult oviposition preference and offspring performance (Scheirs and De Bruyn 2002).

Preference and performance relationships are often studied in a bi-trophic context, particularly regarding the interactions between herbivores and plants (Gripenberg et al. 2010, Thompson 1988), but also interactions between parasitoids and their hosts (Chau and Mackauer 2001, Desneux et al. 2009). However, plants are known to affect predation. For many predatory insects, foraging may involve a hierarchical process of identifying suitable habitats and plants containing prey followed by discrimination among prey. Preferences higher in this hierarchy may exclude species at other levels (Vinson 1976). While there is clear evidence that plant defenses can impair predator and parasitoid performance (Campbell and Duffey 1979, Barbosa et al. 1991, Malcolm 1992, Ode 2006), plant defense traits may also affect predator preferences (Obrycki and Tauber 1984, Staley and Yeargan 2005, Gols et al. 2009). In particular, herbivore-induced plant volatiles (HIPVs) have been documented to affect predator foraging behavior (Dicke and van Loon 2000, de Vos and Jander 2010) and may provide cues that allow predators to evaluate plant quality (Gols et al. 2009, Wei et al. 2011, Kos et al. 2012). In a tri-trophic context, predator preference and performance may be affected directly by plant traits, or indirectly by the effects of plant traits on prey quality, density, growth, or behavior. Despite the documented impacts of plant traits on predators and the practical and ecological significance of a predator's diet breadth, the vast majority of tests of the preference-performance hypothesis have been focused on herbivores.

In this paper, we present evidence from *Aphidoletes aphidimyza*, a generalist aphidophagous dipteran predator, that adult oviposition and larval performance are affected by plants, independent of prey species effects. We previously observed a 45-fold difference in the abundance of predatory fly larvae on two different species of milkweed plants in the field (Chapter 2). We hypothesize that variability in defense-related traits across the two milkweed species contributes to the observed pattern. Here, we reanalyze these data to account for variation in plant growth and prey abundance. Then we investigate the effects of these two milkweed species, using a single species of aphid prey, on adult oviposition and larval performance of *A. aphidimyza* using laboratory choice and no-choice assays. We compare the data from the lab and field studies to infer which processes may have generated the patterns observed in the field, and we interpret the results in light of the evolution of diet breadth.

Methods

Study system

The larvae of *Aphidoletes aphidimyza* are exclusively aphidophagous with a diet breadth that includes at least 80 aphid species (Harris 1973; Yukawa 1998). The species is widely distributed in the northern hemisphere and is used as a biological control agent, primarily in greenhouses (Markkula et al. 1979), but also in the field (Meadow et al. 1985). *A. aphidimyza* are known to forage effectively for aphids, being able to locate a single aphid-infested plant among 75 aphid-free plants (El Titi 1974), with aphid honeydew as the primary cue (Choi et al. 2004). As larval predators, *A. aphidimyza* are themselves subject to intraguild predation (Lucas and Brodeur 1999, 2001, Gardiner and Landis 2007). However, several studies have found that *A. aphidimyza* oviposition behavior is primarily influenced by aphid density, which may serve not only as a food source but also as a means of defense against intraguild predation via a dilution effect (Lucas and Brodeur 1999, 2001, Sentis 2012). Some evidence documents an influence of plant variety on oviposition behavior (Mansour 1975), and the influence of different aphid species on *A. aphidimyza* performance and oviposition has been investigated (Kuo-Sell 1989, Havelka and Ruzicka 1984). However, the joint effects of plants on both preference and performance, especially in the context of plant defenses, are unknown.

Milkweed plants are known for their production of toxic cardiac glycosides. Although tens of herbivore species, including the aphid *Aphis nerii*, have evolved the ability to tolerate and even sequester milkweed toxins, these same toxins are thought to harm a variety of predators, including spiders, lady beetles, hover flies, lacewings, and parasitoids (Pasteels 1978, Malcolm 1986, Malcolm 1989, Malcolm 1992, Helms et al. 2004, Desneux et al. 2009). For all experiments, we used the same herbivore species, *Aphis nerii,* which is a bright yellow aphid that specializes on plants in the milkweed family from which it sequesters toxins.

We previously reported that milkweed species affects abundance and per-aphid density of a community of about 10 generalist predators of *A. nerii* in the field (E. Mohl Chapter 2). Here, we analyze the effects of milkweed size, aphid abundance, and milkweed species on the distribution and abundance of predatory fly larvae specifically. Because the distribution of fly larvae in the field can result from multiple processes, including differential adult oviposition preference, larval survival, or predation, we conducted laboratory studies to test for variable preference and performance under controlled conditions.

Of the two milkweed species we studied, *Asclepias incarnata* is faster growing and putatively less-defended, having fewer trichomes and lower concentrations of cardenolides than *A. syriaca* (Agrawal and Fishbein 2006, Zehnder and Hunter 2007b). Additionally, milkweed species show variation in volatile production, and while there are no records for *A. syriaca, A. incarnata* does produce volatiles when attacked by *A. nerii* (Mooney et al. 2010). Together, the defensive profiles of the two species and the potential for aphid-induced volatiles to affect predator foraging suggest that *A. incarnata* may benefit more from indirect defenses mediated by generalist predators while *A. syriaca* may benefit more from direct defense. Specifically, aphids on *A. syriaca* may be of lower quality, perhaps because they sequester more toxic cardenolides, reducing the performance of predatory fly larvae on this species. Furthermore, cardenolides become concentrated in aphid honeydew (Malcolm 1990). Because adult *A. aphidimyza* are known to forage in response to honeydew (Choi et al. 2004), cardenolides may reduce adult preference for, and both adult and larval performance on, *A. syriaca*. Additionally, *A. syriaca* has more trichomes, which could interfere with oviposition or larval foraging, or protect larvae from larger intraguild predators (Lucas and Brodeur 1998). We predict that these milkweed species will similarly affect adult oviposition and larval performances, with *A. incarnata* favoring both. However, the absence of such a relationship is not uncommon (Thompson 1988).

Field Experiment: Fly larvae abundance

We conducted a field experiment with two milkweed species, *A. incarnata* and *A. syriaca*, exposed to various predator exclusion treatments in the summer of 2009 at the University of Minnesota research plots in St. Paul, MN. Details of the experiment are described elsewhere (Chapter 2), so we summarize the relevant points here briefly. *A. nerii* populations were established on 144 plants of each species, planted into a field setting, and either enclosed in mesh cages to exclude predators, left uncaged to allow predator access, or enclosed in sham cages. Sham cages were identical to predator exclusion cages, except they had slits cut in the sides and top to allow predator access. Every week for 10 weeks of the summer growing season, we recorded plant height, leaf number, and aphid number. We recorded the abundance of aphid predators observed on each of the plants, and we removed any immigrant herbivores. We attempted to minimize disruption of insects during our counts; consequently, many were not identified to species. Nevertheless, we believe that at least two-thirds of the nearly 2000 predatory fly larvae we observed were *Aphidoletes aphidimyza*. A small fraction of the fly larvae were clearly from the family Syrphidae. Half of the caged and uncaged treatments were switched at the midpoint of the season; for this paper, we restrict our analyses to 71 plants which were exposed to predators (either uncaged or in sham cages) during the entire growing season.

Lab Experiments

Laboratory studies were conducted in the fall of 2010 to test for the effect of milkweed species on adult oviposition and larval performance of *A. aphidimyza* when the only prey species available was *Aphis nerii*. The milkweed plants, *A. incarnata* and *A. syriaca*, were grown in the greenhouse and were used in the experiments when they had between 3 and 5 pairs of leaves. Beginning in the spring of 2009 aphid colonies were established and maintained either on *A. incarnata* or *A. syriaca*, and aphids in the experiment were tested on the same host plant on which they were reared. The experiment was conducted in a walk-in growth chamber on a 16L:8D cycle maintained at 24°C, and RH fluctuated between 25-35%. *A. aphidimyza* pupae were ordered from a commercial insectary (IPM Laboratories, Locke, New York, USA), and kept in a rectangular Plexiglas cage (~30x35x40cm) in the growth chamber until adults emerged, usually within 24 hours. Kimwipes were used to wick an approximately 5% honey solution from 1-ounce clear plastic cups (Jet Plastica Industries, Hatfield, Pennsylvania, USA) with lids (Solo Cup Company, Urbana, Illinois, USA) to provide a food source for the emerged adults. Mesh fabric with ~3 mm hole diameter was stretched across the cage to simulate spider webs, where *A. aphidimyza* are known to mate (van Lenteren and Schettino 2003, D. Elliot, personal communication).

To test for the effect of milkweed species on adult *A. aphidimyza* oviposition, we conducted both choice and no choice tests. In all trials, groups of adults were exposed to pairs of plants with aphids. Seedlings were transplanted into 14 cm pots and the soil was covered with a thin layer of sand. We situated plants at the far ends of each cage. Replicates were divided between three treatments: two *A. incarnata* plants ("*A. incarnata* "), two *A. syriaca* plants ("*A. syriaca*"), and one *A. incarnata* and one *A. syriaca* plant together in a cage ("Choice"). Cages with both plant species functioned as a choice treatment. We randomly assigned plants to cages and to location within each cage. The growth chamber held four completely randomized blocks of 3 cages, each on a different shelf, at a time. The experiment was repeated in 4 cohorts, for a total of 16 replicated blocks.

In each cohort, 15 adult aphids were transferred to each plant from the colony reared on the same species of plants. Aphids were allowed to reproduce on the plant, typically for about 2 days, until adult *A. aphidimyza* emerged. Aphids on each plant were counted, and aphids of mixed sizes were added to all of the plants to bring the counts to equal numbers within each block before releasing *A. aphidimyza* into the cages. Aphid counts were always equal within a block, but varied across blocks with a range of 60-160 per plant. Aphids were allowed to settle for at least 1 hour while adult *A. aphidimyza* were collected into vials for release into cages. The number of adults released into cages varied somewhat between blocks based on the numbers of available adults, but was always the same within blocks, typically consisting of a group of 4 females and 1 male. Adults were released from vials placed next to a source of 5% honey solution and directly between the two plants in each cage. Because adults are most active during periods of low light (Markkula et al. 1979), we covered all three cages within each block with a white sheet to diffuse light during the time that adults were present in the experimental cages. After 48 hours, we removed as many adult *A. aphidimyza* from each cage as we could find and counted the number of eggs on each plant.

To measure the performance of *A. aphidimyza* larvae, those plants with eggs were retained in separate microcosms to observe larvae and adult emergence. Development time is temperature-dependent in *A. aphidimyza*, and at 25°C the egg stage lasts for approximately 2 days, the larval stage for approximately 5 days, and the pupal stage for approximately 8 days (Havelka and Zemek 1988). Five days after releasing adults into cages, we counted the number of larvae on each plant. Beginning on the 12th day after the initial release of adults, we checked daily for emerging adults for the first week and then every two or three days during the second and third weeks.

Statistical Analysis

Field experiment: We have count data for predatory fly larvae on 71 plants over 10 weeks; however, many of these counts were zeroes. We calculated the cumulative abundance of fly larvae over time using the formula:

$$\sum_{i=1}^{n} \frac{(x_i + x_{i-1})}{2} \times t_i,$$

where n=number of sampled dates, x_i=count on date *i*, and t_i=the number of days since the previous count (Hanafi et al. 1989). This calculation integrates the abundance over time into a single value representative of the area under the curve (Ruppel 1983, Chacón et al. 2012), which serves to eliminate pseudoreplication and reduce the prevalence of zeroes in the dataset. We used a Wilcoxon test to test for a difference in cumulative fly larva abundance across milkweed species, considering all plants and only those plants on which larvae were observed in two separate tests. We also used a chi-squared test to test whether the presence of fly larvae was independent of plant species. Previous studies have demonstrated that *A. aphidimyza* respond strongly to aphid density (Lucas and Brodeur 1999, Sentis et al. 2012), and also to plant meristems (Jandricic et al. 2013). Because *A. incarnata* and *A. syriaca* have different growth forms and support different numbers of aphids, we wanted to test whether these differences were sufficient to explain the differences in fly larva abundance between species. We used the same formula to calculate cumulative aphid day and cumulative leaf day counts. We used a generalized linear model (glm) in the binomial family to test for effects of cumulative aphid days, cumulative leaf days, milkweed species, and cage treatment on presence or absence of fly larvae on a plant during the course of the season. We chose not to analyze cumulative fly larva abundance with a Poisson glm because the data were highly overdispersed and there was non-constant variance across species. Instead, we analyzed the effects of the same predictors on cumulative fly larva days for only those plants with fly larvae using a linear mixed effects model with a square-root transform of both the response and the continuous predictors and plot number as a random effect. We chose this method because it is possible to account for non-constant variance in the model (Pinheiro et al. 2014), as we did for our previous analyses of similar field data (Chapter 2). A single plant with fast growth and a high value for cumulative aphid days was a highly influential data point in this model, and the implications of excluding this point are described in Appendix A. For both the binomial glm and the mixed effects model of abundance, we began with a model including all 2-way interactions and dropped non-significant terms sequentially to determine the minimal adequate model (Crawley 2007).

Adult oviposition. We first used a chi-squared test to evaluate whether the number of *A. aphidimyza* eggs observed overall on each plant species, and in each experimental treatment, deviated from the proportion of each plant species, or each treatment type, available during the whole experiment. Then, we analyzed the effect of treatment on the number of eggs found per cage in each block. Because many of the experimental cages contained no eggs, we used a variety of nonparametric tests and generalized linear models (glms) to assess and test the effect of treatment on egg count. We report here the results of the nonparametric Friedman test, which accounts for the experimental blocking structure. In Appendix B, we present the analysis using a zero-inflated regression model, which corresponds qualitatively with the results from the Friedman test. We also present a separate analyses of the effect of treatment on the probability of eggs being observed in a cage, and on the number of eggs observed in a cage using only the subset of cages in which at least one egg was found.

Overall, about 25% of the adults in the experiment were found dead at the end of the two-day oviposition period; we used a Friedman test to determine whether treatment affected adult mortality. In two cages from a single block of the experiment, all adult *A. aphidimyza* died during the oviposition period, so we excluded these cages from all analyses and the entire block from the Friedman tests on egg counts. We present results from the analyses including all three treatment levels, *A. incarnata*, *A. syriaca*, and choice, to show how the effects of combining plant species in a small environment affects *A. aphidimyza* oviposition behavior. We also present the analysis of the two no-choice treatments, as this comparison most simply tests the effect of species on oviposition in a no-choice setting.

To test for adult oviposition within the "Choice" treatment, we used a paired Wilcoxon Signed Rank Test to compare the number of eggs per plant on the two different species of milkweeds in the choice cages. We also report results from a quasi-Poisson generalized linear model of the effects of species on the number of eggs laid per plant in the choice cages. The quasi-Poisson method estimates an additional parameter to account for overdispersion.

Larval performance. We consider individual plants as the experimental unit of our performance studies, and we test for an effect of species on the proportion of eggs reaching the larval and adult stages. We analyze these data using both the non-parametric Wilcoxon test and a binomial glm, which allowed us to include the blocking structure in the analysis. For the proportion of eggs reaching the adult stage, we used a quasi-binomial model to account for overdispersion. Although we never observed more emerging adults than eggs on a plant, there were some clear cases of observational error. In one sample, we counted more larvae (1) than eggs (0) on an *A. syriaca* plant, but the larva did not go on to emerge as an adult. In four samples on *A. incarnata* and one sample on *A. syriaca*, we counted more emerged adults than larvae. In each of these cases of experimental error, we back-corrected the counts so that no ratio was greater than one for the analyses.

Distribution of Larvae. Because we have data on the presence and abundance of larvae from both the field and the laboratory, we performed similar analyses to facilitate comparisons between them. We used a chi-squared test with Yates' continuity correction to test whether the presence of *A. aphidimyza* larvae was affected by plant species in the laboratory. For the subset of plants with larvae, we used a Wilcoxon test to determine whether the abundance of larvae differed between plant species.

All analyses were conducted in R version 3.0.1 (R Core Team 2013). For parametric models, we report Likelihood Ratio or Wald Chi Square test statistics based on type II tests calculated using the car package in R (Fox and Weisberg 2011).

