

BIODIVERSITY AND FEAR ECOLOGY: THE CASCADING EFFECTS OF SPECIES
RICHNESS AND NONTROPHIC INTERACTIONS

By

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Abstract

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Food web complexity may arise as much from species richness as from behavioral interactions among species. An important yet underdeveloped area of food web ecology is the degree to which species richness and nontrophic interactions drive community dynamics. To examine these relationships, we isolated the consumptive and nonconsumptive effects of predator assemblages, as well as the effect of predator diversity *per se*. We found that species-rich predator assemblages evoked more frequent antipredator behaviors in their prey than less diverse predator assemblages, while similar numbers of prey were killed at both diversity levels. This effect cascaded to the host-plant of the prey, allowing greater plant biomass at the higher diversity level. Thus, we show that consumptive and nonconsumptive predator effects may operate independently and scale differently with predator diversity. There appeared to be two underlying mechanisms for these findings: several predator species visited the plants more often when among heterospecifics than conspecifics, and on a per-visit basis, these species evoked greater intimidation when within species-rich assemblages. These findings suggest that predator-predator intimidation may be reduced within species-rich assemblages. Given that cascading fear effects may significantly influence the basal resource of a given system, the density of prey transmitting the fear effect should be a significant factor. We investigated this hypothesis by

subjecting varying prey densities to a nonlethal enemy. Nonlethal enemy effects increased linearly with prey density, indicating that cascading fear effects were a function of prey density. Not all nontrophic interactions in communities are predicated on cascading predator-prey interactions. We focused on the nontrophic impacts of a ubiquitous, important ecological phenomenon: insect folivory. In a simple collard system, we found that caterpillar folivory on collard plants dramatically altered leaf architecture, inducing predators to alter their foraging patterns, which ultimately facilitated increased aphid predation. Folivory also affected host plant quality such that aphids fed much less on damaged leaves. We quantified the per capita interaction strength of each link in these indirect interaction pathways to facilitate the parameterization of nontrophic effects in food web models.

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Dedication

This dissertation is dedicated to my mom.

CHAPTER ONE

INTRODUCTION

Food web complexity derives from both trophic and nontrophic interactions among species. Nontrophic interactions are increasingly recognized as important drivers of community structure and dynamics. Chapter 2 explores how the cascading, nontrophic effects of fear are shaped by predator diversity; this chapter has been formatted for submission to *Ecology Letters*. Chapter 3, which investigates the degree to which cascading fear effects are a function of prey density, has been formatted for submission to *Ecological Entomology*. Chapter 4, which examines the nontrophic impacts of insect folivory on a subset of the collard community, has been formatted for submission to *Ecology*.

LITERATURE REVIEW

A multitude of anthropogenic threats are eroding biodiversity (Vitousek et al., 1997), and this global phenomenon has inspired much interest in the relationship between biodiversity and the functioning of ecosystems (Loreau et al., 2002; Hooper et al., 2005). Many researchers are engaged in this line of inquiry because it is the rich functioning of ecosystems that provides invaluable goods and services, such as harvestable biomass, decomposition, nutrient retention, pollination, and pest/disease suppression (Wilby and Thomas, 2002; Kremen et al., 2004; Hooper et al., 2005; Snyder et al., 2006). Ecosystem services are vital to human health (Ostfeld and Holt, 2004), as well as the economy (Daily and Ellison, 2002), and the recognition of this value is one

of the major reasons scientists are seeking efficient methods to conserve biodiversity (Srivastava and Vellend, 2005; Hooper et al., 2005).

Generally, ecosystems process energy and matter in complex yet characteristic ways, and the consequent apportionment of biomass among trophic groups is determined by species interactions (Lawton and Brown, 1994). Understanding the factors driving community structure, therefore, requires information on the direct and indirect effects of species interactions (Paine, 1966; Wootton, 1994; Polis and Strong, 1996). While there may not be a single overarching rule relating biodiversity to ecosystem function (Cardinale et al., 2000), there is evidence to suggest that diversity among *trophic groups* may be very important (Duffy et al., 2007). Thus, the way in which species are arrayed across trophic groups may influence an ecosystem's capacity to provide goods and services.

Food webs provide useful theoretical models with which to approach the subject (Paine, 1980; Pimm et al., 1991), but recent work has suggested that connectance among species should include not just trophic links, but also nontrophic links (Soluk, 1993; Borer et al., 2002; Dill et al., 2003; Bruno et al., 2003; Goudard and Loreau, 2008). Exploration of the various nontrophic links within communities is beginning to illuminate the idiosyncrasies in the relationship between diversity and ecosystem functioning. Under the “banner” of nontrophic interactions, studies of the effects of fear and intimidation are contributing significantly to this line of inquiry (see reviews by Lima and Dill, 1990; Sih et al., 1998; Werner and Peacor, 2003; Schmitz et al., 2004; Preisser et al., 2005; Preisser et al., 2007; Bruno and Cardinale, 2008).

The fear ecology concept

Nontrophic interactions

Nontrophic links within a food web are those interactions between species that are not characterized exclusively by an exchange of matter or energy (Soluk, 1993; Borer et al. 2002). Such interactions may manifest variously as interspecific facilitation (Cardinale et al., 2002), territoriality (Switalski, 2003; Berger and Gese, 2007), behavioral interference (Soluk and Collins, 1988; Peacor and Werner, 1997), apparent predation (Huang and Sih, 1991), and prey intimidation (Sih, 1984; Soluk and Collins, 1988; Peckarsky, 1993; Snyder and Wise 2000). For prey, foraging under predation risk is a ubiquitous and abiding element of growth and reproduction (Sih, 1987; Lima and Dill, 1990). In natural systems, there is much evidence to suggest behavioral, nontrophic interactions can shape community structure (Ripple and Beschta, 2004; Schmitz et al., 2004; Preisser et al., 2005; Byrnes et al., 2006; Heithaus et al., 2007; Berger et al., 2008; van der Merwe and Brown, 2008; Valeix et al., 2009). These phenomena have been termed, “the ecology of fear,” and have been formally defined as “the melding of optimal predator and prey behaviors with the population- and community-level consequences” (Brown et al., 1999). This definition focuses on the behavioral aspect of predator-prey interactions, but prey may also respond to threats via other types of polyphenism (Abrams, 1995). Fundamental to the concept of fear ecology, therefore, is phenotypic plasticity, wherein an organism’s morphological, physiological, behavioral, and life history traits may be altered significantly by the environmental challenges experienced during its ontogeny or adulthood (Dodson, 1989; Stearns, 1989; Werner and Peacor, 2003). Phenotypic plasticity confers evolutionary advantages in that it allows for rapid responses to environmental challenges without necessitating an altered genotype (Agrawal, 2001). Many animal species show evidence of context-dependent behavior, morphology, and/or reconfigured life history traits, often deployed to either avoid encounters with predators or survive unavoidable encounters (Sih, 1987; Werner and Peacor, 2003).