Results

Field study

Over the course of the season, fly larvae were observed on 32 out of 36 *A. incarnata* plants, but only 9 of 35 *A. syriaca* plants, thus they were not distributed independently of species ($\chi^2_{d.f.=1}$=26.5, P<0.0001). Fly larval abundance was significantly higher on *A. incarnata*, with a median value of 290 cumulative fly larva days, compared to a median of 0 on *A. syriaca* (W = 1148.5, P<0.0001; Fig. 3.1A). Even when plants with no fly larvae were excluded, we still found significantly greater cumulative fly larva days on *A. incarnata* than on *A. syriaca* (medians=377 and 7.5 respectively; W = 264.5, P= 0.00016). Cumulative aphid days, cumulative leaf days, and species were retained in the binomial model of fly larva presence-absence on a plant during the 10- week growing season. Fly larvae were significantly more likely to be present on *A. incarnata* than *A. syriaca* (binomial model $\chi^2_{d.f.=1}$=22, P<0.0001), even when the positive effect of cumulative aphid days ($\chi^2_{d.f.=1}$=5.25, P=0.022) and the negative effect of cumulative leaf days ($\chi^2_{d.f.=1}$=7.63, P=0.0057) are accounted for in the model. Additionally, we find that the effect of cumulative aphid days on the presence of fly larvae is more positive for *A. incarnata* than *A. syriaca* (CADxSpecies interaction: $\chi^2_{d.f.=1}$=8.086, P=0.0045). Given the presence of fly larvae on a plant during the season, fly larva abundance, as measured by cumulative fly larva days, appears to be significantly lower on *A. syriaca* than *A. incarnata* ($\chi^2_{d.f.=1}$=5.7448, P=0.0165), even when accounting for aphids and plant growth in the full model (Fig. 3.1B,C). However, excluding the outlying point results in a more complex interpretation (see Appendix A). Sham and uncaged treatments had different effects on both predators and aphids (Chapter 2), and cumulative fly larva abundance also responded to significant interactions between cumulative aphid days and cage treatment ($\chi^2_{d.f.=1}$=7.82, P= 0.0052) and cumulative leaf days and cage treatment ($\chi^2_{d.f.=1}$=10.4, P= 0.0013). Specifically, the relationship between aphids and fly larvae days appears to be positive in uncaged replicates and negative in sham-caged replicates, but the relationship between leaf number and fly larvae is negative in uncaged replicates and positive sham caged replicates (Fig. 3.1B,C).

Laboratory study

Adult oviposition. Very low rates of oviposition were observed throughout the experiment. Of the 48 experimental cages employed through the course of the experiment, eggs were only found in 15 of them. Of the 392 eggs observed, 336 were found on *A. incarnata* plants and 56 were found on *A. syriaca* plants. Across treatments, 282 eggs were found in *A. incarnata* cages, 54 in *A. syriaca* cages, and 56 in the choice cages. Eggs were thus most abundant on *A. incarnata* and unequally distributed across plant species ($\chi^2_{\text{d.f.}=1}=188.1$, $P<<0.0001$) and experimental treatments ($\chi^2_{\text{d.f.}=2}=238.6$, $P<<0.0001$; Fig. 3.2); however, there were high levels of variability within treatments. Adult mortality rates were not significantly different across treatments (Friedman $\chi^2_{\text{d.f.}=2}=3.53$, $P=0.17$). An analysis of the full data set with both choice and no choice treatments revealed a non-significant effect of treatment on the number of eggs laid per cage (Friedman $\chi^2_{\text{d.f.}=2}=4.32$, $P=0.12$). However, comparing the effect of plant species on egg count in just the two no-choice treatments indicates significantly more eggs were found in *A. incarnata* cages than *A. syriaca* cages (Friedman $\chi^2_{\text{d.f.}=1}=4.5$, $P=0.034$). Results of additional analyses reported in Appendix B also frequently found a significant effect of treatment only when the choice treatment is excluded from the analysis.

Only four of the choice replicates were found to have eggs. In three replicates, between 9 and 35 eggs were laid on *A. incarnata* while none were laid on *A. syriaca*. In the remaining case, only 2 eggs were laid on *A. syriaca*. The paired Wilcoxon signed-rank test did not detect a significant effect of species on egg counts ($V=9$, $P=0.25$); however, the quasi-Poisson glm did find a significant effect of species on egg count within the choice treatments (Likelihood Ratio $\chi^2_{\text{d.f.}=1}=6.45$, $P=0.011$).

Larval performance. In our laboratory experiment, *A. aphidimyza* larvae were observed on 16 of 47 *A. incarnata* plants, but only 4 of 45 *A. syriaca* plants, thus they were not distributed independently of species ($\chi^2_{\text{d.f.}=1}=7.13$, $P=0.0076$). Furthermore, on those plants with larvae, they were more abundant on *A. incarnata* than on *A. syriaca* (medians =11.5 and 1.5 respectively; $W = 59$, $P = 0.012$).

Only two adult *A. aphidimyza* were recovered from a total of six *A. syriaca* plants with *A. aphidimyza* eggs, compared to 93 adults emerging from a total of 16 *A. incarnata* plants with eggs. *A. syriaca* significantly reduced the performance of *A. aphidimyza* as measured by the proportion of eggs that reached the adult stage (Fig. 3.3A, Wilcoxon test: W=81, P=0.013; quasi-binomial glm: Likelihood Ratio $\chi^2_{d.f.=1}$=4.49, P=0.034). There was already evidence for the effect of plant species on *A. aphidimyza* survival at the larval stage, five days after *A. aphidimyza* adults were released onto the plants (Fig. 3.3B, Wilcoxon test: W=73, P=0.07; binomial glm $\chi^2_{d.f.=1}$=6.36, P=0.012). In fact, within those first five days, there was 54% mortality on *A. incarnata*, and there was 89% mortality on *A. syriaca*. Larvae on *A. syriaca*, when they were observed, were much smaller than those observed on *A. incarnata* (personal observation). We estimate that most of our experimental error was associated with counting fewer larvae than were actually present, so we regard the estimates of the proportion of eggs to reach adulthood as the most reliable indicator of *A. aphidimyza* performance.

Discussion

In both the field and the laboratory studies, predatory fly larvae were more likely to be present and were more abundant on *A. incarnata* than on *A. syriaca*. These results are consistent with the hypothesis that plant traits on the putatively more-defended *A. syriaca* reduce *A. aphidimyza* oviposition or performance, either directly, or indirectly by affecting aphid quality. Our laboratory data provide strong evidence for reduced performance of *A. aphidimyza* larvae on *A. syriaca* compared to *A. incarnata*. Five days after the adult oviposition period began, we found significantly reduced survivorship on *A. syriaca* compared to *A. incarnata*, a pattern which continued through the adult stage. Although we cannot identify the mechanisms contributing to lower performance on *A. syriaca* in our study, higher trichome densities or elevated toxic cardenolide concentrations in the plant or aphids are likely candidates.

The evidence for *A. aphidimyza* preference is less convincing due to the absence of oviposition in many replicates and high variability between trials. The absence of eggs in many replicates may be due to the highly specialized mating behavior of *A. aphidimyza* (van Lenteren and Schettino 2003), in which egg-laying depends on mating (Markkula 1979), or to the fact that adults may take several days to mature eggs (Havelka and Zemek 1999). We would expect adults to be able to distinguish between plant species; a previous study found an effect of *Brassica* variety and plant species on *A. aphidimyza* oviposition (Mansour 1975). In our experiment, *A. aphidimyza* oviposited more frequently and deposited more eggs on *A. incarnata* plants, an effect that was most clear in comparisons of the no-choice cages. Within the four replicates of the choice treatment in which eggs were found, there was also a trend toward more eggs on *A. incarnata*.

Because *A. aphidimyza* larvae are so immobile and their survival is so dependent upon proximity to suitable prey, we would expect a positive relationship between preference and performance in this species. While we did observe a probable alignment of adult oviposition patterns and larval performance on different plant species with a common prey species, the effect of plant species upon larval performance is much more distinct than the effect on adult oviposition. Interestingly, a previous study using different aphid species on a single host plant species found that adults preferred to oviposit on *Aphis fabae* even though more larvae completed development on *Acyrthosiphon pisum* (Havelka and Ruzicka 1984). One potential explanation for the difference in the relationship between preference and performance on aphids compared to plants could require the integration of our understanding of optimal foraging and optimal oviposition behaviors (Scheirs and De Bruyn 2002). Adult *A. aphidimyza* feed on aphid honeydew, which increases their survival and fecundity (Markkula et al. 1979), and evidence suggests that they orient toward honeydew, possibly in combination with plant cues (Choi et al 2004). Honeydew quantity and quality are likely to vary in response to an interaction between aphid and plant species. For example, when *A. nerii* feeds on the very toxic, high-cardenolide milkweed species *A. curassavica*, nearly half (46%) of the dry mass of its honeydew is cardenolides (Malcolm 1990). If *A. syriaca* affects honeydew (adult resource) and aphid (larval resource) quality in the same way, compared to *A. incarnata,* this might explain the apparent alignment between adult oviposition and larval performance we observed. We note that honeydew quality could have a repellent effect on *A. aphidimyza* foraging behavior, or it could directly affect adult fitness, even though alternate sources of sugar were available in cages. In either case, it is not surprising that the effect of plants on larval performance was stronger than that on adult oviposition because larvae are confined to the site of oviposition, but adults are mobile and can utilize a variety of resources. At the same time, different aphid species on the same host plant may be less likely to covary in the quality of resources they provide to adult and larval *A. aphidimyza*, which could explain the different effects of aphid species on adult preference and larval performance previously observed (Havelka and Ruzicka 1984). If *A. aphidimyza* preferentially alight on plants based on attraction to honeydew signals that promote their own fitness, they may also oviposit on these same plants, even if it does not promote the fitness of their offspring.

Although variability across replicate choice cages frequently resulted in non-significant effects of treatment, the choice cages were most similar to the *A. syriaca* treatments in both numbers of eggs laid per cage as well as the proportion of cages that contained eggs. This suggests that some of the effect of plant species on *A. aphidimyza* oviposition behavior may be generated by a negative effect of *A. syriaca* rather than a positive effect of *A. incarnata*. Previous research has shown that combined cues from plants in choice tests under laboratory conditions may result in insect behaviors that are not observed under no-choice conditions or in the wild. For example, cues from a preferred host may increase oviposition on a less preferred host when they are presented together in laboratory tests (van Driesche and Murray 2004). A similar process can generate associational susceptibility, or conversely resistance, in a field setting (Barbosa et al. 2009). Given the potentially suppressive effect of *A. syriaca* on oviposition by *A. aphidimyza*, we suggest that further investigations of its effects on adult survival and performance, as well as adult preference, are necessary to fully understand the mechanisms by which these plant species affect the distribution of *A. aphidimyza* larvae in the laboratory and the field. We suspect that effects of host plant mediated by *A. nerii* honeydew are important. Alternatively, other characteristics of plants, such as trichomes or unmeasured volatile compounds could affect *A. aphidimyza* oviposition behavior.

Although *A. aphidimyza* displayed an apparent preference for trichome-rich regions of potato plants (Lucas and Brodeur 1999), the milkweed species with lower trichome densities was apparently preferred in our experiment.

Based on a comparison of the distribution of fly larvae across milkweed species in the lab and the field, we expect that acceptance behavior is similar across environments, but that performance differences and/or oviposition behaviors are more extreme in the field. The ratio of plants of each species with fly larvae in the field (32 *A. incarnata:* 9 *A. syriaca*) was remarkably similar to the ratio of plants with *A. aphidimyza* larvae in the lab (16 *A. incarnata:* 4 *A. syriaca*). Furthermore, we observed a similar proportion of *A. syriaca* plants with fly larvae in separate field experiments in 2009 and 2010 (E. Mohl unpublished data). This suggests that acceptance of *A. syriaca* occurs across different environments and is not simply a function of artificial conditions in the laboratory. As a generalist aphid predator, *A. aphidimyza* adults may not be able to discriminate between the quality of different host plants for their offspring, or they may be willing to lay a fraction of their eggs at available sites, even if they are of poor quality. Optimal foraging theory suggests that poor-quality oviposition sites should be accepted when insects are limited in the time available to lay eggs (Jaenike 1990), a situation which could have occurred in both the laboratory and the field for different reasons.

There was a 50-fold difference in the abundance of predatory fly larvae on *A. incarnata* and *A. syriaca* in the field (median cumulative fly larvae days per plant =377 on *A. incarnata*; 7.5 on *A. syriaca*), but just an 8-fold difference in the number of *A. aphidimyza* larva across species in the lab (median larvae per plant=11.5 on *A. incarnata*; 1.5 on *A. syriaca*). A number of non-mutually exclusive hypotheses might explain the greater effect of species on fly larvae abundance in the field. First, the effects of plant traits on *A. aphidimyza* larval performance may be exacerbated under more extreme and variable field conditions compared to those in the lab. Second, *A. aphidimyza* is known to vary its clutch size in response to aphid density both in the field (Sentis et al 2012) and the lab (Lucas and Brodeur 1999). In contrast to our laboratory experiment where per-plant aphid densities were held constant across species, aphid abundance was higher on the faster-growing *A. incarnata* plants in the field, although per-leaf aphid density was higher on *A. syriaca* (Chapter 2). Consequently, adults may have increased their clutch size on, or aggregated to, *A. incarnata* plants in the field, amplifying the difference in larval abundance across species. Third, *A. aphidimyza* larvae are themselves subject to predation (Lucas and Brodeur 2001, Gardiner and Landis 2007). If the overall greater aphid abundance on *A. incarnata* plants served to reduce the impact of larval predation via a dilution effect, this too could amplify the difference in the abundance of fly larvae across plant species. On this point, however, it seems likely that per-leaf aphid density, which was higher on *A. syriaca*, might also be important for reducing predation. Consequently the effects of plant species on predation risk mediated by aphid density could be highly dependent on the foraging behavior of the top predator in the system (see also Lucas and Brodeur 2001). Finally, the laboratory and field experiments studied different *A. aphidimyza* populations which may exhibit different preference and performance traits.

Taken together, the evidence suggests that host plants can have a strong effect on predator performance, even when the aphid species and aphid densities are the same. In both the laboratory and the field, *A. aphidimyza* was more prevalent and abundant on *A. incarnata* than on the putatively better-defended *A. syriaca*. The strong negative effects of *A. syriaca* on performance in the lab coupled with weaker evidence that *A. syriaca* may suppress oviposition suggests that the observed distribution of predators in the field may be due more to reduced oviposition, growth, and survival on *A. syriaca* than to any attraction to *A. incarnata*. For *A. aphidimyza*, performance seems to be more variable across plant species than acceptance. Despite the observation that larvae almost never survive to adulthood on *A. syriaca* in the lab, *A. aphidimyza* adults continue to oviposit on *A. syriaca* plants in both the lab and the field. Such a pattern may not be uncommon among polyphagous consumers. For example, the preference and performance of the aphid parasitoid *Binodoxys communis* are generally associated with the phylogeny of the aphid hosts but not their host plants; however, host-plant mediated toxicity is thought to underlie a strong mismatch between preference and performance on a single aphid host species (Desneux et al 2009, Desneux et al 2012). In contrast, other studies report some evidence that parasitoid responses to plant cues align with the performance of their offspring on those hosts (Gols et al 2009). An important research goal should be to elucidate the conditions that favor an effect of plants on the relationship between preference and performance in predatory insects. Based on this study, we hypothesize that plants may generate a positive correlation between preference and performance when they affect adult and larval resources in the same way.

Our observations of the relationship between adult oviposition and larval performance in the laboratory cannot account for ecological factors affecting the history of evolution in the predatory fly population such as the relative abundance the two milkweed plants in the environment and the effects of predation across species. However, given that the population has been reared in a laboratory culture for many generations, a more parsimonious explanation for a weaker effect of plant species on adult oviposition than on larval performance may simply be that adults cannot detect and respond to specific plant cues, an example of the neural constraints hypothesis (Bernays 2001). When preference and performance do not align, the physiological machinery necessary to detect relevant cues and associated preferences could be under continued selection to align with performance. In this case, *A. aphidimyza* could evolve the ability to avoid ovipositing on *A. syriaca*, given strong enough selection and suitable genetic variation. Alternatively, continued oviposition on *A. syriaca* could select for increased tolerance to the conditions on these plants and improved larval performance. If *A. syriaca* affects adult and larval performance in a similar manner, this could further select for a narrower diet breadth over time. At the same time, joint expression of host preference and avoidance behaviors can, under certain conditions, facilitate specialization and speciation (Feder and Forbes 2008). Further research into the genetic basis of preference and both adult and larval performance in this species would help to predict the potential for diet breadth evolution.