The term, “trait-mediated interaction” (TMI), emerged as an encapsulation of the wide array of significant interactions between individuals that do not involve changes in the densities of the interacting species (Abrams, 1995; Peacor and Werner, 1997). Further, when the interactions between two species are nontrophically-mediated by a third, the trait-mediated interactions are clearly indirect, and this has been referred to as trait-mediated indirect interactions (TMII). Such indirect interactions are increasingly recognized as important drivers of community structure (see reviews by Lima and Dill, 1990; Werner and Peacor, 2003; Schmitz et al. 2004; Preisser et al. 2005). Much of this empirical work has involved partitioning the effect of an enemy species into its component parts—lethality and non-lethality—and this has allowed researchers to unmask and isolate the non-lethal effects of predators on their prey (Beckerman et al., 1997; Schmitz et al., 1997; Trussell et al., 2003; Byrnes et al., 2006; Griffin and Thaler, 2006). Significant overlap between lethal and non-lethal components has been documented, as well (Peacor and Werner, 2001; Griffin and Thaler 2006), suggesting that the two components do interact and likely generate non-additive effects, which is intuitive given that the consumption of a prey item precludes its subsequent intimidation, and that strong anti-predator behaviors will minimize the likelihood of capture.

Predator-prey interactions

There is much evidence to suggest that predator species exert significant non-lethal effects on prey (Sih, 1980; Werner et al., 1983; Sih, 1984; Ives and Dobson, 1987; Soluk and Collins, 1988; Lima, 1998; Snyder and Wise, 2000; Relyea, 2003; Magalhães et al., 2004; Castellanos and Barbosa, 2006; Byrnes et al., 2006; Griffin and Thaler, 2006; Trussell et al., 2006; Heithaus et al., 2007; van der Merwe and Brown 2008; Valeix et al. 2009). “Fear effects”

may emerge when victim species deploy and modulate anti-predator behaviors to balance feeding needs with predation risk (Sih, 1987; Werner and Peacor, 2003). In essence, prey pay the cost of reduced predation risk by assuming reduced individual fitness (Ives and Dobson, 1987). Nonlethal predator-prey interactions have the potential to generate trophic cascades equal (or greater) in strength to those caused by changes in the density of intermediate species (Luttbeg et al., 2003; Schmitz et al., 2004; Lima and Steury, 2005). Analyses confined to predator and prey demographics, therefore, may significantly underestimate the effects of predators on community structure (Scheffer, 1997; Brown et al., 1999, van der Merwe and Brown, 2008; Valeix et al., 2009).

Antipredator defenses may be categorized as pre- or post-encounter with predators (Sih, 1987). Before an encounter, prey may reduce their proximity to predators by confining their foraging to effective refuges, or by restricting their activities in space and time. Enduring environmental stressors, or exploiting ephemeral resources, can also facilitate predator avoidance. If prey cannot preempt spatial coincidence with their natural enemies, then the prey may rely on morphological crypsis, behavioral vigilance, and general inactivity to avoid discovery when within close proximity to a predator (Sih, 1987). Upon discovery, prey may invoke various antipredator defenses, which can be broadly categorized as rapid evasion, unpalatability, threatening postures, or the deployment of weapons in an active defense. In turn, predators often counter prey defenses with their own responses—generally, this may manifest as strategic movement, designed to maximize spatial coincidence (Sih, 1984; Lima, 2002; Schmitz et al., 2005). To optimize net energetic gain from their prey, higher-order consumers often attempt to aggregate where their prey are most abundant or accessible (Sih, 1987).

Some vertebrate prey species, in the process of trading relative safety for feeding opportunities, strike interesting balances. For example, sparrows opt for complex, brushy habitats that subject them to ambushes from accipiter hawks (Lima, 1995), but this appears to be favorable considering the threat of falcon attacks in the open. Conversely, on the African plains, many grazing and browsing herbivore species opt for open habitats, given the ambushing tactics of lions (Valeix et al. 2009). Invertebrate species have also been shown to modulate their antipredator responses relative to the particular threat being presented (see reviews by Sih, 1987; Lima and Dill, 1990; Sih et al., 1998; Lima and Steury, 2005). Caterpillars can discern and respond accordingly to the particular substrate-borne vibration signatures of wind, conspecifics, wasps, and hemipteran predators (Castellanos and Barbosa 2006). Cucumber beetles parse the threats posed by different spider species (Snyder and Wise 2000), and mayfly and stonefly naiads variously respond to interspecific, intraspecific, and size differences among predation threats in aquatic systems (Peckarsky, 1980; Shaffer and Robinson, 1996). While many species strike a balance that allows for a degree of predation risk, other species may have antipredator responses that are essentially reflexive suicide when confronted with a predation threat. In the presence of non-lethal predators, pea aphids dropped from their host plant in such high numbers—and then failed to reestablish on the plant—that the colony-wide growth rate was significantly reduced (Nelson et al., 2004). Thus, trait-mediated interactions between predators and prey may also indirectly reduce prey densities.

Behavioral TMI, TMII

Behavioral antipredator defenses represent some of the earliest evidence of trait-mediated interactions (Sih, 1980, 1984; Soluk and Collins, 1988; Peckarsky, 1991; Peckarsky et al. 1993).

Most of these examples are associated with “fear” responses, in which prey respond to a threat of predation. Among animal species, these changes are often induced by specific cues, and if the cue is perceived to be significant, a response can be invoked that either eliminates or mitigates the immediate (or chronic) threat (Sih, 1987). Plasticity in defensive behavior can be modulated according to the nature of the threat. The capacity to tailor the response occurs in both vertebrate and invertebrate species (Lima and Steury, 2005).

Prey species may effectively reduce perceived threats via spatial shifts—typically, dispersing from the risky area and/or abruptly seeking cover within a refuge (Sih, 1980; Schmitz et al., 1997; Magalhães et al., 2002; Wang and Keller, 2002; Trussell et al., 2006). Alternatively, prey may reduce foraging activity and deploy vigilant behaviors as long as the threat persists (Sih, 1984; Soluk and Collins 1988; Snyder and Wise, 2000; Prasad and Snyder, 2006; van der Merwe and Brown, 2008). Finally, potential prey may actively confront their enemies via posturing and attacks (Shaffer and Robinson, 1996), or various manifestations of unpalatability (Sih, 1987). These responses may be further modulated relative to prey state/stage (Luttbeg et al., 2003), competition (Werner, 1991), prey health (Heithaus et al., 2007), resource level (Wojdak and Luttbeg, 2005), and resource identity (Trussell et al., 2008).

The effects of behavioral trait shifts have generated a wide range of interesting ecological interactions (Lima and Dill, 1990; Schmitz et al., 2004; Preisser et al., 2005). For example, Soluk and Collins (1988) showed asymmetrical interference between two aquatic predators (sculpins and stoneflies), the cascading effects of which were further modified by the differing antipredator responses in their prey (*Baetis* and *Ephemerella* mayflies). The sculpins intimidated their fellow predators, the stonefly naiads, keeping them from hunting for mayflies around all rock surfaces. The stoneflies’ avoidance of exposed sites on rock topsides allowed one prey

species, *Baetis*, to indirectly benefit because this prey species tended to be distributed around all rock surfaces; hence, it suffered less stonefly predation on rock topsides. Importantly, the sub-additive effect of the two predators emerged even when the sculpins were rendered non-lethal to the stoneflies; thus, the indirect, cascading predator effects were behaviorally mediated.