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Figures

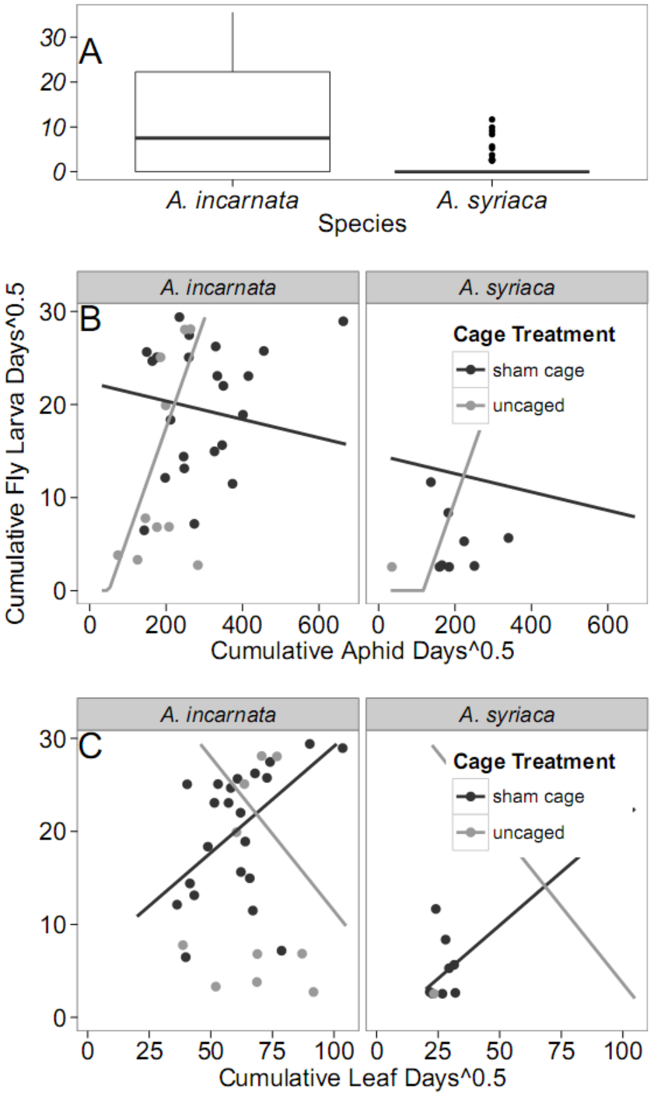

Figure 3.1. Distribution of fly larvae in the field. The distribution of cumulative fly larva days on plants of the two milkweed species in 2009 (A). For only those plants with fly larvae, we show cumulative fly larva days as a function of (B) cumulative aphid days and (C) cumulative leaf days. Response and predictors are square-root transformed in all plots. Shown are the predicted linear regression lines at the mean value for CLD (B) or CAD (C). Lines are truncated when predicted values of Cumulative Fly Larva Days ≤ 0.

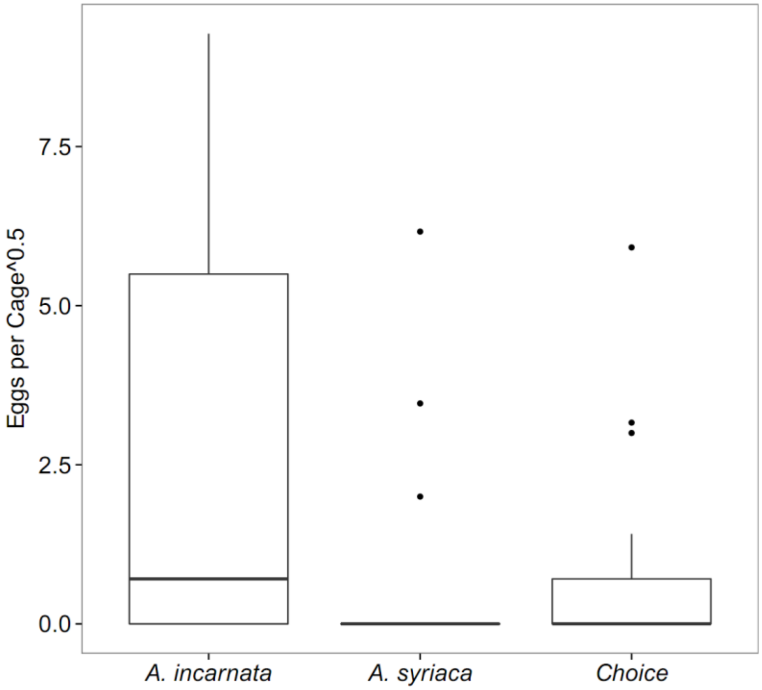

Figure 3.2. ***Aphidoletes aphidimyza* preference.** We measured preference as the number of eggs in each treatment. Box plots show the distribution of the data with outlying points; the weighted line shows the median value, and the box shows the 25-75th percentile of the distribution.

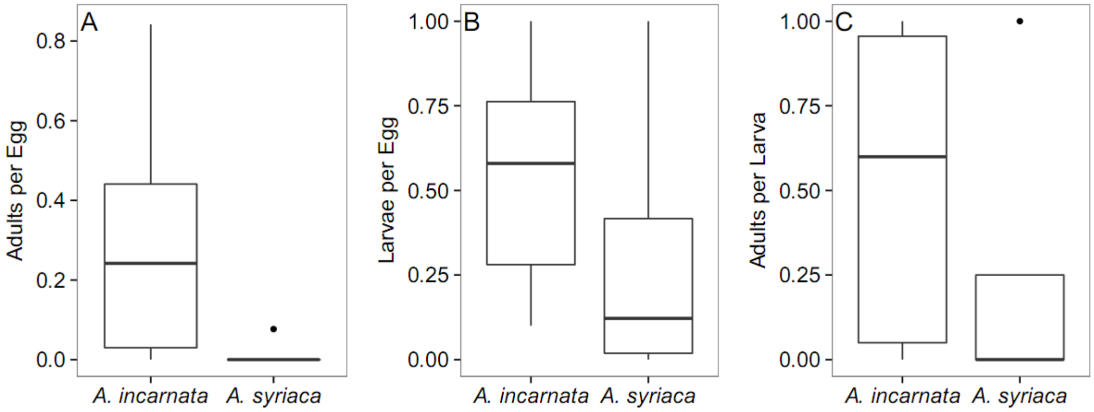

Figure 3.3. ***Aphidoletes aphidimyza* performance**. We measured predator survivorship at different life stages from egg to adult on each species of host plant. A. The distribution of the ratio of adults to eggs on those plants on which eggs were found (n =16 *A. incarnata* and 6 *A. syriaca*). B. The ratio of larvae to eggs found on the same set of plants. C. The ratio of adults to larvae from those plants on which larvae were observed (n=16 *A. incarnata* and 4 *A. syriaca*).

Supplementary Material

Appendix A. Analysis excluding influential point

Our analysis of the effect of plant species on the abundance of fly larvae in the field was sensitive to a highly influential point, so we repeated the analysis after excluding that point. As with the analysis of the full data set including all plants on which fly larvae were found during the season, we found a significant main effect of species on cumulative fly larva days ($\chi^2_{d.f.=1}$=6.59, P =0.0102). However, after excluding the outlying point, the slopes and intercepts changed. The overall intercept for cumulative fly larvae days (CFLD) on *A. incarnata* is more negative than that for *A. syriaca*, a result that differs from our analysis of the full dataset in which CFLD was higher on *A. incarnata*. This may occur in part because slopes and intercepts are related, and because plant species alters the slope of the relationship between cumulative fly larvae days and cumulative aphid days (Species x CAD interaction: $\chi^2_{d.f.=1}$=9.59, P=0.0020) such that the cumulative fly larva days accumulate at a faster per-aphid day rate on *A. incarnata* than on *A. syriaca* (Fig. 3SA1). This result is consistent with our analysis of the complete data set: fly larvae respond to aphids more on *A. incarnata* than they do on *A. syriaca*.

As with the analysis with all data points, we found that sham and uncaged treatments had different effects on the relationship between fly larvae and aphid or leaf accumulation. The slope of the relationship between cumulative fly larva days and cumulative aphid days was more positive in uncaged replicates (Cage x CAD interaction: $\chi^2_{d.f.=1}$=15.6, P<0.0001). However the slope of the relationship between cumulative fly larva days and cumulative leaf days was more positive in sham cages (Cage x CLD interaction: $\chi^2_{d.f.=1}$=15.91, P<0.0001). Thus the abundance of *A. aphidimyza* larvae seems to relate more to aphid density in uncaged replicates and more to plant growth in the caged replicates.

When the influential data point is removed, cumulative fly larva days appears to respond to an interaction between cumulative aphid days and cumulative leaf days (CADxCLD interaction: $\chi^2_{d.f.=1}$=9.02, P=0.0027) such that the cumulative aphid days slope declines at higher values of cumulative leaf days (Fig. 3SA1). If *A. aphidimyza* larval abundance is greater on small plants with many aphids and on large plants with fewer aphids, this could be a result of adult oviposition preference, differential larval survival, or a combination of these processes.

Overall, using cumulative measures of insect abundance resolves some of the challenges of this data set posed by non-independence and a high frequency of zeroes; however, aspects of the analysis are sensitive to a highly influential point. Excluding the influential point changes the relative location of the cumulative fly larvae days intercept for the two milkweed species in a manner that is surprising, given the results of our laboratory studies. Excluding the influential point also indicates a significant interaction between cumulative aphid days and cumulative leaf days may affect cumulative fly larvae days, a result that we believe requires further investigation to confirm. However, both analyses (with and without the influential point) indicate that the relationship between cumulative fly larvae days and cumulative aphid days is steeper on *A. incarnata* than on *A. syriaca*, a pattern consistent with our laboratory observations that *A. aphidimyza* appears to prefer and perform better on *A. incarnata*. Furthermore, both analyses indicate cumulative fly abundance appears to respond positively to cumulative leaf abundance in sham cages but to cumulative aphid abundance in uncaged replicates. One possible interpretation of these patterns is that sham cages alter *A. aphidimyza* oviposition behavior by providing a shelter that is absent in the uncaged replicates. Alternatively, larval survival could be differentially affected, potentially by different risks of intraguild predation, in the two environments. Our laboratory studies are not relevant to these hypotheses; further investigation is necessary to identify the mechanisms that are behind these patterns.

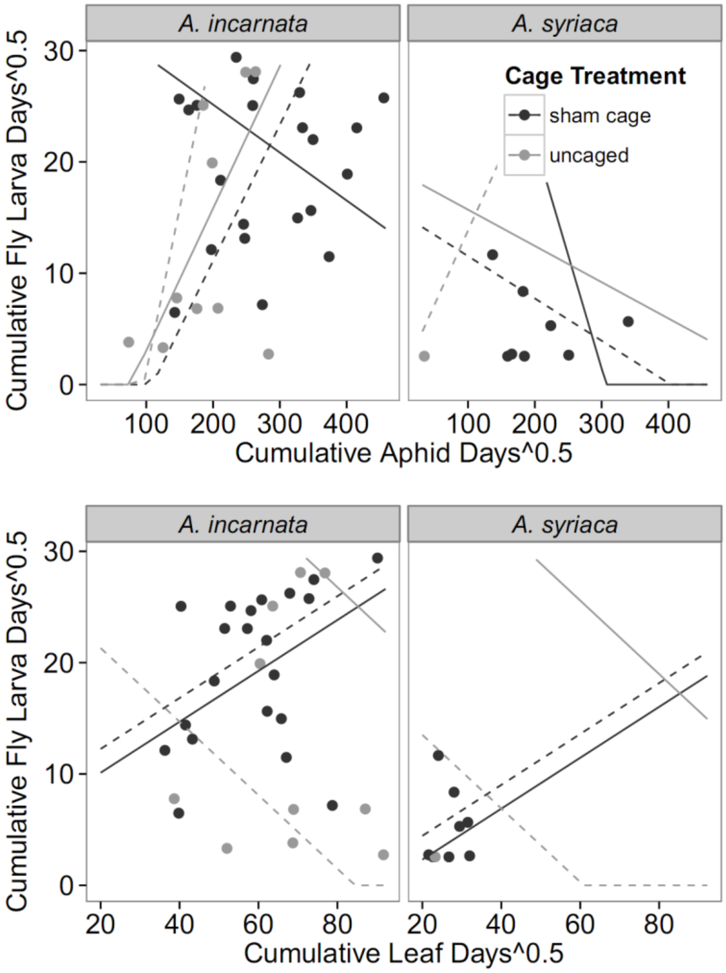

Figure 3SA1. Field data excluding outlier. For only those plants with fly larvae, and excluding an influential data plant with fast growth and abundant aphids, we show cumulative fly larva days as a function of cumulative aphid days (top panel) and cumulative leaf days (bottom panel). Response and predictors are square-root transformed in all plots. Because our model found an interaction between two continuous variables, two lines are shown for each treatment combination. Solid lines represent the relationship between plotted variables at one untransformed standard deviation above the mean of the continuous covariate not shown (cumulative leaf days for top panel; cumulative aphid days for bottom panel). Dashed lines represent the relationship between plotted variables at one untransformed standard deviation below the mean of the continuous covariate not shown. Lines are truncated when predicted values of Cumulative Fly Larva Days ≤ 0.

Appendix B. Alternative models for the analysis of adult oviposition

We considered a variety of analytical methods to assess the effect of treatment on *A. aphidimyza* oviposition given the high frequency of cages with zero eggs in our study. In general, we find a tradeoff between nonparametric methods that are conservative and appropriate for small sample sizes and more powerful models that account for the experimental blocking structure but require larger sample sizes. For completeness, we present all analyses not described in the text here.

Zero-inflated regression models account for an excess of zero counts by combining two types of models. The model assumes that there are two categories of experimental units that contribute to the zero counts: those that come from a process that generates a standard count distribution, such as the Poisson or negative binomial, and those that come from a separate process. In our study, the absence of eggs could be due to female choice, or due to females being physiologically unable to lay eggs due to unmated status or delayed egg maturation, and a zero-inflated regression model can account for these separate processes. We separately analyzed the effect of treatment on the presence or absence of eggs in a cage using both Fisher's exact test for analysis of a contingency table and a generalized linear model (glm) in the binomial family, which allowed us to account for the blocking structure. Finally, we used analysis of variance to test for an effect of treatment on square-root-transformed egg counts in only those cages where eggs were observed. In all of these analyses, we exclude two cages in which all of the adult *A. aphidimyza* released into the cage were found dead, suggesting that they did not make any choices about where to lay eggs.

For the zero-inflated regression analyses, we used the pscl (Zeileis et al. 2008) and lmtest (Zeileis and Hothorn 2002) packages in R. We modeled block and treatment as predictors for the female choice process resulting in the response of egg count, and we used the binomial component to estimate an intercept parameter for the probability that a female would be in a physiological state capable of laying eggs. Vuong tests indicated whether zero-inflated negative binomial models improved the fit over zero-inflated

Poisson models; in all cases the zero-inflated models fit better than the Poisson glm. We used likelihood ratio tests to assess the significance of terms in the model. For the full dataset including both choice and no-choice treatment levels, we found a zero-inflated negative binomial fit the data best; whereas, a zero-inflated Poisson model was used for the analysis of the no-choice treatment levels only.

More eggs were found in *A. incarnata* cages than in the other treatments, but the significance of this result varies depending upon the method of analysis. We observed similar results with the zero-inflated Poisson to those of the Friedman test. When all treatment levels were included, we found no significant effect of treatment (Likelihood Ratio $\chi^2_{d.f.=2}=1.57$, $P=0.46$). However, when we analyzed only the two no-choice treatments, we found a highly significant effect of plant species on *A. aphidimyza* oviposition (Likelihood Ratio $\chi^2_{d.f.=1}=54.94$, $P<<0.0001$). Although 50% of the "Incarnata Only" cages had eggs compared to 27% of the cages with both species and 20% of the "Syriaca Only" cages, the presence or absence of eggs in a cage was distributed independently of treatment (Fisher's Exact Test, $P=0.22$). However, binomial glms show a marginally significant effect of all three treatment levels on the presence or absence of *A. aphidimyza* eggs (Likelihood Ratio $\chi^2_{d.f.=2}=5.15$, $P=0.076$), and a significant effect of species in no-choice treatments on the presence of eggs (Likelihood Ratio $\chi^2_{d.f.=1}=5.82$, $P=0.016$). When we exclude cages that did not have any eggs, we did not find a significant effect of all three treatment levels ($F_{2,12}=1.55$, $P=0.25$), or of the two no-choice treatment levels ($F_{1,9}=1.18$, $P=0.31$), on the square-root transformed number of eggs per cage.