However, the other prey species, *Ephemerella*, suffered increased predation in the presence of both the sculpin and stoneflies because this prey species tended to reside underneath the rocks, and while evading stoneflies, it was readily caught by sculpins. In another study, Huang and Sih (1990) demonstrated “apparent predation” between two non-competing prey species:

salamanders and freshwater isopods. Salamander larvae and isopods directly influenced each other’s behavior within a shared refuge. In the presence of a shared enemy (green sunfish), the salamander larvae stayed within their refuge more often, but only when the isopods were present. In contrast, the salamanders tended to induce the isopods to leave the refuge, causing them to suffer increased mortality in the presence of the predator. The result was that the direct, nonconsumptive relationship between the two prey species caused one of the species to suffer greater predation (Huang and Sih, 1990).

The “landscape of fear” has been defined as a spatial map of an animal’s perceived predation risk, measured as reductions in foraging rate due to this risk (Laundré et al., 2001). In a study designed to physically map a landscape of fear, researchers arranged depletable food patches in a grid across a prescribed area containing many Cape ground squirrel burrows (van der Merwe and Brown, 2008). In this work, the degree to which a food patch was depleted represented the relative safety of the immediate area surrounding the food patch, and when these measures were mapped across the entire grid, it was apparent that there were gradations of fear across the area being studied. The squirrels tended to spend more time at a food patch and

deplete it to a greater degree when the patch was near a burrow entrance. Among food patches initially far from burrow entrances, the squirrels subsequently created burrow entrances near these remote food sites, an example of ecological engineering (Lawton, 1994). By mapping food consumption, the costs of predation—in terms of sacrificed energy resources—can be quantified in space for a victim species, and in the case of the Cape ground squirrel, this species endured high foraging costs in 31-92% of the area surrounding their colonies.

Morphological TMI, TMII

Predator-induced changes in prey morphology have been documented for both vertebrate and invertebrate species (Dodson, 1989; Stearns, 1989). Morphological shifts in *Daphnia* were some of the earliest evidence of predator-induced polyphenism (Stearns, 1989). In this work, *Daphnia* exposed to predation cues early in their ontogenetic development would often develop large, pointed “helmets,” or a ring of spines around their heads (“neck teeth”) (Dodson, 1989). These defenses reduced the capacity of predaceous phantom midge larvae to easily consume *Daphnia*, and would thereby dissuade the phantom midges from targeting them. Even the progeny of adult *Daphnia* experiencing predator stressors showed evidence of neck teeth.

Predators have been shown to influence the average prey phenotype via three processes: induction, thinning, and selection (Relyea, 2002). Induction of phenotypic plasticity in prey is often the result of visual and chemical cues associated with the predators’ presence, while the thinning of the prey reduces the degree of exploitative competition. Selection is simply the non-random form of thinning (selecting out certain phenotypes). Ontogenetic changes in larval anurans (tadpoles) have been documented where the anurans were exposed to waterborne predation cues (Relyea, 2002, 2003). Early in their ontogeny, tadpoles experiencing evidence of

predation relied primarily on behavioral defenses such as reduced activity and frequent hiding, but later in their development, they relied on a combination of increased mass, deeper tails, and shorter bodies as their defense strategy (Relyea, 2003). This morphological shift allowed the older tadpoles to move more abruptly, which conferred an advantage to the surviving tadpoles in an environment ostensibly populated by many predators. This work corroborated previous findings with newts under predation pressure from dragonfly naiads (Van Buskirk and Schmidt, 2000). Here, larval newts developed longer tails and bigger heads in the presence of the dragonflies, and the long-tailed phenotype allowed much higher survival among the newts, but it also slowed their development. Relyea (2004) showed that the effects of predation and competition interactively mediated tadpole morphology. At low levels of competition, predator-induced effects were always larger, and while low levels of predation amplified the effect of competition, the effect was larger only when the induced traits were in the same direction. In this example, it was apparent that phenotypically plastic traits were fine-tuned to a variety of environmental conditions.

Physiological, life-history TMI

Prey physiology and life-history strategies can be altered by exposure to predation risks. These effects may involve expedited (Black, 1993) or delayed development (Peckarsky and McIntosh, 1998) to minimize predation threats along a temporal continuum. However, changes in physiology may also be a means of indirectly affecting behavior, increasing the stress level and thus the wariness of potential prey (Scheuerlein et al., 2001; Monclús et al., 2009). *Daphnia* exposed to notonectid predators (backswimmers) did not develop neck teeth but rather experienced rapid juvenile growth, followed by little adult growth and high reproductive output

(Black, 1993). The antipredator response is clearly not aimed at reducing predation. Instead, it would seem that this strategy allows *Daphnia* to outpace the effects of predation by shortening its generation time and increasing its fecundity.

In another aquatic system, mayflies in flow-through, artificial stream tanks reduced feeding rates when exposed to the predation cues of trout and stoneflies, causing significant reductions in adult mayfly size and fecundity (Peckarsky and McIntosh, 1998). Thus, the mayflies traded future reproduction for immediate survival, based entirely on perceived predation risk. Further, it appeared that the stoneflies had a greater nonlethal effect on the mayflies than the trout, given that the mayflies experienced a significantly delayed development time in the presence of stoneflies. This would have increased the mayflies' exposure to subsequent predation and parasitism, but again, the trade-off between subsequent fitness and immediate survival seemed to strongly favor immediate survival (Peckarsky and McIntosh, 1998).

In terrestrial systems, vertebrate prey species show evidence of increased corticosterone levels in the presence of predation threats (Scheuerlein et al., 2001; Monclús et al., 2009). The annual fecundity and defensive behaviors of stonechats (a tropical bird species) were investigated in the presence and absence of shrikes, one of their primary natural enemies. Stonechats in the presence of shrikes were less likely to initiate a second brood, and after fledging their young, male stonechats were more likely to produce distraction calls when a human entered their territory. Corticosterone level, a measure of stress, in male stonechats was significantly higher in the presence of shrikes, suggesting the shrikes were causing chronic stress in the male stonechats. Similarly, rabbits in temperate zones have been shown to experience increased corticosterone levels depending on the degree of predation risk perceived (Monclús et

al., 2009). That the stress hormone modulated relative to predation risk may suggest that in social species, agitated behavior is a means of communicating the likelihood of imminent threats to the group, which may invoke heightened wariness behaviors.