Chapter 4

Parasitoid adaptation to plant-derived defenses in their hosts: a potential resolution to the lethal plant defense paradox

Summary

When specialist herbivores are better adapted to plant-derived defenses than their natural enemies, plants may be subject to a tradeoff between direct and indirect defense. However, such a tradeoff may be mitigated if natural enemies also become adapted to plant traits. By studying a generalist aphid parasitoid that was introduced from North America to Europe for biological control, we test the hypothesis that parasitoid populations have adapted to the local chemically-defended host plant in their region. We find evidence consistent with reduced overall performance of introduced parasitoid populations and with local adaptation of parasitoids to the host plant common in the region of origin. Our experimental design accounts for differentiation in host aphid populations and provides indirect evidence that aphid populations are also locally adapted to their host plant species. Our results indicate that parasitoids may evolve in response to trait-mediated indirect interactions with plants. If this is a general phenomenon, natural enemy evolution in response to plant defenses may help to resolve the lethal plant defense paradox.

Introduction

When specialist herbivores are adapted to plant defenses that negatively impact generalist natural enemies, they may gain enemy-free space, ameliorating their own predation and reducing the fitness of their plant host (Price et al. 1980, Ode 2006). This is thought to be particularly common when herbivores sequester toxins from their host plants, generating the lethal plant defense paradox (Malcolm 1992). A number of studies have investigated whether direct defenses, like toxins, trade off with indirect defenses provided by natural enemies. The evidence in ant-plant systems is mixed (Heil and McKey 2003), as it is in other systems. Some find evidence consistent with a tradeoff (Bartlett 2008, Wei et al. 2011); whereas, others do not (Thaler et al. 2002, Gols et al. 2008), and the outcome may depend on exactly what is measured. For example, plant defenses may have strong effects on natural enemy survival without a corresponding effect on plant growth (e.g. Havill and Raffa 2000). Some authors have proposed that reduction of plant defenses in response to herbivory by specialists may resolve the lethal plant defense paradox (Malcolm and Zalucki 1996), and others have suggested that plants may evolve reduced levels of defense in environments with resistant specialist herbivores but susceptible natural enemies (Ode et al. 2004, Berenbaum and Zangerl 2006, Agrawal and Fishbein 2008). However, an alternative resolution could occur if generalist natural enemies evolve tolerance of plant-derived defenses and effectively consume sequestering herbivores.

Chemical defenses are typically associated with tight coevolutionary relationships between prey and highly specialized consumers. For example, the phenotype matching of the parsnip webworm and wild parsnip plant chemotypes in many populations is indicative of coevolution (Berenbaum and Zangerl 1998). However, tight coevolutionary interactions are likely to occur only at certain times and places (Thompson 2005); in particular, they may be disrupted when they take place in larger interaction webs with competitors or predators (Berenbaum and Zangerl 2006). When selection upon consumers is diffuse (as defined in Strauss et al. 2005), as is most likely the case with a polyphagous consumer, the consumer population's evolutionary response to a chemically defended prey species will depend upon genetic correlations with performance on other prey species. If consumer fitness on different prey species is negatively genetically correlated, as is often assumed, this will constrain the evolutionary response of a predator population to either prey species (Futuyma and Moreno 1988). Even in the absence of negative genetic correlations, the presence of alternative prey should result in relaxed selection for performance on chemically defended prey simply by reducing exposure (Kawecki 1994, Whitlock 1996, Snell-Roode et al. 2010). Given additive genetic variation for tolerance to chemical defenses, polyphagous consumers are expected to evolve tolerance to chemical defenses at a slower rate than highly specialized consumers. Nonetheless, there is evidence that polyphagous consumers can become locally adapted to chemically defended prey. For example, sea urchins are generalists that consume chemically defended seaweeds within part of their geographic range, and populations in this region show increased tolerance to these defenses (Craft et al. 2013).

Coevolutionary studies focus primarily on two species that directly interact, although there is evidence that the involvement of additional species can change the nature or "temperature" of a coevolutionary relationship (Thompson 2005). Many additional studies demonstrate evolution of one species in response to another: for example, studies have demonstrated the potential for natural enemies to evolve in response to their prey species (Henry et al. 2008, Antolin et al. 2006). Although density- and trait-mediated indirect interactions are known to be important in ecological interactions (Wootton 1994), the potential importance of evolution in response to indirect interactions is an emerging area of study (Biere and Tack 2013, Walsh 2013). Additionally, an extensive literature on tri-trophic interactions has shown that plant traits not only affect the fitness of herbivores, but also predators (Price et al. 1980, Ode 2006, Desneux et al. 2009). Consequently, given appropriate genetic variation, we expect predators to have the potential to evolve in response to plant defenses. Although we are not aware of any examples of predator or parasitoid evolution specifically in response to host plant defenses, there are cases where parasitoid divergence parallels host–plant associated divergence in herbivores (Stireman et al. 2006, Forbes et al. 2009). Cascading plant-associated divergence of hosts and parasitoids is not universal, however (Hufbauer 2001, Bilodeau et al. 2013).

We currently lack the ability to predict when indirect interactions should strongly affect evolution; however, several examples demonstrate that when direct interactions alter the traits of a focal species, evolution in response to trait-mediated indirect interactions is possible. For example, some herbivorous insects are locally adapted to changes in plant traits caused by soil biota (Bonte et al. 2010). Furthermore, the food plants of insect hosts have been shown to influence local adaptation of parasites (Cory and Myers 2004) and to have the potential to affect pathogen virulence evolution (de Roode et al. 2011). These studies provide emerging evidence that trait-mediated indirect interactions, in which the phenotypic response of a focal species to a second species alters its interactions with a third species, can influence evolution and may drive patterns of local adaptation.

Local adaptation occurs when a genotype by environment interaction generates divergent selection in spatially structured populations (Kawecki and Ebert 2004). In a tri-trophic system, each of the interacting species has the potential to be locally adapted to both of the other species, though local adaptation between plants and natural enemies will be indirect. Even with just two interacting species, models show that a number of factors interact to affect patterns of local adaptation. Gene flow degrades local adaptation, and asymmetric, maladaptive gene flow from large populations can cause smaller "sink" populations to become maladapted and go extinct (Ronce and Kirkpatrick 2001). A species can also appear to be maladapted to another species if it is "losing" in the coevolutionary relationship. For example, parasites commonly show local adaptation to their hosts, but cases of parasite local maladaptation also occur (Gandon 2002, Lajeunesse and Forbes 2002, Hufbauer and Roderick 2005) and may result from time lags in coevolutionary cycling or from local adaptation of host defenses to the resident parasite population. Altogether, time lags in frequency-dependent selection, generation time, the availability of genetic variation, migration rates, mutation rates, population size, and the strength of selection can all interact to affect the pattern of local adaptation observed between two interacting species (Gandon 2002, Kawecki and Ebert 2004). Because suites of traits tend to co-occur, predictable patterns emerge. For example, large populations of fast-reproducing, highly dispersive parasite populations tend to become locally adapted to longer-lived, less dispersive hosts with smaller population sizes (Kawecki and Ebert 2004). Furthermore, parasites with a broad host range appear to be less locally adapted to their hosts than those with narrow host ranges (Lajeunesse and Forbes 2002). The challenge is to apply these theoretical predictions to populations in a multi-trophic context and to test them with empirical data.

To test the hypothesis that insect natural enemies are locally adapted to the plants on which their hosts feed, we investigated two populations of the polyphagous aphid parasitoid, *Lysiphlebus testaceipes*. The populations, from southern France and north-central United States, have been separated since at least the 1970s when the *L. testaceipes* was introduced from Cuba into France for biological control. *Lysiphlebus testaceipes* has a broad host range, having been reared from more than 120 different species of aphids

(Shufran et al. 2004). We studied its performance on the bright yellow aposematically colored aphid, *Aphis nerii*. *A. nerii* feeds on plants in the Apocynaceae, from which it sequesters toxic cardenolides for its own defense (Mooney et al. 2008). Although it likely originated in Eurasia (Foottit et al 2006), *A. nerii* is now widely distributed in warmer climates (Blackman and Eastop 2008). In France and Cuba, the predominant host plant for *A. nerii* is oleander (*Nerium oleander)*, while milkweeds (*Asclepias* spp.) are the dominant host plant in the north-central region of the United States. Toxic cardenolides are more concentrated in oleander, a shrub, than in milkweed, a forb, by an order of magnitude. We expected plant-derived defenses to influence parasitoid performance. At the same time, aphid populations may show variation in the chemicals they sequester from the plants, in their innate defenses (e.g. Hufbauer and Via 1999), or in the composition of their defensive endosymbionts (reviewed in Oliver et al. 2010). In order to test whether parasitoid populations were locally adapted to the local aphid or plant populations, we conducted a factorial experiment in which parasitoid origin (French or American), aphid origin (French or American), and plant species (*A. incarnata* or *N. oleander*) were fully crossed and aspects of parasitoid performance were measured under each condition. Additionally, to control for broad differences in parasitoid performance across populations, we assayed parasitoid performance on a standard host-plant combination: *Rhopalosiphum maidis* on barley. Our experimental design allows us to infer the relative importance of direct and indirect interactions for parasitoid local adaptation, and to gather indirect evidence about the local adaptation of aphids to the host plants in their range.

If parasitoid populations are locally adapted to the host plant common in the region of origin, independently of aphid population origin, that would demonstrate that polyphagous populations can, in fact, evolve in response to plant traits. Because *A. nerii* sequesters toxins from its host plants, parasitoid local adaptation to host plants is likely to be a trait-mediated indirect effect transmitted through the aphid. This would suggest that polyphagous natural enemies can evolve tolerance of plant-derived defenses in their specialist herbivore hosts, consistent with an evolutionary resolution to the lethal plant defense paradox. Furthermore, evidence for local adaption would indicate a less than perfect positive correlation in performance across host plants.

We now combine the theoretical predictions for local adaptation described above with our knowledge of the biology and history of *L. testaceipes* interactions to generate a series of predictions about patterns of performance likely when different processes dominate. All else being equal, we would predict parasitoid populations to perform better on the host plant native to the region of collection than on the novel host plant (the home vs. away comparison, sensu Kawecki and Ebert 2004). However, oleander contains more concentrated cardenolides than milkweed. If the limited evidence for a positive relationship between cardenolides concentrations in plants and aphids (Malcolm 1990, Malcolm 1992) holds here, we might predict both parasitoid populations to perform better on milkweed than on oleander, with only a difference in their relative performance on each species. In this case, we predict the local population to outperform the foreign population on each host plant (the local vs. foreign comparison, sensu Kawecki and Ebert 2004), still providing strong evidence for local adaptation. Population history, however, could further alter predictions. We know the French *L. testaceipes* population experienced a bottleneck during the process of introduction (Stary et al. 1988a). If the genetic bottleneck resulted in inbreeding depression, then we might expect the French population to perform less well in all assays, which would limit our ability to draw strong conclusions about local adaptation. In contrast, we might predict the French population, which was collected entirely from *A. nerii* prior to introduction (Stary et al. 1988a), to outperform the American population on *A. nerii*, but not on *R. maidis*, if maladaptive gene flow from parasitoids on more abundant aphid species prevents them from locally adapting to *A. nerii* in the American population.

Predictions for local adaptation between aphids and parasitoids, and between aphids and plants are challenging because we lack critical information. We might speculate that the rapid rate of aphid reproduction would generate ample genetic variation via mutation to allow aphids to adapt locally to both plants and parasitoids. However, *A. nerii* typically does not reproduce sexually (Blackman and Eastop 2008), which could constrain its relative rate of evolution. Furthermore, the population structure or rate of gene flow among populations at each trophic level, the nature of diffuse coevolution, and the presence of endosymbionts are all likely to influence evolutionary outcomes. The patterns of parasitoid performance we observe provide some evidence about which processes may dominate in the evolution of these populations and suggest areas for further research.

Methods

Natural History

Aphid parasitoids lay a single egg inside each aphid host. The egg develops through a larval stage into a pupal "mummy", and then emerges as an adult. In the 1970s, *L. testaceipes* mummies were collected from *A. nerii* on oleander in Cuba, reared for over a year on several different hosts, and then released in France for biological control (Stary et al. 1988a, Stary et al. 1988b). The introduced population of *L. testaceipes* cannot effectively parasitize one of the aphids it was intended to control in much of the Mediterranean region (*A. citricola*); nevertheless, it has widely expanded its host range in Europe, where it continues to parasitize *A. nerii* on *N. oleander* (Stary et al. 1988b, Stary et al. 2004). Preliminary experiments with an American population demonstrated that its performance varies across host aphid and host plant species (Mohl unpublished). *Rhopalosiphum maidis*, the corn leaf aphid, was chosen as an alternative host for *L. testaceipes* because it can be found both in France and Minnesota, USA, in the regions where experimental populations were collected, as well as in Cuba, the original source of the French *L. testaceipes* population (Stary 1981). Barley (*Hordeum vulgare L.*) is one of many grasses suitable for *R. maidis*. *Asclepias incarnata* is a common host plant for *A. nerii* in Minnesota, and was chosen for its very low levels of toxic cardenolides (Martel and Malcolm 2004, Agrawal and Fishbein 2006, Zehnder and Hunter 2007b) and the fact that its growth form, while herbaceous, is similar to that of *N. oleander* in that they both have long leaves without trichomes.

Collection and Rearing

Plants. We grew all plants in the greenhouse with potting soil (Metromix Sunshine MVP). Prior to planting, we cold stratified *A. incarnata* seeds (Prairie Moon Nursery, Winona, MN) in wet sand at 5°C for at least one week. We propagated cuttings of *N. oleander* (Hardy Red variety, Purplegard4me, Dallas, TX) by dabbing them in rooting hormone (indole-3-butyric acid, 0.1%, Hormodin), placing them in vermiculite and misting every five minutes until they were ready to be transplanted individually into potting soil. Oleander plants were fertilized initially and one month prior to use with water soluble NPK 20-10-20 fertilizer (426 ppm N, Scotts, Maryville, OH) to facilitate new growth necessary to support aphid populations. Most plants were approximately 20 cm tall during the bioassays, but barley plants were up to 40 cm from soil to leaf tip.

Insects. *Aphis nerii* first appeared in France April 26-29, 2011. During this time, we collected aphids from 5 separate oleander bushes within a 5 km range near Antibes, France, the site of the original release of *L. testaceipes*. Single individuals from each of these clones were combined into a single colony on May 2 and reared in the lab on cut oleander branches with young leaves. On July 2 and 4, 2011, several hundred mummies were collected from French *A. nerii* populations on approximately 35 different oleander trees distributed across four locations within 25 km of Antibes, France. Mummies were maintained at 8 °C until they were shipped, along with *A. nerii*, to the quarantine lab at the University of Minnesota on July 5, 2011. Approximately 65 individuals (45F and 22M) emerged, and the population was maintained for several generations while the identity of the emerged parasitoids was confirmed. We initially split the French parasitoid populations between *R. maidis* and *A. nerii* on *A. incarnata*, but we switched to *R. maidis* after two generation because of higher productivity in those cages. Offspring from 13 mated pairs confirmed to be *L. testaceipes* were mixed into a single population on September 16 and thereafter reared in cages on a mixture of French *A. nerii* and *R. maidis* for the duration of the experiment. Because both French and American *A. nerii* grew slowly on oleander, we reared them separately in the lab on *A. incarnata*.

Mummies first appeared on *A. nerii* in Minnesota, USA in early August, 2011. We collected individual aphids from 5 different aphid clones on 5 different plants within a 5 km range of the St. Paul campus of the University of Minnesota. On August 5, we combined a single individual from each of the clones into a colony. Between August 1 and August 20, the earliest mummies of the season were collected from approximately 30 different milkweed plants (*A. syriaca*) distributed across three different locations within about 7 km of the St. Paul campus of the University of Minnesota. Approximately 40 individuals emerged (31 F and 9 M), and the population was maintained for several generations on *R. maidis* or American *A. nerii* on *A. incarnata*. On Sept. 19, offspring from 20 mated pairs confirmed to be *L. testaceipes* were mixed into a single population and reared on a mixture of *A. nerii* and *R. maidis*.

French and American parasitoid populations were reared in the lab for 10 and 8 generations, respectively, prior to beginning the experiment, during which time the identity of the parasitoids in each populations were confirmed. In order to avoid mixing individuals from two populations, they were reared in separate rooms under 16h:8h light:dark cycle and ambient temperature, approximately 20+/- 3°C. Although we did not keep daily records, we did notice temperature differences between the rooms on several dates, and we address these issues in the results and discussion. The populations were also handled in separate rooms during assays and measurement, and they were never handled by the same person on the same day.

Performance Assays

We conducted bioassays in microcosms to measure the performance of individual parasitoids from the French and American populations on each of four factorial aphid-plant combinations: French and American aphids on *N. oleander* and *A. incarnata*, and also on *R. maidis* on barley. Each of our 10 treatment combinations was replicated approximately 12 times, spanning three temporal blocks. To avoid contamination, we kept the French and American parasitoid populations separate for nearly all phases of the experiment. Plants and aphids were randomly assigned within parasitoid treatments, but assays for each parasitoid population were always conducted in separate rooms until the point of mummy collection. We acknowledge that this confounds the population differences of interest with differences between growth rooms, noting that concern about the risk of contamination outweighed the concern about confounded effects of environment, which were held the same, as nearly as possible. After collection, mummies were stored in the same growth chamber.

Each assay involved exposing an individual mated female to 100 aphids on the designated host plant. To reduce the potential for environmentally determined maternal effects to mask genetic differences between parasitoid populations, we reared both populations on *R. maidis* for one "transfer" generation prior to conducting the assays. We transferred 15 pairs of parasitoids from the stock population into a cage with four *R. maidis*-infested barley plants. We collected mummies from the transfer generation into 0.65 mL microcentrifuge tubes (dotscientific.com) provisioned with a drop of honey for the emerging adult, and retained the mummies in a growth chamber at 25°C, 16h:8h light:dark, and approximately 65% humidity. As adults emerged from these mummies, they were sexed and pairs were mated for at least four hours prior to experimental assays. Female parasitoids were less than 24 hours old when assays began.

We conducted assays in individual microcosms consisting of a plant in a 14 cm round pot covered with an inverted clear acrylic cup (10 cm diameter, 21 cm tall, 58 oz, Pioneerplastics.com). Fifteen 2-cm holes in the tube were covered with fine nylon filtration screening (mesh opening 105 μ, componentsupplycompany.com) to allow ventilation but prevent insect migration, and the soil was covered with plaster to control other insects. Cuttings with the appropriate variety of aphids were applied to plants prior to the assay, and aphids were allowed time to transfer to the experimental plants. Prior to the beginning of each assay, we removed excess aphids so that each experimental plant supported 100 individual apterous (non-winged) aphids of mixed instars. Assays began when we released a single mated pair into a microcosm for 24 hours, at which point the parasitoids were recovered. Each assay microcosm was retained to allow the development of parasitoid offspring. After 10 days, we collected mummies into individual wells of a 96 well plate to track emergence. We continued to check plants daily, and any mummies developing after 10 days were collected into microcentrifuge tubes and followed for emergence as well. Plants were checked until no new mummies appeared for 20 consecutive days. We recorded the number of mummies resulting from each assay and the date and sex of emergence of each offspring. Ten days after mummies were collected, unemerged mummies and emerged offspring were frozen until we could slide-mount up to three males and three females from each of the experimental bioassays to measure hind tibiae. Body size and fitness are frequently related in parasitoids (Jervis et al. 2007 Chapter 2) and adult hind tibia length explains about 50% of the variation in egg load in *L. testaceipes* (A. Biondi unpublished data). We measured the most visible hind tibia of each specimen at 50x magnification on a Leica MZ8 stereomicroscope.

Statistical Analysis

All analyses were conducted in R version 3.0.1 (R Core Team 2013). Our assays allowed measurements of several dimensions of parasitoid performance on each plant-aphid combination, including mummification rate, emergence rate, development time to adulthood, sex ratio, and adult size in the offspring brood. For each response variable, we conducted separate analyses for the factorial reciprocal transplant experiment (with two levels for each of the three factors: plant species, aphid origin, and parasitoid origin) and the assays on *R. maidis* (with parasitoid origin as the predictor). We present values calculated from the raw data in the text and figures and indicate the significance of patterns based on analysis of our models. Unless otherwise noted, all models included temporal cohort as a blocking factor and excluded the highest order interaction, which was never significant at α=0.05. We present Likelihood Ratio or Wald Chi Square test statistics based on Type II tests calculated in the car package (Fox and Weisberg 2011).

Since aphid number was standardized prior to each assay, we analyzed the effects of our predictors on mummy counts using generalized linear models (glms) in the quasi-Poisson family which incorporate an overdispersion parameter. Fourteen of the 120 replicates were excluded from these analyses because the female died or was lost during the 24-hour trial. We used binomial glms to analyze the effects of predictors on the rate of adult emergence from those replicates with mummies, employing quasi-likelihood in the reciprocal transplant analysis to account for overdispersion. For the analysis of emergence rates on *R. maidis*, three replicates from the American population were excluded because the adults escaped from their cells in the 96-well plates, precluding an accurate count. Excluding these three replicates did alter the outcome, so the analysis of the full dataset is shown in Appendix 1. For those replicates in which at least one female offspring emerged, indicating that the experimental female was successfully mated, we used quasi-binomial glms to analyze the effects of our predictors on offspring sex ratios.

For each assay in which adults emerged, we calculated the average number of days between the start of the trial and the offspring emergence as adults. We log-transformed the averages and analyzed these data as a function of parasitoid origin using a linear model for development time on *R. maidis*. Because there was nonconstant variance across plant species, we used a linear mixed effects model from the nlme package in R for our analysis of the reciprocal transplant experiment on *A. nerii*, which allowed us to account for non-constant variance across plant species in the model structure (Pinheiro et al. 2014). In this model, average time to adulthood was log-transformed, temporal block was a random effect, and two-way interactions between each of our three predictors were included. We also used linear mixed effects models from the nlme package to analyze the effects of our predictors on adult hind tibia length in those trials where adult offspring emerged. In these models, sex, parasitoid origin, aphid origin, and plant species were crossed fixed effects, and offspring individual hierarchically nested within trial within temporal block were modeled as random effects.

Results

Mummy number

More mummies were found in trials on milkweed than on oleander ($\chi^2_{d.f.=1}=9.909$; $P<<0.001$), in trials with American aphids than with French aphids ($\chi^2_{d.f.=1}=4.209$; $P=0.040$), and in trials with American parasitoids than with French parasitoids, both on *A. nerii* (Fig. 4.1; $\chi^2_{d.f.=1}= 17.238$; $P<0.001$) and on *R. maidis* (Fig. 4.1; $\chi^2_{d.f.=1}=17.973$; $P<0.001$). Indeed, we observed the highest mummy counts when American parasitoids were attacking American aphids on milkweed (Fig. 4.1). However, we did not find a significant interaction between plant species and parasitoid origin (plant x parasitoid origin interaction $\chi^2_{d.f.=1}$=0.233; P= 0.629), so we do not have evidence for parasitoid local adaptation directly to plants in terms of mummy number. We did find a significant plant by aphid origin interaction ($\chi^2_{d.f.=1}$=4.379; P= 0.036) such that, on a given plant species, parasitoids from both populations produced more mummies when attacking the local rather than foreign aphid to the plant. On oleander, trials with French aphids averaged slightly more mummies (mean +/- SE = 16.4+/-2.9) than trials with American aphids (13.7+/-2.3). On milkweed, however, trials with American aphids averaged about 50% more mummies (47.5+/-7.7) than trials with French aphids (29.1+/-3.3). Although the three way interaction was not significant ($\chi^2_{d.f.=1}$=0.044; P= 0.83293), this trend appears to be driven largely by the higher mummy numbers in trials with American parasitoids on American aphids on milkweed plants. Additionally, we found a marginally significant interaction between *A. nerii* origin and parasitoid origin on the number of mummies produced: American parasitoids produced twice as many mummies as French parasitoids on American aphids compared to just 30% more on French aphids ($\chi^2_{d.f.=1}$=3.024; P=0.082). Interestingly, the French parasitoid population appears to produce an average of 19 mummies, regardless of aphid origin; however, the American parasitoid population produced an average of 26 mummies on French aphids and an average of 42 mummies on American aphids.

Emergence Rate and Sex Ratio

We found that parasitoid origin changed the effect of plant species on emergence rates in a manner consistent with "local vs. foreign" local adaptation to host plant (parasitoid origin by plant interaction: $\chi^2_{d.f.=1}$=14.701; P<0.001). Somewhat surprisingly, French and American parasitoids emerged from mummies on the putatively more-toxic oleander at relatively high rates: 74% and 69%, respectively, compared to their much lower emergence rates on milkweed: 9% and 30% respectively (main effect of plant species $\chi^2_{d.f.=1}$= 167.210; P<<0.001). Although American parasitoids emerged at a higher rate than French parasitoids on *A. nerii* overall ($\chi^2_{d.f.=1}$=15.723; P<0.001), this effect appears to be driven by its emergence on milkweed. We found no effect of parasitoid origin on emergence rates on *R. maidis* ($\chi^2_{d.f.=1}$=0.847; P=0.357), which was approximately 83% for both populations. Sex ratios were strongly female biased: between 30-35% of the offspring from both parasitoid populations were male on *R. maidis* and on *A. nerii*. There was no effect of parasitoid origin on sex ratios on *R. maidis* ($\chi^2_{d.f.=1}$=0.0127; P=0.91), nor were there any strongly significant effects of any of our predictors on sex ratios on *A. nerii*. However, there was a marginally significant trend toward more males on French aphids (40%) compared to American aphids (30%) ($\chi^2_{d.f.=1}$=3.337; P=0.0677), and a marginally significant interaction between plant species and parasitoid origin such that there was a greater proportion of male parasitoids from French populations on oleander and a greater proportion male parasitoids from American populations on milkweed ($\chi^2_{d.f.=1}$=2.7880; P=0.095).

Development time and size of offspring

Plant species strongly affected development time for both parasitoid populations, independently of aphid origin (Fig. 4.2; $\chi^2_{d.f.=1}$=58.76, P<<0.001): it took parasitoids an average of 20.4 days to reach adulthood on oleander but just 13.8 days on milkweed. Interestingly, aphid origin also had a small effect: development time was less than a half-day faster on French aphids compared to American aphids (Fig. 4.2; $\chi^2_{d.f.=1}$= 5.292; P=0.0214). We also observed differences in development time across parasitoid populations, although this may be most attributable to temperature differences (up to 4°C) between the rooms in which they were reared. On *A. nerii*, average development time was faster for American parasitoids (16.3 days) than for French parasitoids (18.8 days; Fig. 4.2; $\chi^2_{d.f.=1}$= 39.933; P<<0.001). On *R. maidis*, American parasitoids reached adulthood in an average of 13.3 days, faster than the average of 14.9 days for French parasitoids although this could again be due to temperature differences between the rearing rooms (Fig. 4.2; $F_{1,19}$= 46.07; P<<0.001).

With respect to offspring size, parasitoids emerging from milkweed had hind tibiae that were 30% longer, on average, than those emerging from oleander (Fig. 4.3; $\chi^2_{d.f.=1}$=222.041; P<< 0.001). Furthermore, parasitoid origin modulated the effect of plant species on hind tibia length ($\chi^2_{d.f.=1}$=5.3313; P=0.021). In this case, the pattern was not consistent with local adaptation; instead, French parasitoids appeared to show more plasticity in body size than American parasitoids, generating a pattern of local maladaptation to host plant. Offspring from French parasitoids were larger on milkweed and smaller on oleander than were American parasitoids (Fig. 4.3). As expected, females were bigger than males on both *A. nerii* (Fig. 4.3; $\chi^2_{d.f.=1}$= 49.411; P<<0.001) and *R. maidis* (Fig. 4.3; $\chi^2_{d.f.=1}$=13.232; P<0.0001). Additionally, we observed an interaction between parasitoid offspring sex, plant species, and aphid origin (Three way interaction; $\chi^2_{d.f.=1}$=5.845; P= 0.0156) such that male parasitoids grew bigger on milkweed than on oleander regardless of aphid origin but female parasitoid size depended on an interaction between aphid origin and plant species. Consistent with local adaptation of aphids to their native host plant, females, on average, were larger when they emerged from French rather than American aphids on oleander, but they were slightly larger when they emerged from American rather than French aphids on milkweed. Although we observed no main effect of parasitoid origin on the hind tibia length of offspring emerging from *A. nerii* ($\chi^2_{d.f.=1}$=0.0443; P=0.833). French parasitoids were bigger than American parasitoids on *R. maidis* (Fig. 4.3; $\chi^2_{d.f.=1}$=11.736; P<0.001), with hind tibiae that averaged 6% longer.

Discussion

Local adaptation

For emergence rates, the fraction of parasitized aphid mummies from which adults emerge, our data are consistent with local adaptation of parasitoid population to the host plant in the region of origin. Although the absence of replicated populations in our experiment limits our ability to conclude with certainty that local adaptation is due to divergent selection, we nonetheless consider it likely that *L. testaceipes* populations have evolved in response to trait-mediated indirect interactions with plants for several reasons. First, emergence rates are an intimate component of parasitoid fitness, so we think it likely that this trait is under directional selection in each environment. Additionally, we know of only one introduction of *L. testaceipes* to Europe that occurred about 40 years ago, suggesting that gene flow has not occurred between populations since at least that time. Since the French population was founded from individuals collected on oleander in Cuba (Stary et al. 1988a, Stary et al. 1988b), selection for emergence rates on oleander could have occurred for a much longer period of time. Finally, we observed that the local population emerged better than the foreign population on each plant species. Although the American parasitoid population produced more mummies on each plant, French parasitoids emerged at a slightly higher rate on oleander, and American parasitoids emerged at a higher rate on milkweed, which suggests that the two plants impose divergent selection on parasitoid emergence rates (Kawecki and Ebert 2004). The absence of overall differences in emergence rates between parasitoid populations, as observed on *R. maidis,* facilitates interpretation. The "local vs. foreign" pattern of performance on the more defended *Aphis nerii* is indicative of local adaptation. Even in the absence of population-level replication, our data show that, at the very least, there is variation in emergence rates on each host and a genotype by environment interaction such that the genotypes that emerge best on oleander are not the same genotypes that emerge best on milkweed. We cannot decipher whether the divergence is due to selection or drift, but we suspect both processes may be involved.

Emergence rate was the only trait showing a significant interaction consistent with local adaptation of parasitoids to host plant; however, other traits show divergence suggestive of parasitoid local adaptation to aphid population and aphid local adaptation to host plant. American parasitoids produced more mummies on American aphids than on French aphids, and it is tempting to attribute the lack of a similar home advantage in the French population to the relatively novel association between the French parasitoids and French aphids. At least one study has demonstrated that younger populations of parasites are less locally adapted to their hosts than older populations (Mopper et al. 2000). However, further replication, either in space or time, would make this inference more convincing. Testing with additional populations would allow us to distinguish the effects of overall aphid susceptibility from patterns associated with local adaptation that we simply cannot identify with just two populations (Blanquart et al. 2013). Alternatively, repeated tests of adaptation of the French parasitoids to the local aphid population over time could provide more support for the hypothesis that the French parasitoid population performs equally well on both aphid populations due to its recent introduction. We note that, in our study, female offspring from the French parasitoid populations showed greater plasticity in body size in response to plant species than those from the American population. At the same time, the French populations appear to be less plastic in terms of mummy number on aphids from different populations. This might be adaptive if plasticity in size allows the parasitoid access to a greater range of hosts in a novel environment, and stability in mummy number ensures fitness across those hosts. Evidence from plants supports the idea that invasive species are more plastic than native species (Davidson et al. 2011), and there is some evidence that introduced plant populations are also more plastic than their native counterparts (Bossdorf et al. 2005). Further investigations into the adaptive nature of plasticity in introduced parasitoid populations are warranted.

One challenge with using mummy number to test for local adaptation is that the American parasitoid population always produced more mummies than the French population on both *A. nerii* and *R. maidis*, suggesting differences in the “genetic quality” (sensu Blanquart et al. 2013) of these populations that may mask the pattern of local populations outperforming foreign populations expected under divergent selection. Other studies suggest that inbreeding depression may be an issue any time an introduced population has experienced a genetic bottleneck (Hufbauer and Roderick 2005). For example, introduced populations of the parasitoid *Aphidius ervi* showed reduced neutral genetic variation (Hufbauer et al. 2004) and reduced mummification rates on some aphids (Hufbauer 2002) compared to native populations of the parasitoid, despite relatively large population sizes during introduction. Unfortunately, the pre-mummification effects of parasitoid population in our experiment are confounded with the different rearing rooms. Although the rooms were next to each other, we noticed temperature differences between the rooms that were likely sufficient to affect development times (Royer et al. 2001; Hughes et al. 2011); however, we do not expect temperatures in this range to affect the functional response (Jones et al. 2003), the fecundity of females (van Steenis 1994), or the sex ratios of the offspring (Royer et al. 2001) for *Lysiphlebus testaceipes*. We can conclude that either differences between the rooms or genetic differences between the populations resulted in lower mummification rates in assays with the French population of *L. testaceipes.*

Two traits provided indirect evidence consistent with local adaptation of aphid populations to the host plant in their region of origin. We did not directly measure aphid performance; however, development of aphid parasitoids is intimately tied to aphid growth (Cohen et al. 2005, Henry et al. 2006). Consequently, parasitoid performance measures such as mummy number, development time, and offspring size are influenced not only by parasitoid adaptations, but also by aphid fitness and growth. For these measures of parasitoid performance, we interpret an appropriate interaction between plant species and aphid population as indirect evidence consistent with adaptation of aphid populations to their local host plant. Based on the patterns of interactions we observed for parasitoid mummy number and female parasitoid offspring size, parasitoids from both populations showed a trend for higher performance on a given host plant when the aphid population was local to the plant. This may be one of the first studies to generate indirect evidence for local adaptation, and our results demonstrate the implications of excluding plant species from a study of local adaptation of parasitoids to aphid populations. For example, if we had conducted our study only on milkweed plants, not only would we have missed the evidence for local adaptation of parasitoids to plants, but we would also have inferred that American aphids produce bigger females, which is true only on milkweed.