Predator-predator interactions

All animals are consumers, thus at a very fundamental level, all animal species must endure predation threats—that is, virtually no animal species is entirely safe from being killed and eaten by another (Lima 2002). Species must trade-off their need to eat with that of safety or defense (Sih, 1987). This balance may be heightened among carnivores, because these species generally have many adaptations for catching and killing organisms. Given the opportunity, many carnivores may readily turn this lethal capacity upon fellow carnivores. Raptorial forelegs, claws, talons, sharp teeth, stingers, venom, powerful limbs, and long ovipositors may facilitate either cannibalism or intraguild predation. Cannibalism and intraguild predation among carnivorous species is not uncommon and can influence the cascading impacts of predators on their respective communities (Rosenheim et al., 1993; Snyder and Ives, 2001; Finke and Denno, 2004, 2005; Griffen and Byers, 2006; Rudolf, 2008). Empirical and theoretical findings in the last two decades suggest that the effects of multiple predator species may also arise via behavioral interactions among predators (Soluk and Collins, 1988; Peckarsky, 1991; Abrams, 1995; Sih et al., 1998; Werner and Peacor, 2003; Preisser et al., 2007; Goudard and Loreau, 2008). When behavioral interference occurs within a given guild of consumers, the interaction may be referred to as intraguild intimidation. Though less frequently explored than intraguild predation, intraguild intimidation is an important subset of predator-predator interactions (Peckarsky, 1991; Sih et al., 1998; Switalski, 2003; Berger and Gese, 2007; Berger et al., 2008).

Studies focusing on pair-wise interactions among invertebrate predators have shown that prey tend to benefit when predators interfere with or intimidate one another (Soluk and Collins, 1988; Sih et al., 1998; Siddon and Witman, 2004; Björkman and Liman, 2005; Vance-Chalcraft and Soluk 2005). In an aquatic system, two dragonfly species that prey on damselfly naiads were much less efficient at capturing their prey when together than when alone, and much of this effect was shown to derive from predator-predator intimidation (Wissinger and McGrady, 1993). In marine systems, consumption of barnacles by snails may be reduced in the presence of predator risk cues generated by a crab species that targets the snails (Trussell et al., 2003). Ground beetles prey upon various soft-bodied prey in *Brassica* systems, but when a larger beetle was coincident with smaller species, the smaller beetles became less active, affording their prey a release from predation (Prasad and Snyder, 2006). Intraguild prey (mesopredators) may use substrate-borne, chemical cues to assess the likelihood of intraguild predators, and not surprisingly, the mesopredators avoid such patches (Magalhães et al., 2004). These findings have been corroborated by observations of vertebrate predators in the field (Berger et al., 2008). In the Greater Yellowstone Ecosystem, pronghorn fawns experienced a four-fold increase in predation by coyotes in the absence of wolves, yet coyote abundances were similar in wolf-free and wolf-abundant sites (Berger et al., 2008). Wolves are known to consume coyotes, and antipredator behaviors by coyotes are strongly affected by the presence of wolves (Switalski, 2003; Berger et al., 2007). This suggests that increased pronghorn fawn survival in the presence of wolves was a function of changes in coyote foraging strategies, induced by the threat of predation by the apex predator, wolves (Berger et al., 2008). Prey species may, in theory, benefit from being in areas frequented by predators—referred to as “dangerous habitats” (Lima, 1992). For example, curlews have been shown to benefit from establishing nesting sites near the nests of avian natural

enemies (Norrdahl et al., 1995). Here, curlews were subjected to kestrel predation, but the suite of other predators that were effectively rebuffed by the presence of the kestrels provided the curlews a marginal benefit; thus, the curlews seemed to benefit by enduring the least of many potential “evils.”

Biodiversity and the ecology of fear

Cascading biodiversity effects

It is increasingly evident that species diversity within and across trophic groups significantly influences community structure and ecosystem functioning (Tilman et al., 1996; Schmitz et al., 2000; Terborgh et al., 2001; Ives et al., 2005; Cardinale et al., 2006; Snyder et al., 2006; Duffy et al., 2007; Stachowicz et al., 2007; Goudard and Loreau, 2008). Early biodiversity work showed that among floral species, plants tended to be more productive when among heterospecifics than conspecifics (Tilman et al., 1996; Loreau and Hector, 2001). The mechanism was shown to be mostly attributable to complementary resource use, or niche differentiation, among the competing plant species. Positive diversity effects can also derive from interspecific facilitation, or synergism (Cardinale et al., 2002). Finally, a selection effect, or identity effect, may also influence the diversity effect, as evident when a dominant species in polyculture performs particularly strongly or poorly in monoculture (Loreau and Hector, 2001; Straub and Snyder, 2006; Stachowicz et al., 2007). Among higher-order consumers, it has been well-documented that greater predator diversity may enhance the exploitation of prey (Losey and Denno, 1998; Cardinale et al., 2003; Aquilino et al., 2005; Snyder et al., 2006), mirroring the consumer-resource relationships observed at other trophic levels (Tilman et al., 1996; Tilman et al., 1997; Loreau and Hector, 2001). Conversely, antagonism and/or redundancy among

predators may dilute the effects of increasing predator diversity (Soluk and Collins, 1988; Lawton and Brown, 1994; Polis and Strong, 1996; Snyder and Ives, 2001; Crumrine and Crowley, 2003; Rosenheim et al., 2003; Finke and Denno, 2004). Whether the effects of predator diversity are positive, neutral, or negative, there is growing evidence that diversity effects may cascade through multiple trophic levels, generating significant impacts on basal resources (Power, 1992; Strong, 1992; Sih et al., 1998; Cardinale et al., 2003; Aquilino et al., 2005; Bruno and O'Connor, 2005; Hooper et al., 2005; Byrnes et al., 2006; Cardinale et al., 2006; Snyder et al., 2006).

It is not surprising, therefore, that among studies isolating the effects of diversity *per se*, the effects of predator diversity at the community-scale have been relatively idiosyncratic: species-rich predator assemblages may confer greater protection upon plants by imposing greater mortality rates on herbivorous prey (Wilby et al., 2005; Snyder et al., 2006), or such assemblages may dampen the cascading effects of predators via intraguild predation (Finke and Denno, 2005), strong identity effects (Straub and Snyder, 2006), and omnivory (Bruno and O'Connor, 2005). Recent biodiversity work suggests that strict partitioning of resources (Finke and Snyder, 2008), functional diversity within a predator guild (Ramirez and Snyder, 2008), and the degree of competition for prey (Griffin et al., 2008) are strong determinants of the cascading effects of biodiversity. Finke and Snyder (2008) controlled for innate phylogenetic differences among competing parasitoids while examining how resource partitioning permitted the parasitoids to more efficiently exploit their resource—aphids. In effect, a given parasitoid taxon was successfully parsed into three different niches, and in so doing, conspecifics experienced less redundancy in their resource requirements, allowing them to compete less. Ramirez and Snyder (2008) used natural enemies inhabiting above- and below-ground spatial zones to achieve a

similar result—here, species richness did not, in itself, generate positive diversity effects; rather, it was the functional differences between the above- and below-ground carnivore species that permitted greater prey suppression. Griffin et al. (2008) showed conclusively that increasing predator density along with species richness allowed any differences in resource use among predator species to be accentuated—that is, positive diversity effects were not evident until the predators were essentially forced to partition a limited resource. The implication of this finding is that the effects of resource partitioning may not manifest until the resource is somehow limited—when the resource is abundant, predator species can be relatively redundant and still find adequate food (producing similar prey suppression as the complementary predator assemblages), but if prey are limited, then redundancy among predators will constrain overall prey consumption. Thus, only when the prey base is sufficiently limiting can species-rich predator assemblages exploit more prey than species-poor assemblages (Griffin et al., 2008).