Host-Parasite coevolution

Together, our results suggest that aphids are locally adapted to their host plants while the relationship for parasitoids is more complicated. We have better evidence for parasitoid adaptation to local host plant than we do for parasitoid adaptation to local aphid population. Time may be the best explanation for these patterns. *A. nerii* has a cosmopolitan distribution. Various references place its origin in east Asia (Foottit et al.

2006), the Mediterranean (Harrison et al. 2011), or Europe (Essig 1958); however, it has been in the United States since at least 1879 (Foottit et al 2006). Consequently, the aphid populations have likely been evolving on different hosts for well over a century. If we account for the availability of oleander in Cuba, where the French population of parasitoids originated, the two parasitoid populations have also been exposed to different host plants for a long time. However, the association between the French parasitoids and the French aphid population is novel since the introduction. Additional factors could also contribute to the differences in the nature of local adaptation of aphids and parasitoids. First, more host specific parasites are more likely to be locally adapted to their hosts (Lajeunesse and Forbes 2002), and *A. nerii* may be more host specific than *L. testaceipes*. Additionally, the relative rate of insect evolution is likely to be faster than the relative rate of plant evolution (as described for parasites and hosts in Kawecki and Ebert 2004), which may help to explain the pattern of aphid and parasitoid local adaptation to plant species. If the rate of parasitoid evolution does not greatly exceed that of aphids, this could limit the potential for local adaptation in coevolving populations. Finally, the plant species used in our study are presumably separated by much greater phylogenetic distance than the aphid populations in our study, so selection across plants could vary more than across aphids.

By comparing studies of local adaptation in similar aphid-parasitoid systems, we can consider the role of factors like time, host specificity, evolutionary rate, and phylogenetic distance in local adaptation more generally. In the pea aphid-parasitoid system, where aphid local adaptation to host plant was directly assayed (Via 1991), aphids are also more locally adapted to plants than parasitoids are to aphids. Pea aphid populations adapted to different plants are differentially susceptible to parasitism by *Aphidius ervi*, but these effects are apparently mediated by a heritable mechanism of physiological resistance and not by host plants or behavior (Hufbauer and Via 1999). *A. ervi* was introduced into the United States from Europe for biological control but shows no local adaptation to aphid populations on different host plants within the United States (Hufbauer 2001), in France, or between the United States and France (Hufbauer 2002). In contrast to the lack of local adaptation to populations of the same aphid species on different host plants, several studies have documented adaptation of parasitoids to locally abundant aphid species (Antolin et al. 2006), including *A. ervi* (Henry et al. 2010, Emelianov et al. 2011). These patterns suggest that, at least for *A. ervi*, phylogenetic distance between hosts may greatly facilitate local adaptation; whereas, even the indigenous French populations showed no local adaptation to pea aphids on different crops, despite a potentially long history of coevolution.

Consistent with evidence for local adaptation to different host species, *A. ervi,* long thought of as a generalist species, may actually be a complex of variously divergent host races (Emelianov 2011). However, there is no evidence for host-plant associated divergence, as shown in its pea aphid hosts (Bilodeau et al. 2013). In other parasitoid species, host-plant associated divergence has been absent (Lozier et al. 2009) or supported (Stireman et al. 2006, Forbes et al. 2009). For *Lysiphlebus testaceipes,* host-plant associated divergence has not been tested, but European populations are structured with geographic distance but not aphid host species (Mitrovic et al. 2013). This suggests that geographic separation between our populations may have been a prerequisite for the pattern of local adaptation to plant species that we observed.

Recent research has shown that facultative endosymbionts in aphids mediate interactions with host plants and parasitoids (reviewed in Oliver et al. 2010) and may contribute to the evolution of local adaptation in aphids. Endosymbionts, however, may also contribute to the coevolutionary responses of plants and parasitoids. Interestingly, the parasitoid *Lysiphlebus fabarum* has the potential to rapidly evolve local adaptation to particular genotypes of an endosymbiont in aphids (Rouchet and Vorburger 2014). This raises the question of whether and when phylogenetic distance corresponds to trait divergence in ecologically relevant ways.

Notably, we found no patterns consistent with local maladaptation of a consumer to its resource, which suggests that in this tri-trophic system, the consumers are "winning" the diffuse coevolutionary interactions. The only case of local maladaptation we observed was that, for each plant studied, parasitoid offspring from the novel population were bigger than those from the local population. We do not expect local maladaptation to be a result of a coevolutionary arms race in this indirect interaction;

instead, we speculate that the appearance of local maladaptation is related to differences in plasticity of the two parasitoid populations. Specifically, we suspect that French parasitoids, more than American parasitoids, are affected by changes in aphid growth mediated by host plant, although this hypothesis requires further testing. We also found no evidence that French parasitoid populations performed better on *A. nerii* than American populations did, so we have no support for the idea that maladaptive gene flow constrains the evolution of *L. testaceipes* performance on *A. nerii.*

Indirect effects and Evolution

We found strong main effects of plant species on mummy number, emergence rates, development time, and offspring size, but we did not find similar main effects of aphid origin. Thus, in contrast to the pea aphid system (Hufbauer and Via 1999), plant-mediated effects on parasitoids of *A. nerii* appear to be more important than physiological resistance for local adaptation. However, aphid resistance might mediate the aphid x parasitoid interaction we observed for mummy number. Despite the clear effect of plants on parasitoids, the pattern of effects is somewhat puzzling. As expected, the putatively more toxic plant, oleander, negatively affected most parasitoid performance traits: mummification rates were lower, development time was longer, and offspring size was smaller. However, emergence rates were surprisingly higher on oleander, suggesting that plant traits affect parasitoids differently at different life stages. Most research on local adaptation in parasitoids focuses on mummification rates. For example, in *A. ervi*, emergence rates show little genetic variation, but mummification rates are much more variable (Hufbauer and Via 1999, Henry et al. 2008). Aphid defenses against parasitism are likely to have a stronger influence on mummification rate because this reflects the survival of the parasitoid larva during the time the aphid is alive; however, plant-derived defense traits may affect parasitoid development even after the aphid has died. It is possible that compounds from oleander have a more immediate toxic effect on parasitoid larvae, but that those that mummify have a high probability of successfully developing. In contrast, milkweed compounds may confer a low-level but chronically toxic environment that impacts parasitoid development over time. However, these patterns clearly require more investigation to understand, including measurements of the cardenolides sequestered in aphids on each plant and serial dissections to track the development of parasitoid larvae inside aphids on each plant.

One of the challenges of studying trait-mediated indirect local adaptation is that effects may be propagated through the system in a number of ways. We anticipated that plant species would generate trait-mediated changes in aphids that would generate selection on parasitoids. It appears plant species generated at least two types of changes in aphids that affected parasitoid performance and potentially local adaptation. First, plant species has been shown to affect the population growth rates of *A. nerii* (Agrawal 2004, Mooney et al. 2010). In our study, both aphid populations appeared to grow at a lower rate on oleander than on milkweed. If plant species also affected the growth rate, size or survival of individual aphids, these effects likely contributed to the differences in mummy number, development time, and offspring size observed between plants. At the same time, if aphids sequester different compounds from different plants, we might expect these compounds to affect any of those parasitoid traits, as well as emergence rates, without affecting aphid fitness. We cannot tease apart the relative contribution of effects mediated by aphid fitness and effects mediated by sequestered compounds on any of the traits we observed. However, we suspect sequestered compounds to be primarily responsible for differences in emergence rates since aphids are not alive after the point of mummification. This may help to explain why we only found a pattern of parasitoid local adaptation to plant species for emergence rates.

Conclusion

We know of just two other studies demonstrating evidence consistent with local adaptation of native and introduced populations of biological control agents to their local hosts (described in Hufbauer and Roderick 2005), and this is the first, to our knowledge, that demonstrates local adaptation to plant species. In fact, this is one of relatively few studies to examine the role of a third player on the evolution of a pairwise interaction (Biere and Tack 2013, Walsh 2013), especially in a tri-trophic context. Additionally, we have shown that introduced populations of *L. testaceipes* show reduced performance on at least two aphid species. In cases where replicating demes is impractical, we demonstrate the value of assaying parasite populations on a common host as a way to distinguish differences in the overall performance of each population. While differences in habitat quality are frequently distinguishable, knowledge of differences in population performance on a common host can point to effects of alternate processes, like genetic drift and inbreeding that can interact with divergent selection to obscure a local vs. foreign pattern of local adaptation. Together with other studies (Cory and Myers 2004, de Roode et al. 2011), our data demonstrate that plants can and do affect local adaptation of parasitoids, predators, or pathogens. The next steps will be to understand how common these evolutionary responses are and under what conditions they are likely to occur. Importantly, when populations are adapted to traits induced by other organisms or environmental contexts, we may fail to find evidence of local adaptation when assays are performed in an artificial context (see Nuismer and Gandon 2008). This is one commonly overlooked explanation for failure to find evidence of local adaptation.

Based on our results and theory of local adaptation, we expect local adaptation in response to trait-mediated indirect interactions to occur when the relative rate of evolution in the target species occurs quickly enough that the indirect interaction remains relatively stable over time, and when indirect interactions provide strongly divergent selection pressures. Plant traits may frequently modify herbivore traits in a way that meets these criteria. Evolution of predators, parasitoids, and pathogens is one mechanism by which the lethal plant defense paradox can be resolved. Our work in the milkweed system has shown that plant traits do affect the preference and performance of some predators (Chapter 3). However, plant effects on predators may be less important than plant tolerance of herbivory in mediating plant responses to top-down effects (Mooney et al. 2010, Chapter 2). This may be in part because some predators have evolved tolerance of plant-derived defense traits in herbivorous prey. This study suggests that for *L. testaceipes*, adaptation to plant traits can and has occurred.

Acknowledgements

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Figures

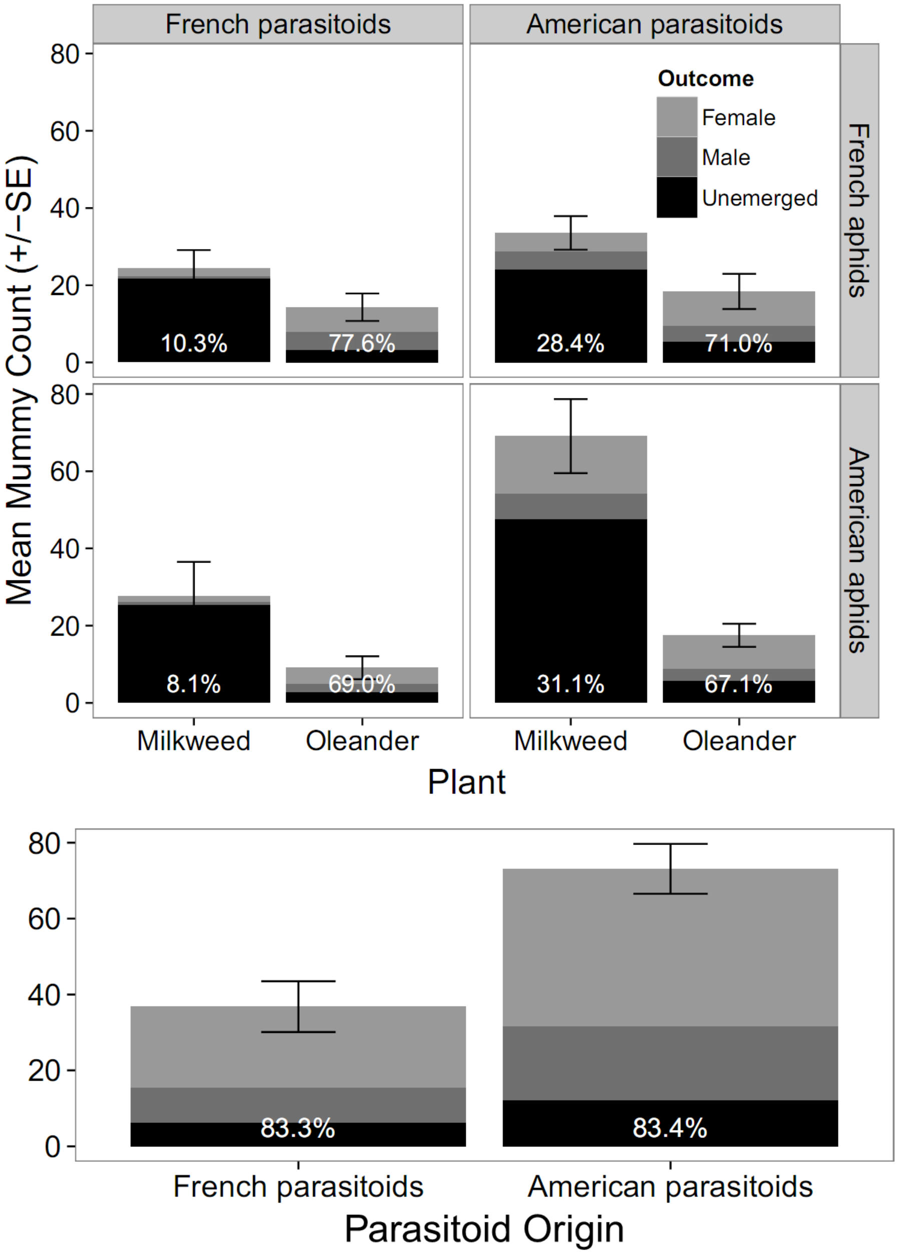

Figure 4.1. Mummy number and emergence rates. Bar height represents the mean mummy count (+/- SE) of each parasitoid population. Each bar is divided to indicate the proportion of mummies in each treatment that produced adult females, adult males, or remained unemerged. Values on each bar indicate the emergence rate: the percent of mummies from which adults emerged across all replicates within the treatment. Shown are data for parasitoid performance on *A. nerii* as a function of plant species and aphid origin (top) on *R. maidis* (bottom).

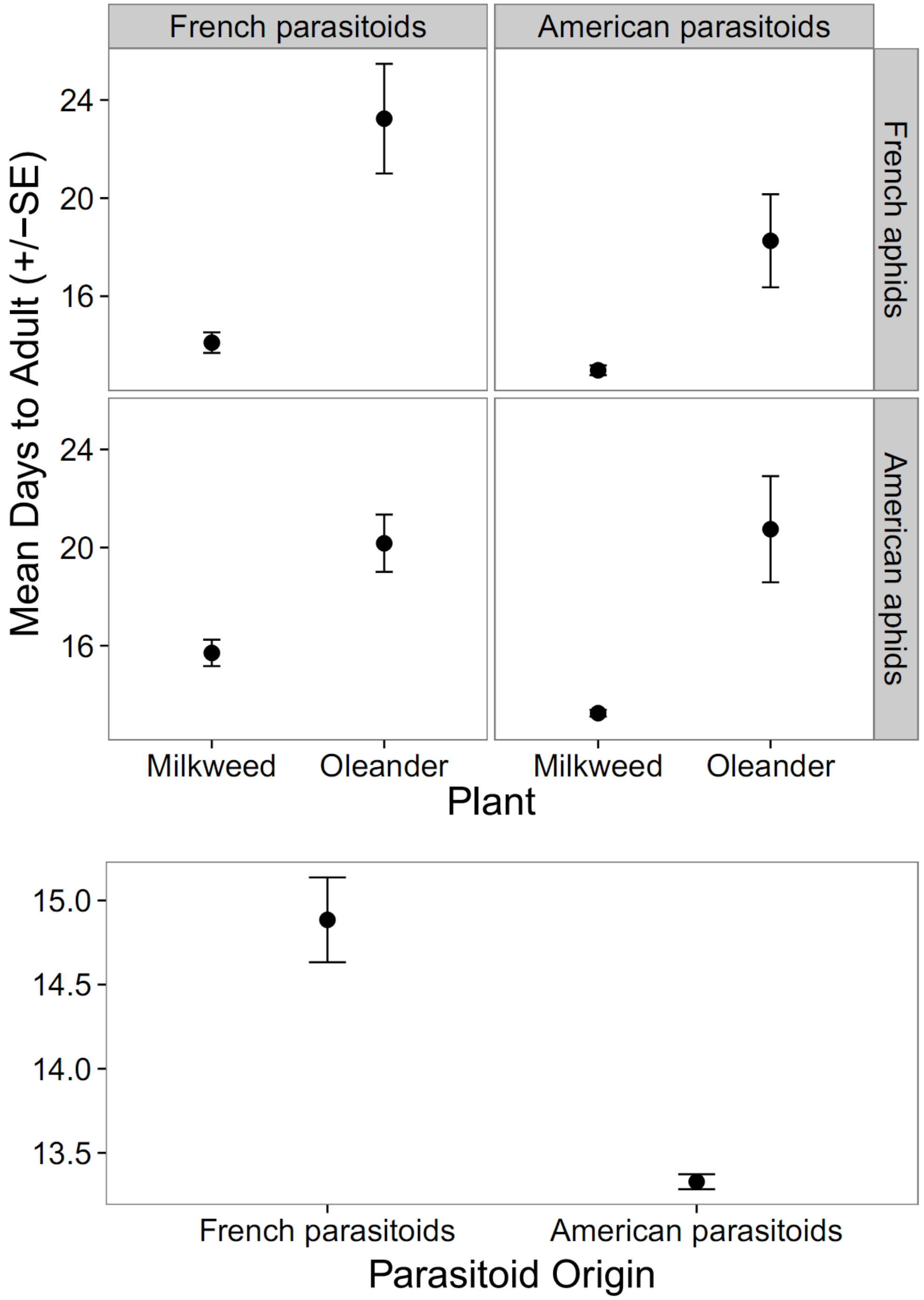

Figure 4.2. Development time. Points show the mean days to adulthood (+/- SE) for parasitoids from each population developing on different populations of *A. nerii* on milkweed and oleander (top) and on *R. maidis* (bottom). Values are means across the average development time for individuals in each replicate.

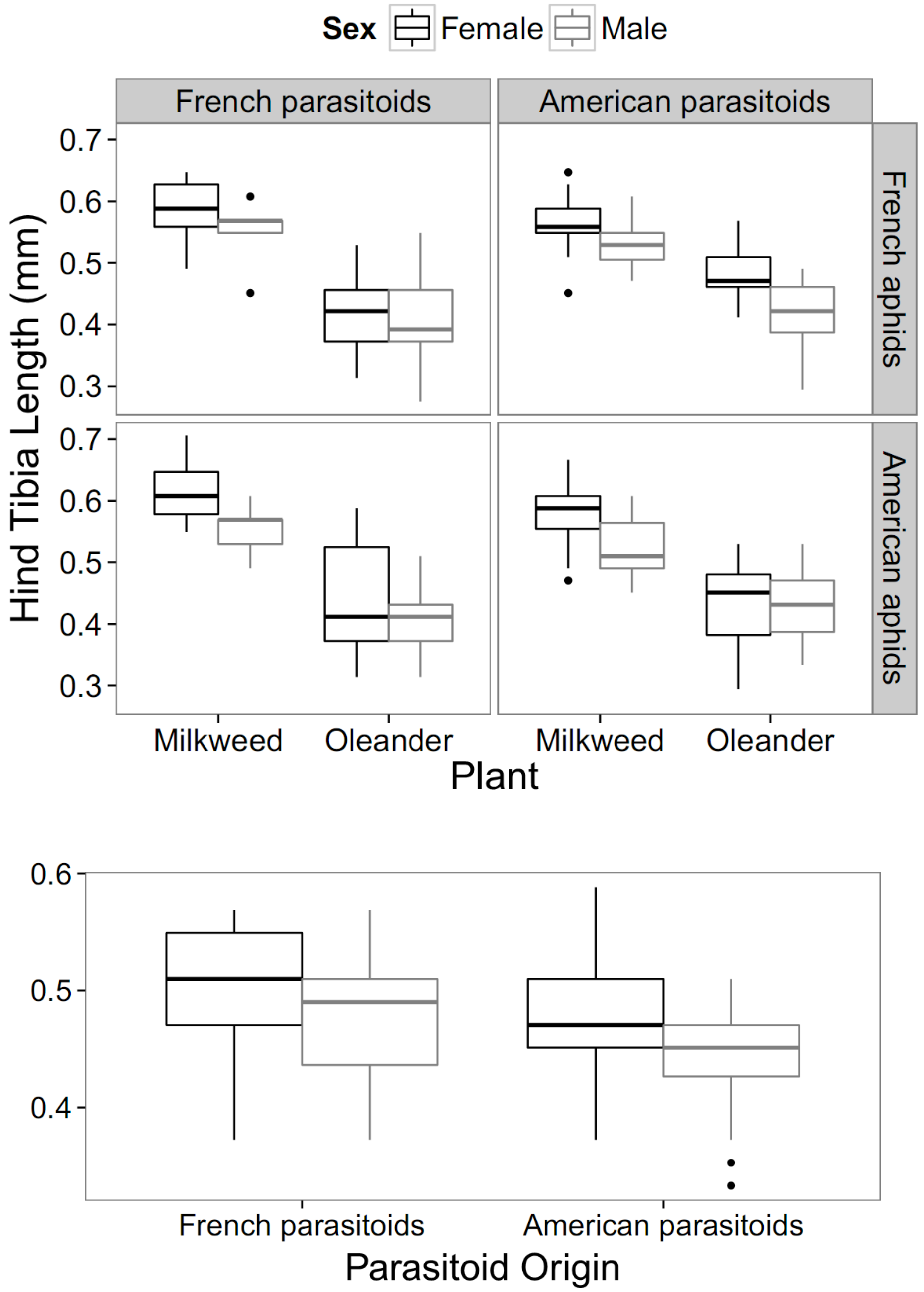

Figure 4.3. Offspring size. The distribution of hind tibia lengths, a proxy for parasitoid size, for adult parasitoids of both sexes from each population. Boxes show the interquartile range and median of the distribution; "whiskers" show the range. Shown are the size distributions of adults emerging from *A. nerii* as a function of aphid origin and host plant species (top), and from *R. maidis* (bottom).

Appendix A: Analysis of emergence rate data including outliers

For our analysis of the effects of parasitoid origin on emergence rates on *R. maidis*, we excluded three replicates from the American population because the adult parasitoids escaped from their cells in the 96-well plates, precluding an accurate measurement of emergence rate. These three replicates all had low emergence rates compared to the range of measured values. Including these replicates lowers the average emergence rate for American parasitoids from 83% to 75% and results in a significant effect of parasitoid origin on emergence rate ($\chi^2_{d.f.=1}$=4.3502; P=0.037). We believe these three points are outliers that are not representative of the performance of parasitoids from American populations. However, if it is true that the French population emerges better than the American population on *R. maidis*, then we have a pattern of crossing reaction norms because the American population emerges better on *A. nerii*. At this time, we have no good explanation for this pattern.

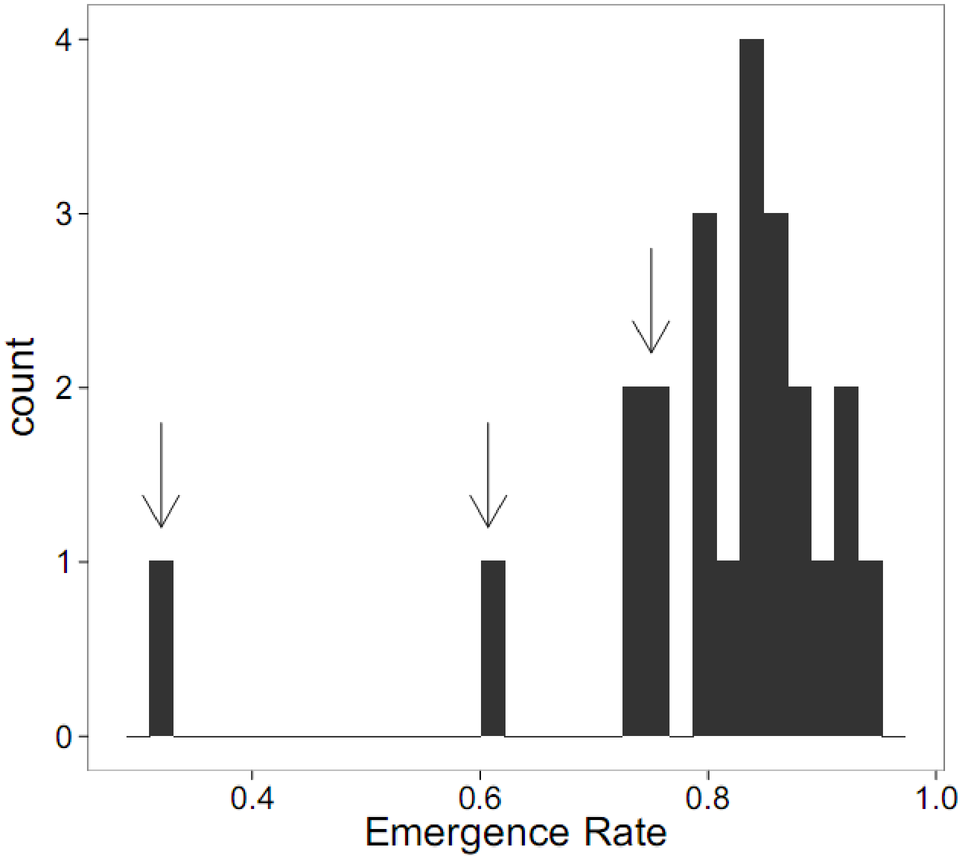

Figure 4SA1. Histogram of the distribution of emergence rates in trials on *R. maidis*. The arrows indicate the values of the three points excluded from the analysis in the main text. The excluded replicates were all from the American parasitoid population, and in each case the adults escaped the wells in the 96-well plates, precluding an accurate count of adults necessary to calculate emergence rate.

Conclusion

One of the fundamental premises of natural selection is that the ecological context in which a species lives and interacts affects the relationship between traits and fitness. Recently, explanations for variation in plant defense have expanded from focusing primarily on models of resource allocation to models that allow for ecological costs of defense traits. My dissertation research begins to test some of the assumptions and predictions of a model for variation in optimal defense that incorporates a multi-trophic perspective.

Given appropriate curvature in one of the defense curves for herbivory, growth, or predation, variation in optimal defense can result from variation in predation pressure or the relative susceptibility of herbivores and predators in the tri-trophic model of optimal defense. I used the aposematic sequestering specialist aphid, *Aphis nerii*, as the focal species in my investigations. *A. nerii* grows on a variety of plants in the Apocynaceae, including milkweeds (*Asclepias* spp.), that have been shown to vary in their defense traits and investment (Agrawal 2004; Agrawal and Fishbein 2006). At the same time, it is attacked and consumed by a variety of polyphagous insect predators. I expected the choice of a sequestering specialist herbivore consumed by generalist predators to maximize the potential for ecological costs of defense to become apparent; however, the benefits of defense in this context are questionable. If plant defenses disrupt predation, I predicted that more defended phenotypes should have higher fitness in the absence of predators and less defended phenotypes should have higher fitness in the presence of predators.

In the trophic cascade study, we found that the putatively less-defended species always grew more and the putatively more-defended species always had a higher rate of survival. Unfortunately, we do not have an integrated measure of fitness for the plants in this study. However, predator exclusion always had a greater magnitude of effect for the putatively less-defended *A. incarnata*, suggesting that it benefitted more from predators. Additionally, the predator *Aphidoletes aphidimyza* preferred and performed better on *A. incarnata* than *A. syriaca* in both the laboratory and the field. These patterns were consistent with my predictions; however, a closer inspection of the data revealed that the effects of predators on aphids did not reliably differ across plant species. Consequently, we cannot conclude that a pattern consistent with our predictions was generated by the mechanism we expected. Instead, we inferred that differential tolerance of aphid herbivory may explain the differential benefits of predation between the two milkweed species studied. Thus, like other authors (Carmona et al. 2011), I conclude that plant traits other than secondary compounds may be more important for plant fitness in the face of herbivory. In a separate study, we found patterns consistent with adaptation of aphid parasitoid populations to the local host plant in the region of origin. We interpret these patterns to mean that natural enemies of herbivores have the potential to evolve in response to plant-derived defense traits. Such a pattern suggests that the ecological costs of secondary compounds in tri-trophic interactions will decline with the evolution of tolerance in predator populations.

Altogether, we have limited evidence that milkweeds incur significant ecological costs through reduced predation. Instead, our evidence demonstrates that predators and plants both have the potential to tolerate *A. nerii* to some degree. We interpret this to mean that the "lethal plant defense paradox" may not be a stable phenomenon. Nonetheless, we do find considerable evidence that differences between plant species affect the preference and/or performance of aphid natural enemies, and that aphids can strongly affect plant growth and survival. Consequently, the lethal plant defense paradox may be an important, if temporary, phenomenon during some phases of coevolutionary interactions. For example, the tri-trophic niche concept argues that predators associated with specific habitats or host plants promote the diversification of herbivorous insects, which find enemy free space on novel host plants, especially those with novel chemical compositions (Singer and Stireman 2005). In this theory, plant defenses have ecological costs when herbivores shift to plants with novel defenses to which relatively specialized predators or parasitoids are not yet adapted. Diet expansion or diversification of the third trophic level would be expected to follow host shifts by herbivores, emphasizing the temporary nature of the ecological costs of plant defense in a tri-trophic context.

However, the tri-trophic niche concept does not specifically address predictions about plant evolution in response to herbivores and predators.

I hypothesize that the evolution of tolerance in both plants and natural enemies is consistent with one or more coevolutionary processes (Thompson 2005). In classic "escape and radiate" theory, novel or increased chemistry in plants allow initial escape from herbivores, facilitating diversification of plants through some unknown process (Futuyma and Agrawal 2009). Suppose a plant species acquires a novel chemical in a tri-trophic context, and an herbivore population specializes on the novel defense, facilitated by enemy free space. This is the stage during which the lethal plant defense paradox should be important. If plants with higher concentrations of the chemical experience less herbivory, coevolutionary escalation may occur between the newly specialized herbivore and the plant, especially if producing the chemical has limited fitness costs. In this scenario, predation must be either unaffected by the chemical or the defense must be effective enough that predation does not benefit the plant. In a coevolving polymorphism scenario, the herbivore is so well adapted to the defense that it is not effective, and negative frequency dependent selection should favor other defense types until the well-adapted herbivore becomes rare. In a coevolving polymorphism, a defense trait that disrupts predation should impose ecological costs that increase the strength of selection against the common type and the frequency of cycling. This in turn may reduce the potential for predators to evolve tolerance of the specific plant trait. Alternatively, if the novel defense trait coexists with tolerance for the specialist herbivore, the evolution of increased tolerance in the plant should allow increased herbivore abundance but reduce the costs of the defense trait and the frequency of cycling. As the herbivore becomes more abundant, this should increase selection on predator populations to evolve tolerance of the novel plant trait. Selection on plants may favor reduced concentrations of the defensive chemical to mitigate ecological or allocation costs, especially if low concentrations effectively limit herbivory by non-specialized herbivores. The evolution of reduced defenses and increased tolerance of herbivory both appear to have occurred during the diversification of milkweeds (Agrawal and Fishbein 2008). Depending on the predator population structure, predators may then specialize, as the herbivore did, or they may function as generalists, attacking a range of hosts. As predators suppress herbivore populations, selection for tolerance of herbivory in the plants, and tolerance of the plant trait in generalist predators, may be relaxed. Although these changes would be expected to have different effects on herbivore population size, they would both result in a greater impact of herbivory on plant fitness, potentially favoring novel defenses. If secondary chemistry does not predict susceptibility of plants to herbivory in the long term (Carmona et al. 2011), it may be an important temporary component of coevolutionary diversification.

Below I further discuss my dissertation research in light of the tri-trophic model of optimal defense, identify areas requiring further research, and consider new research questions in light of the potential role of tri-trophic interactions in coevolutionary dynamics.