Nontrophic interactions in diverse food webs

Variability in the net effect of predator diversity may derive, at least in part, from the suite of nonconsumptive interactions among predator species (see reviews by Sih et al., 1998; Bruno and Cardinale, 2008). In studies involving more replete predator communities, there appears to be substantial variability in the strength and nature of the interaction between lethal and non-lethal effects (Vance-Chalcraft and Soluk, 2005, Byrnes et al., 2006; Prasad and Snyder, 2006). For example, greater intimidation among predator species may yield countervailing effects between lethal and nonlethal predator effects (Vance-Chalcraft and Soluk, 2005; Prasad and Snyder, 2006). Further, complementarity among fear effects may manifest when a predator assemblage encounters prey species sensitive to the particular predator species present in the

assemblage (Byrnes et al., 2006). Considering that it is the prey's response to a predator that determines the ferocity of the predator (Brown et al., 1999), the "ecology of fear" is likely a function of both predator and prey diversity. The myriad interactions between diversity, predator lethality, and prey intimidation appear to be important determinants of community dynamics, yet the community-level consequences of such interactions are still poorly understood.

In terms of predator-predator intimidation, there is not a clear trend as to whether interspecific interactions tend to differ from intraspecific interactions. Some studies suggest that behavioral interference among heterospecific predators is more severe than that among conspecifics (Soluk and Collins, 1988; Peckarsky, 1991; Huang and Sih, 1990; Wissinger and McGrady, 1993); one study suggests the opposite (Björkman and Liman, 2005), while others have found inter- and intraspecific interference to be essentially equal (Shaffer and Robinson, 1996; Vance-Chalcraft et al., 2004; Vance-Chalcraft and Soluk, 2005). Thus, based on past studies of pair-wise interactions among predators, it is difficult to predict how changes in predator diversity might influence the nature and strength of intraguild intimidation. Theory suggests that competing individuals should experience less competition among heterospecifics than among conspecifics, given that speciation processes should amplify character displacement among heterospecifics, allowing different species to coexist (Darwin 1859; MacArthur 1958). Assuming an upper bound to total predator abundance in a community, each species will become increasingly rare with increasing species richness, and the frequency of interactions among conspecifics should decrease accordingly. Thus, to the extent that intraspecific competition is more severe than interspecific competition, increasing diversity should provide a benefit to each species.

Implications of species loss

The way in which species are distributed among trophic groups may influence an ecosystem's capacity to provide goods and services (Duffy, 2002). As habitats are lost, the localized extirpation of functionally efficient species and the cascading impacts of these losses can rapidly reduce the quality of a given service, as in adequate pollination, for example (Larsen et al., 2005). This suggests that not only are certain species important within a given trophic group, but that the trophic group is important within its community. Indeed, it is the diversity of trophic groups that maintain community structure, and ecosystem functioning can be profoundly hobbled by the loss of a trophic group (Terborgh et al. 2001; Duffy et al., 2007; Bruno and Cardinale, 2008). In effect, conservation efforts may need to be aimed as much at community structure as the constituent species.

Recent reviews of community-scale fear effects strongly suggest that the structure and dynamics of a community are largely a function the nontrophic interactions among species (Dill et al., 2003; Schmitz et al., 2004; Preisser et al., 2005; Bruno and Cardinale, 2008). Behavioral interactions may dial up or down trophic relationships, and these effects can be further shaped by the identities of interacting species (Byrnes et al., 2006), the level of competition (Werner, 1991), and variability in the quality or quantity of resources (Preisser et al., 2009). Thus, conservation efforts need to incorporate behavioral, nontrophic species interactions into the decision-making calculus. Excellent empirical studies towards these ends have investigated the minimum natural area required to maintain a given ecosystem service (Kremen et al., 2004), and pushed for the inclusion of ecological parameters in the design of wildlife reserves (Aïramé et al., 2003).

Collard agroecosystem

The community

The most common herbivores observed on collard plants *Brassica oleracea* L. (Brassicaceae), as well as other cruciferous plants in the U.S., are the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), imported cabbageworm *Artogeia rapae* (L.) (Lepidoptera: Pieridae), cabbage looper *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), flea beetle *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae), and the cabbage maggot *Delia radicum* (L.) (Diptera: Anthomyiidae) (Wyman, 1992). The animal consumers in the collard community are relatively insular, which has been attributed to the glycoside, sinigrin, and mustard oils common to brassicaceous plants (Root, 1973). A wide variety of natural enemies exploit these herbivores, including various coccinellid beetles (Coleoptera: Coccinellidae), damsel bugs (Hemiptera: Nabidae), big-eyed bugs (Hemiptera: Lygaeidae), lacewings (Neuroptera: Chrysopidae), and several braconid (Hymenoptera: Braconidae), ichneumonid (Hymenoptera: Ichneumonidae), and chalcidoid (Hymenoptera: Chalcidoidea) parasitoid species (Harcourt, 1960; Root, 1973; Snyder et al., 2006). Recent biodiversity research in the collard system has shown that with increasing predator diversity, the exploitation of prey increased, the degree of interference among predators decreased, and plant biomass was conserved (Snyder et al., 2006). This represented strong evidence of complementary interactions among higher-order consumers in the collard system. Subsequent explorations into the mechanisms by which the complementary interactions may have emerged suggested that there was a degree of spatial partitioning of the collard leaf surface by some of the more common enemy species in the collard system (Straub and Snyder, 2008).

An ideal system for studies of fear ecology

In biodiversity studies, a species-rich assemblage of natural enemies offers a large pool of potential species, thereby facilitating manipulations of various levels of diversity. The collard community provides such a community. Additionally, the leaf surfaces of the collard plant present a flat, relatively smooth and uniformly colored surface, which allow for rapid, accurate counts of the fauna in the collard canopy. In studies of fear ecology, it is helpful to have discernable antipredator behaviors. Diamondback moth caterpillars are known to deploy a distinct antipredator behavior—dropping from leaf undersides by a silk, and hanging until the perceived threat is gone (Wang and Keller, 2002). This behavior is effective at providing an immediate, mid-air refuge for the caterpillars, but since the caterpillar must forego feeding while suspended and also haul itself back up to the plant, there is likely a very real cost in terms of its growth rate. This defense may be induced by a wide variety of perceived threats (S.A. Steffan, *personal observation*), but the highly specialized, primary parasitoid, *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) represented the most significant threat to diamondback moth caterpillars in the field (S.A. Steffan, *unpublished data*). Congeners of *Diadegma* have been shown to be highly attracted to caterpillar-damaged plants (Ohara et al., 2003), and to concentrate their foraging time on plants with higher densities of caterpillars (Waage, 1983; Wang and Keller, 2002). In fact, *Diadegma* is known to wait at the site of the caterpillar's silk attachment, in anticipation of the return of a suspended diamondback moth caterpillar (Wang and Keller, 2002), which can induce repeated silk-drop defenses (S.A. Steffan, *personal observation*). This easily recognized antipredator behavior, coupled with the diversity of natural enemies that could evoke the response, made the collard system an ideal one for

studies of cascading fear effects. Using this system, we examined three primary questions: 1) do nonconsumptive predator effects scale with predator diversity differently than consumptive predator effects?, 2) are cascading predator effects a function of prey density?, and 3) does caterpillar folivory generate significant nontrophic impacts within the collard community?

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CHAPTER TWO

**CASCADING DIVERSITY EFFECTS TRANSMITTED EXCLUSIVELY BY
BEHAVIORAL INTERACTIONS**

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Abstract

Consumer diversity generally increases resource consumption. Consumers can also impact other species by altering their behavior, but it is unclear how such nonconsumptive effects scale with diversity. We independently manipulated predator species richness, and the lethal and non-lethal effects of predator communities, to measure the role of each factor in protecting *Brassica oleracea* plants from caterpillar herbivory. Plant biomass was greatest when diverse predator assemblages induced antipredator behaviors in herbivores, an effect not further strengthened when predators could also kill caterpillars. Predators within diverse communities were more likely to forage on plants, and to disrupt herbivore feeding, reflecting greater aversion to foraging among conspecific than heterospecific competitors. Predator diversity, therefore, initiated behavioral changes at the predator and then herbivore trophic levels, both to the benefit of plants. Our results indicate that strong, emergent species richness effects can be transmitted entirely through behavioral interactions, independent of resource consumption.

Keywords: biodiversity, predator-prey, ecosystem function, nontrophic interaction, interference competition, intraguild intimidation, trait-mediated

INTRODUCTION

The performance of diverse communities generally exceeds that of species-poor communities (Cardinale *et al.* 2006). In most cases this is attributed to niche partitioning among species, or interspecific facilitation of resource capture (Hooper *et al.* 2005). In either case, diversity effects are generally envisioned as operating through the consumption of resources. However, when the consumers are animals, multi-species interactions can be mediated by changes in behavior, which may be reflected in biomass changes that are one or more trophic levels removed from the inciting species (Turner & Mittelbach 1990; Schmitz *et al.* 1997). For example, predator avoidance behaviors by herbivores often come at the cost of lost feeding opportunities, leading to reduced plant damage even when herbivore numbers do not change (Lima & Dill 1990; Werner and Peacor 2003; Preisser *et al.* 2005). Such behaviorally-mediated indirect effects can be as strong as those induced by the actual consumption of intermediary species (Schmitz *et al.* 2004). Analyses confined to predator and prey demographics, therefore, may significantly underestimate the effects of predators on community structure (Brown *et al.* 1999; Byrnes *et al.* 2006; Goudard & Loreau 2008).

For predators, there is good reason to expect complex interactions between consumer diversity and the relative strengths of nonconsumptive and consumptive effects. Behavioral interactions are particularly important in predator communities (Sih *et al.* 1998), perhaps contributing to the widely varying diversity-consumption relationships observed at this trophic level (Ives *et al.* 2005; Bruno & Cardinale 2008). Prey consumption can decline at higher

diversity levels when intraguild predation is common (Finke & Denno 2004), but increase when predators partition prey (Wilby *et al.* 2005; Bruno & O'Connor 2005; Finke & Snyder 2008) or facilitate one another's prey capture (Soluk & Collins 1988). Thus, interactions between predator diversity and predator consumptive effects themselves often are non-additive (Ives *et al.* 2005). Similarly, the lethal and non-lethal effects of single predators can be positively or negatively non-additive (Peacor & Werner 2001; Griffin & Thaler 2006).

In terrestrial and aquatic systems, there is evidence to suggest that nonconsumptive effects could largely underlie predator diversity effects (e.g., Sih *et al.* 1998; Byrnes *et al.* 2006; Prasad & Snyder 2006), but the relative scaling of lethal and non-lethal effects across diversity levels has yet to be examined empirically (Borer *et al.* 2002; Goudard & Loreau 2008). This omission is unfortunate given that in nature, higher-order consumers are exerting lethal and non-lethal effects while imbedded in diverse communities (Switalski 2003; Werner & Peacor 2003; Siddon & Witman 2004; Berger *et al.* 2008; Trussell *et al.* 2008). Understanding the relationship between nonconsumptive effects and diversity, per se, is of particular importance given global declines in biological diversity (Hooper *et al.* 2005) and the bias toward extirpation among higher-order consumers (Duffy 2002).

In the field within a community of predatory insects, we independently manipulated lethal and non-lethal predator effects, across two levels of predator diversity, and within a fully-factorial design. We then measured the resulting effects on predators, herbivores, and the basal resource of the system, *Brassica oleracea* L. plants. Nonconsumptive effects were isolated by replacing caterpillars killed by predators, and consumptive effects were isolated by hand-removing caterpillars in the absence of predators. These manipulations were catered to reproduce the magnitude of these effects in predator communities differing both in species richness and

species composition, ensuring a factorial manipulation of predator nonconsumptive, consumptive, and diversity effects. We found that diverse predator communities promoted increased plant growth by reducing herbivory, but that this emergent diversity effect was transmitted entirely through a chain of nonconsumptive interactions at the predator and then herbivore trophic levels. Thus, when compared to species-poor predator assemblages, the more diverse predator assemblages increased system productivity without increasing herbivore mortality. Our results indicate that behaviorally-mediated effects can interact with diversity quite differently than, and independent from, those due to resource consumption. This supports the contention that nonconsumptive interactions warrant greater attention in biodiversity studies (Borer *et al.* 2002; Schmitz *et al.* 2004; Bruno & Cardinale 2008; Goudard & Loreau 2008).

METHODS

Natural history

Our study focused on the diverse community of predators attacking *Plutella xylostella* L. caterpillars on *B. oleracea* plants. These caterpillars are the dominant chewing herbivore on *B. oleracea* in the northwestern region of the United States (Biever *et al.* 1992). When threatened by a predator, *P. xylostella* caterpillars drop from the plant, anchored by a silk thread affixed to the leaf underside (Wang & Keller 2002). This behavior is readily observed, and the larvae may remain suspended for minutes to hours on this thread before climbing back up to the plant (Wang & Keller 2002). Since they cannot feed while dangling from the plant, deployment of this antipredator behavior carries the cost of lost feeding opportunities.

Locally common predators attacking these caterpillars include the ladybird beetle *Hippodamia convergens* Guérin-Méneville, the predatory bugs *Geocoris pallens* Stål and *Nabis*

alternatus Parshley, the golden-eyed lacewing *Chrysopa oculata* Say, and the specialist endoparasitoid *Diadegma insulare* (Cresson). This predator community encompasses a wide variety of body sizes, feeding rates, hunting modes, and foraging domains, of the type that has been demonstrated to yield complementary impacts on shared prey (Snyder *et al.* 2006; Straub & Snyder 2006; Preisser *et al.* 2007). At the same time, intraguild predation has been documented among several community members: lacewing larvae may succumb to *Geocoris* and *Nabis* attacks (Rosenheim *et al.* 1999), and *Geocoris* and *Nabis* prey upon one another (Snyder *et al.* 2006). Further, prey may stand to benefit from various manifestations of intraguild intimidation among predators in more diverse communities (e.g., Berger *et al.* 2008).