Plants and the tri-trophic model of optimal defense

Although our results do not support the idea that plant defenses impose sustained ecological costs via reduced predation, our research does uphold some of the assumptions of the tri-trophic model of optimal defense. The trophic cascade study confirms that aphid abundance and density are reduced by allowing predators to access plants, and that the effects of predator exclusion cascade down to affect plant growth and survival. From field data and laboratory studies, we have shown that plant species affects the preference and performance of the predator *Aphidoletes aphidimyza* such that both appear to be reduced on the putatively more defended milkweed species. These studies are limited in that we are comparing just two species that vary in many traits, including defenses. Additionally, we have not been able to show that these effects translate into differences in aphid suppression across plant species. It appears that the effects of plant species on aphid natural enemies do not correspond directly with their effects on aphids or plants, and this type of non-additivity in interactions suggests we should use caution when making inferences about indirect effects based on evidence of specific direct effects.

Evidence for other model assumptions is less consistent. We do not have convincing evidence from our studies that plant defenses reduce herbivory (aphid density) or plant growth. Other studies in the milkweed system have found that growth and defense are negatively correlated across species (Mooney et al 2010), or uncorrelated (Agrawal and Fishbein 2008), or that the tradeoff is found in the absence but not the presence of arbuscular mychorrizal fungi (Vannette et al. 2013). Additionally, studies report that milkweed defenses reduce herbivore abundance and monarch larvae growth (Agrawal 2005a) as well as aphid population growth rates (Agrawal 2004). These studies suggest that plant defenses may be beneficial, even in the presence of sequestering specialists like *A. nerii*. However, the shapes of the defense curves and the net effects of these processes on plant growth or fitness are areas of potential future study.

The choice to study aphid herbivores makes it difficult to determine whether herbivory removes a proportion or a constant amount of plant biomass because leaf area removal is not a valid measure. Furthermore, the fact that we added a constant number of aphids to all plants initially removes the natural colonization process. Nevertheless, if there is a positive relationship between aphid number and the amount of biomass consumed, then our data from the trophic cascade experiment demonstrate that herbivory is unlikely to represent a constant amount of plant biomass over the whole season. At the end of 10 weeks in that study, cumulative leaf days was a significant predictor of cumulative aphid days, demonstrating that plant growth has a positive effect on aphid populations. However, the relationship is affected by species and predator exclusion treatment and appears to change over time. After five weeks, cumulative leaf number was only a significant predictor of cumulative aphid days for *A. incarnata* in predator-exclusion cages (Chapter 2, Appendix F). Together, our data suggest that there may be conditions under which it is true that aphids consume a constant amount of plant biomass, for example early in the season on *A. syriaca* or in the presence of predators. However, we need to investigate whether these results hold when herbivores are allowed choices about which plants to colonize. Ultimately, it is unlikely that this assumption holds under all conditions. We need to further investigate the consequences of relaxing this assumption for variation in optimal defense under both the resource availability model and the tri-trophic model of plant defense.

In the resource availability model of plant defense, faster growing plants have lower optimal defense because herbivory reduces growth by a constant amount. This result is typically interpreted to mean that faster growing plants, which evolved in more resource rich environments, have short leaf lifetimes and fast leaf turnover, traits which allow these plants to tolerate herbivory and reduce the need for defenses. However, our data suggest that the faster-growing *A. incarnata* is less tolerant of aphid herbivory than *A. syriaca*. There are several hypotheses that could explain this result. First, there may not be a correlation between tolerance to chewing herbivores, tolerance of clipping, and tolerance of aphid herbivory. Although several studies have shown that milkweed species and genotypes vary in their ability to tolerate clipping (Hochwender et al. 2000, Agrawal and Fishbein 2008), only one other study links tolerance to aphid herbivory. Mooney et al. (2010) also indirectly inferred that those milkweed species that respond most to fertilization, which are also the faster growing species, are less tolerant of aphid herbivory. Thus it is possible that the relationship between growth rate and tolerance to aphid herbivory is opposite that predicted for growth rate and tolerance under the resource availability model. Alternatively, for milkweeds, root:shoot allocation across species or genotypes may predict both tolerance to herbivory (Hochwender et al. 2000, Agrawal and Fishbein 2008) and growth rate. Under this model, species or genotypes which allocate more to roots should be both slower growing and more tolerant of above-ground herbivory in general, although they may be less tolerant of below-ground herbivory. In this case, slow growth rates could be associated with greater defense, as in the resource availability model, if tolerance and defense are effective against different types of consumers (Nunez-Farfan et al. 2007), or operate at different life history stages (Boege et al. 2007).

We lack clarity about the traits and mechanisms that confer tolerance of herbivory (Tiffin 2000, Fornoni 2011), which constrains our ability to predict when tolerance will be expressed. Whereas the resource allocation model of plant defense predicts fixed traits associated with fast growth to confer tolerance, different types of resource limitation may also affect the ability of plants to tolerate different types herbivory (Wise and Abrahamson 2005). Under this model, traits that affect which resources limit a plant interact with the environment to determine tolerance. However, as with other types of defenses, tolerance may also involve induced responses. For example, *A. syriaca* has been shown to increase nitrogen allocation to stems in response to root and leaf herbivory (Tao and Hunter 2013) and clipping damage results in lower root:shoot ratios than observed (Bryant et al. 1983) in control plants (Hochwender et al. 2000), suggesting re-allocation of resources in response to damage. When plants have induced tolerance responses, traits that affect resource storage and allocation may alter the predictions of other models. Thus slow growing species that store resources may be inherently growth-limited, but they may also tolerate leaf herbivory better than fast growing species with "cheaper" tissues. Additionally, species or genotypes might differ in their innate root:shoot allocation and in their induced tolerance responses, meaning that neither measure alone will be a sufficient predictor of tolerance to herbivory. Furthermore, physiological responses to low nutrient conditions may pleiotropically facilitate tolerance to herbivory. In *A. syriaca*, allocation to root tissues correlated with better tolerance of clipping and greater fitness when damaged under low nutrient conditions, but growth rate predicted fitness when damaged under high nutrient conditions (Hochwender et al. 2000). Although it would have been interesting to determine whether the genotypes that grew the fastest under high nutrient conditions were also the least tolerant under low nutrient conditions, there was evidence for a tradeoff between growth and tolerance under low nutrient conditions (Hochwender et al. 2000). Given the complex pathways to expression of tolerance, and the mixed support for simple tradeoffs between tolerance and resistance (Leimu and Koricheva 2006, Nunez-Farfan et al. 2007), the role of tolerance in the patterns predicted by the resource availability hypothesis deserves careful examination. If faster-growing plants are not always the most tolerant of herbivory, we may need to refine our explanations for growth-defense tradeoffs.

It seems that there is good support for a tradeoff between growth and differentiation (Herms and Mattson 1992), leading to a common trade-off axis of associated leaf traits (Wright et al. 2004) and support for growth-defense tradeoff across species (Coley et al. 1985, Fine et al. 2006). Such a trade-off is analogous to a powerefficiency tradeoff described for some aquatic grazers in which growth rates in low resource environments trade off with higher maximal growth rates under resource rich conditions (Tessier et al. 2000), although the mechanisms behind the tradeoff are likely to be different. If slower growing plants are also longer lived, then the risk of herbivory may be greater, increasing the value of both defense and tolerance. Perhaps what we now need to better understand is how various traits and processes involved in growth or differentiation interact and tradeoff. Do plants with chemical defenses also evolve tolerance in response to ecological costs imposed by specialist herbivores? Is tolerance specific to different types of herbivores, or is there a correlated response to all herbivores? Is the growth-defense tradeoff in milkweeds explained by different mechanisms than that observed for tropical trees? If so, is that due to different growth forms of the plants, to different limiting resources, to different herbivores studied, or to some combination of these factors?

Herbivores

I chose to study *A. nerii* because its host range and susceptibility to predation allowed me to test for effects of different plant species on the preference and performance of aphid natural enemies. However, *A. nerii* is thought to have been introduced into North America, the source of most of the milkweed and natural enemy populations used in my studies. With well over 100 years since the first records of *A. nerii* in North America (Foottit et al. 2006), these populations have had time to evolve in response to each other. Nevertheless, introduced species often lose host specific natural enemies, and *A. nerii* in our studies was attacked primarily by generalist aphid predators. Consequently, we predicted ecological costs to be particularly great for plants attacked by *A. nerii*. Instead, ecological costs seemed to have been tempered by the ability of milkweeds to tolerate some degree of aphid herbivory and by a mismatch between predator abundance and effectiveness across plants in my study. My inferences are limited in that my experiments reduced the herbivore community to a single species, rendering me unable to detect benefits or ecological costs of plant traits that are mediated through interactions with different herbivores. Nevertheless, failure to observe strong evidence for ecological costs with *A. nerii* suggests that the tri-trophic model of plant defense may have limited applicability, at least in milkweeds.

Although *A. nerii* now has a cosmopolitan distribution, it was probably not present during the radiation and diversification of the milkweeds. Furthermore, its abundance is highly variable across years and some authors identify herbivorous beetles as the primary drivers of selection on milkweeds (Agrawal 2005a). Consequently, *A. nerii* was most likely not the sole or even primary selective agent in the evolution of milkweed defense across species. Nevertheless, it is one of a suite of herbivores that can tolerate and even sequester toxic cardenolides. Although its feeding mode may be different, its ecological interactions may not be so different from other herbivores that escape predation by sequestering defenses. For example, evidence that more toxic milkweeds reduce the virulence of pathogens of monarch larvae (de Roode et al. 2008) suggests that ecological costs of defense may be expressed across multiple herbivores. This means that *A. nerii* may be a follower rather than a driver of defense evolution in milkweeds, benefitting from the tolerance that some milkweeds have evolved in response to specialist herbivores. It will be important to investigate the specificity of tolerance and the ecological costs of defense when milkweeds are exposed to the beetles that are putative drivers of evolution in milkweeds in order to determine the degree to which ecological costs and the evolution of tolerance may be general across feeding guilds. Additionally, the longhorn beetles in the genus *Tetraopes* are thought to have diversified in relatively tight correspondence with milkweed species (Farrell and Mitter 1998), suggesting a potentially tight coevolutionary relationship. It is possible that the introduction of aphids could disrupt such tight coevolutionary interactions, a question which is also worthy of further research.

From our reciprocal transplant study, there is indirect evidence that *A. nerii* may be locally adapted to their host plants, but this finding awaits confirmation using more direct measures. If aphids do demonstrate local adaptation to milkweed species, then there is potential to investigate the role of predation on the degree of local adaptation among populations of aphids and milkweeds. Such studies would shed light on the role of enemy free space in specialization of herbivores on host plants, a question that has limited support (Stamp 2001). The existence of local adaptation among populations of highly mobile herbivores would also raise questions about the patterns and mechanisms of aphid preference for different host plants.

Predators

Although I found that the milkweed species I studied affected the abundance, preference, and performance of predators, I was unable to demonstrate that predators had different effects on aphid suppression across the two plant species. The fact that predator effects on aphids were uncorrelated with predator effects on plants in a similar study across 15 milkweed species (Mooney et al. 2010) suggests that plants may affect predators, but that these effects do not drive trophic cascades. However, it is an important result to confirm given that it drives my inferences about plant tolerance. The most abundant predator in our field studies, *Aphidoletes aphidimyza*, also showed strong responses to plant species. However, other predator species, including the introduced Asian ladybeetle *Harmonia axyridis*, may have been more important for aphid suppression. Given its invasive status, it would be interesting to investigate whether *H. axyridis* is indeed the most effective predator of *A. nerii*, and to evaluate its preference of and performance on different host plant species. Unlike the parasitoid, *Lysiphlebus testaceipes*, which is native to North America and had the potential to be exposed to plant cues from milkweeds prior to its association with *A. nerii*, *H. axyridis* may be truly naïve to the plants, but not the aphids, prior to the introduction. While most of the predators found attacking *A. nerii* in our studies are considered aphid generalists, it would be worth investigating their preference hierarchies or avoidance responses among milkweeds and other host plants to determine if there is a relationship between toxicity and preference. Theory of coevolutionary alternation posits that consumers will preferentially attack the least defended of the prey types, and that this will select for evolution of increased defense in those prey populations (Thompson 2005). If *A. nerii* derives its defenses from host plants, and if predators use host plant cues when foraging, then it is possible that predator preferences may exert selection on plant defenses and/or aphid preferences or sequestration patterns.

We found evidence consistent with local adaptation of parasitoid populations to the host plant common in their region of origin. While this evidence suggests that parasitoid populations can evolve in response to host plants, it is also notable that parasitoids always performed less well on *A. nerii* than they did on an alternate aphid host, *R. maidis*. Both the preference and the performance of a consumer on different hosts impact its diet breadth, and understanding the factors that constrain the evolution of parasitoid performance on *A. nerii* is necessary in order to identify the full potential for predator evolution to resolve the lethal plant defense paradox. Additionally, as we better understand the community of predators attacking *A. nerii*, it will be useful to test whether there are qualities of aphid natural enemies that predict their sensitivity to host plant traits. At a more mechanistic level, the pattern of higher parasitoid emergence rates on the putatively highly toxic oleander plants remains a puzzle still requiring explanation. Although evidence suggests that there is a positive but saturating relationship between concentrations of toxic cardenolides in plants and aphids (Malcolm 1990), this would be worth confirming with the species used in our studies.

Just as predators may evolve in response to plant traits, predators may also exert selection indirectly on plants. Under the tri-trophic model of plant defense, lower levels of defenses should be favored when predators are more susceptible and/or more common. However, among the milkweeds, where variation in top-down effects may be determined more by plant tolerance than by defenses, our predictions change. When predators are more abundant, we would expect reduced selection for plant tolerance to herbivory. Anthropogenic changes that fragment habitats and threaten top predators are changing ecological dynamics in many parts of the world, and agroecosystems are no exception. Under conventional agricultural practices, reduced plant diversity, spraying of insecticides, and other practices may significantly reduce predator abundance and diversity. For example, landscape diversity is associated with increased biological control of soybean aphids by naturally occurring natural enemies, principally ladybeetles (Gardiner et al. 2009). Similarly, when plants and herbivores are introduced into novel environments, they are also predicted to be exposed to fewer specialist predators in the introduced region. These kinds of anthropogenic impacts generate opportunities to study the influences of predators on coevolution between plants and herbivores across geographic space. For example, half of the cases where host plant resistance was antagonistic to biological control in agricultural systems involved soybean, which may be attributed to the lack of a long-term coevolutionary history between soybeans and the herbivore and natural communities in North America (Hare 2002).

Significance

Plants and their associated insects comprise a large fraction of the described biodiversity on Earth (Berlocher and Feder 2002, Futuyma and Agrawal 2009). Theory predicts that coevolutionary interactions between taxa contribute to the origins and maintenance of this diversity (Thompson 2005), and there is evidence that predators may contribute to these processes. Some of the best evidence consistent with sympatric speciation comes from phytophagous insects (Berlocher and Feder 2002). Insect natural enemies that are generalists, or those that have associations with particular plants, may substantially influence host shifts in phytophagous insects that acquire enemy free space on novel host plants (Bernays and Graham 1988, Singer and Stiremen 2005). Consequently, cycles of "escape and radiate diversification" may occur across multiple trophic levels. Furthermore, antagonistic interactions can maintain diversity through negative frequency dependent selection (Thompson 2005). However, predators are conspicuously absent from one of the classic examples of coevolving polymorphisms between insects and plants. In the introduced range, the frequency of wild parsnip chemical profiles varies across sites, but the frequency of parsnip webworm detoxification phenotypes is well matched to the distribution at most sites. Because there appears to be no temporal trend in most components of the chemical profiles, this evidence is consistent with a coevolving polymorphism (Berenbaum and Zangerl 1998). However, in the native range, the presence of alternate host plants and parasitoids of the webworms may select for reduced levels of some chemical defenses (Berenbaum and Zangerl 2006). There is good reason to suspect that multi-trophic interactions influence coevolutionary processes that generate and maintain diversity, but we are still a long way from a predictive framework that identifies the conditions necessary for different types of direct and indirect interactions to influence populations.

Human activities are changing the ecological landscape by introducing novel species into communities, causing extinctions of species, and changing the abiotic environment in ways that affect species range limits and population structure. A framework that would allow prediction about the ways such changes will impact particular populations of interest, both ecologically and evolutionarily, would be very useful. Such a framework would inform ecological risk assessments that attempt to characterize the risks associated with the introduction of a novel species as well as ecological risk management plans. In my dissertation work, I have investigated the potential for interactions between plants and the natural enemies of herbivores to generate ecological costs of plant defense. Instead of finding strong support for this hypothesis, evidence suggests that plants and predators can evolve tolerance of specialist sequestering herbivores. Thus, chemically mediated interactions among plants, herbivores, and their predators may not be good predictors of optimal defense investment overall. Instead, plant chemistry and tolerance of herbivory may both play important roles in the coevolutionary dynamics of tri-trophic systems.

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