Experimental design

In the field, four factors— predator diversity (*Low, High*), predator nonconsumptive effects (*Fear+*, *Fear-*), predator consumptive effects (*Lethal+*, *Lethal-*), and temporal block (1, 2) — were manipulated within a fully crossed, $2 \times 2 \times 2 \times 2$ factorial design, yielding 16 unique treatment combinations, each of which was replicated five times (total $N = 80$; Fig. 1). Our independent manipulation of these factors allowed us to calculate the main and interactive effects of each. Experimental units were $60 \times 60 \times 60$ -cm field cages (BugDorm-3120, MegaView Science Education Services Co., Taiwan), enclosed on three sides by polyester netting (96×26 threads per cm mesh) and on the fourth with a clear plastic panel that allowed easy viewing of cage occupants. In the middle of the clear front panel, a mesh sleeve allowed access to the interior of the cage. Each cage housed, in addition to predators, two *B. oleracea* plants and 20 *P. xylostella* caterpillars (see *Experimental details* below). The bottom edge of each cage was buried under 5-10 cm of packed soil to block arthropod movement.

Manipulation of predator diversity

Replicates of the *Low Diversity* treatment consisted of each of the five predator species in separate monocultures, while the *High Diversity* treatment included each of the unique draws of four species from our pool of five predator species (i.e., one species was absent from each polyculture) (Snyder *et al.* 2006). This design controls for the effects of species composition and identity by ensuring that no single composition or species was over- or underrepresented (Tilman 1997; Straub & Snyder 2006). Each of these species compositions was included once at each level of consumptive/nonconsumptive effect manipulation (Fig. 1). Predator diversity was manipulated within a substitutive design, with *Low Diversity* cages receiving four individuals of the same species, and *High Diversity* cages receiving one individual of each species.

Manipulation of nonconsumptive predator effects

We isolated nonconsumptive predator effects by systematically replacing caterpillars that had been killed. Each cage in this treatment (*Fear+*, *Lethal-*) included predators, assembled according to diversity level and species composition, that were allowed to forage freely. Each of these cages was randomly paired with a no-predator control (*Fear-*, *Lethal-*) cage, and every day, caterpillar densities were restored to no-predator levels in the *Fear+*, *Lethal-* cage by replacing any dead or missing caterpillars. This system of prey replacement allowed us to virtually eliminate prey losses in excess of background mortality, thereby neutralizing the effect of predator lethality in these cages. Every replaced larva was ensured to be the equivalent instar as that of its corresponding control cage, and all replacement larvae were chosen randomly from the same cohort used to originally populate the cages. Our methods obviated the need for physical

manipulation of predators or the confinement of un-manipulated predators, and by allowing predators to kill prey, the system preserved the cues associated with predation, such as tactile vibration signatures (Castellanos & Barbosa 2006) and substrate-borne residues (Magalhães *et al.* 2005). Such cues have been shown to initiate antipredator behaviors in terrestrial communities.

Manipulation of consumptive predator effects

To isolate consumptive effects, a system of manual prey culling was employed in cages where predators were absent (as in Griffin & Thaler 2006). Each replicate of the *Fear-*, *Lethal+* treatment (predators absent) was paired with the *Fear+*, *Lethal+* replicate (predators present) with the same predator species composition. Each day of the experiment, we recorded caterpillar densities within *Fear+*, *Lethal+* cages and then duplicated these densities within their corresponding *Fear-*, *Lethal+* cages by manually removing caterpillars. Because the *Fear-*, *Lethal+* cages did not contain predators, the prey experienced density reductions in the absence of any predation cues. To ensure that the culling of prey accurately mimicked predation in the *Fear+*, *Lethal+* treatment (as suggested by Okuyama & Bolker 2007), the developmental stages (instars) of surviving larvae were recorded as well, allowing us to not only duplicate the population trajectory of the prey, but also the particular age structure of the survivors.

Methodological details

Experiments were conducted at the Washington State University Tukey Horticulture Orchard in Pullman, WA. Block 1 was initiated 27 July 2007, and Block 2 was initiated 25 August 2007. First, two *c.* 16-cm-tall *B. oleracea* plants (3-4 unfurled true leaves) were planted in each cage.

We released 20 green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) onto each plant, as this herbivore is nearly always present on *B. oleracea* plants at our study site and serves as an important prey for many of the predators (Snyder *et al.* 2006).

We allowed three days for the plants and aphids to establish, and then released 10 *P. xylostella* caterpillars (3rd instars, reared on *B. oleracea* under the same greenhouse conditions) onto each plant in each cage (= 20 caterpillars per cage). This is well within the range of *P. xylostella* densities locally under open-field conditions (Biever *et al.* 1992). On the same day, all enemy treatments were randomly applied to the cages, and the predators released. *Hippodamia*, *Geocoris*, and *Nabis* individuals were collected on adjacent vegetation within 48 h of the experiment's start, and only adult females were used. *Diadegma* females and larval *Chrysopa* were collected from greenhouse colonies.

Three response variables were measured every day, in each cage, during the course of this experiment: the number of surviving caterpillars, the number of caterpillars displaced from plants (i.e., silk-drops or otherwise displaced from plants), and the number of each predator species present on the plants. At this time, any missing predator was replaced in order to maintain predator density and diversity levels. Caterpillars were exposed to the predator treatments for 4 days, at which time caterpillars began spinning cocoons, and the experiment was ended. Cages then were carefully searched and all remaining arthropods collected. *B. oleracea* plants were harvested, dried for at least 4 d at 90° C, and weighed.

Data analyses

Final plant biomass and the total number of caterpillars observed off of plants were analyzed as 2 x 2 x 2 x 2 fully-factorial ANOVAs, including the factors species richness (high, low),

nonconsumptive effects (+, -) consumptive effects (+, -), and temporal block (1, 2). Total numbers of predators observed on plants was analyzed as a three-way ANOVA including the factors diversity treatment, species identity, and block. Caterpillar densities across the four days of each trial were analyzed using repeated measures MANOVA including the factors block (1, 2) and predators (absent, low diversity, high diversity). Here, only *Fear+*, *Lethal+* and *Fear-*, *Lethal-* cages were included in the analysis because caterpillar densities were directly manipulated in the other treatment combinations.

We tested for emergent biodiversity effects (overyielding) in the plant biomass and herbivore data, as transmitted through either consumptive or nonconsumptive channels. We calculated D_T , a metric of polyculture performance relative to the average of all constitutive species when in monoculture, and D_{max} , a metric of polyculture performance relative to that of the single most effective species in monoculture (Petchey 2003). In these calculations values significantly different than zero were taken as evidence of emergent diversity effects. One-sample *t*-tests were used to determine whether the respective D_T and D_{max} means differed significantly from zero. All analyses were conducted in SYSTAT (Systat Software, Richmond, CA, USA) (SPSS 1999).

RESULTS

Because effects on plants of our predator manipulations represented the sum of direct and indirect effects through all channels, we first present the plant biomass data. We then work our way up through the other two trophic levels, presenting behavioral and density data for herbivores and then predators.

Plants

Final plant biomass was greatest where diverse predator communities initiated predator-avoidance behavior by caterpillars (predator diversity \times nonconsumptive effects interaction, $F_{1,64} = 6.88$, $P = 0.011$; Fig. 2a). Consumption of prey also increased plant biomass (predator consumptive main effect, $F_{1,64} = 20.44$, $P < 0.001$; Fig. 2a), but this effect was not further strengthened with either greater predator diversity or the co-occurrence of nonconsumptive effects (all interactive consumptive effects $P > 0.05$; Appendix 1a; Fig. 2a). Nonconsumptive effects were stronger in *Block 2* than *Block 1* (nonconsumptive \times block interaction, $F_{1,64} = 10.12$, $P = 0.002$), but block effects otherwise did not interact with other model terms (Appendix 1a, Appendix 2a).

For plants, only the nonconsumptive effects of predators in high diversity assemblages significantly exceeded expectations, based on the summed per-capita performances of the constituent predator species in monoculture: the mean D_T value for the indirect, non-lethal effect channel was significantly greater than zero ($t = 7.43$, $df = 9$, $P < 0.001$; Appendix 3; Fig. 3a), while that of the lethal effect channel was not significantly different from zero ($t = 0.157$, $df = 9$, $P = 0.878$). The mean D_{max} values for the indirect lethal and non-lethal effect channels did not differ from zero (lethal: $t = -1.614$, $df = 9$, $P = 0.141$; non-lethal: $t = -0.069$, $df = 9$, $P = 0.946$), suggesting that in terms of either consumptive or nonconsumptive effects, the average performance of polycultures did not exceed that of the single most effective predator species (Fig. 3a; Appendix 3).

Herbivores

Mirroring the results for plant biomass, herbivore displacement from the host plant was greater among treatments coupling nonconsumptive effects with diverse predator communities (diversity

× nonconsumptive effects interaction, $F_{1,64} = 8.51$, $P = 0.005$; Fig. 2b). Again, nonconsumptive predator effects were stronger in *Block 2* than *Block 1* ($F_{1,64} = 5.30$, $P = 0.025$; Appendix 1b, Appendix 2b), though the relationship between diversity and caterpillar behavior did not change across blocks (diversity × nonconsumptive × block interaction, $F_{1,64} = 0.212$, $P = 0.647$; Appendix 1b). Displaced caterpillars were observed almost exclusively in cages bearing predators, indicating that the caterpillars generally did not stray from their host plants unless induced by predators (Fig. 2b).

The magnitude of predator-induced caterpillar mortality grew through time (predator × time interaction; $F_{3,108} = 29.65$, $P < 0.001$; Appendix 1c; Appendix 4a-c), but herbivore suppression was not further strengthened with greater predator diversity (Appendix 1d; Appendix 4a-c). Overall caterpillar mortality was significantly higher in *Block 1* than *Block 2* (Appendix 1c).

For herbivores, only nonconsumptive predator effects significantly exceeded what would be expected based on the summed per-capita performances of the constituent predator species in monoculture: the mean D_T value for the direct, non-lethal effect channel was significantly greater than zero ($t = 2.67$; $df = 9$, $P = 0.026$; Appendix 3; Fig. 3b), while that of the lethal effect channel was not significantly different from zero ($t = 1.788$, $df = 9$, $P = 0.107$). The mean D_{max} value for the direct lethal effect channel was significantly less than zero ($t = -4.047$, $df = 9$, $P = 0.003$; Appendix 3; Fig. 3b), while that of the non-lethal effect channel was not significantly different from zero ($t = -1.781$, $df = 9$, $P = 0.109$). This indicates that the consumptive effects of predators in polyculture were significantly less than, and nonconsumptive effects no different than, the single most voracious or intimidating predator species in monoculture (Appendix 3; Fig. 3b).

Predators

Predator species differed in how diversity impacted their likelihood to forage on plants (predator diversity \times species identity interaction; $F_{4,20} = 3.80$, $P = 0.019$; Fig. 4a; Appendix 1e). This interaction appeared to be driven by the responses of two species, the lady beetle *Hippodamia* and the parasitoid *Diadegma*, both of which exhibited higher per-capita visitation of plants when foraging within diverse communities (Fig. 4a); plant visitation by other predators was unaffected by diversity treatment. Overall, plant-visitation by predators was significantly higher in polyculture than in monoculture ($F_{4,20} = 8.33$, $P = 0.009$; Appendix 1e).

Predator species differed in their likelihood of dying during the experiment ($F_{4,20} = 7.14$, $P = 0.001$; Fig. 4b), but there was no difference in mortality, for any species, between *Low* and *High Diversity* treatments (diversity \times species identity interaction, $F_{4,20} = 1.91$, $P = 0.147$; Fig. 4b Appendix 1f). Thus, there was no evidence that predator diversity influenced predator mortality rates.

When in monoculture, predator species differed both in their likelihood of triggering caterpillar displacements ($F_{4,15} = 4.71$, $P = 0.012$; Fig. 4c) and in the total caterpillar mortality that they exerted ($F_{4,15} = 4.74$, $P = 0.011$; Fig. 4d). The lady beetle *Hippodamia* and the parasitoid *Diadegma* were most likely to evoke displacement (Fig. 4c), while the lacewing *Chrysopa* was the most lethal predator (Fig. 4d). In diverse predator communities, displaced and dead caterpillars could not be attributed to a particular predator species.

DISCUSSION

Nonconsumptive interactions are increasingly recognized as ubiquitous and important ecological phenomena, influencing not only how species interact but also how communities function (Lima

& Dill 1990; Sih *et al.* 1998; Schmitz *et al.* 2004; Preisser *et al.* 2005). However, it has yet to be resolved how the effects of behavioral interactions scale with changes in species diversity. Our fully-factorial manipulation of predator diversity and predator impacts operating through nonconsumptive (behavioral) and consumptive (lethal) channels, allowed us to explore the independent and interactive effects of these factors. We found that trophic cascades were strengthened through an interaction between predator diversity and predator nonconsumptive effects, with caterpillars significantly more likely to engage in antipredator behavior when facing diverse than single-species predator communities (Fig. 2a, b). While predators also protected plants by killing herbivores (Fig 2a; Appendix 4a-c), predator effects through the lethal channel were not influenced by predator diversity or the occurrence of antipredator behavior. In monoculture, two predator species were particularly strong disruptors of caterpillar feeding, the parasitoid *Diadegma* and the lady beetle *Hippodamia* (Fig. 4c), and these same two predator species were more likely to forage on plants when embedded within diverse communities (Fig. 4a). Together, these results suggest that predator diversity effects were conducted through a chain of behavioral interactions, with the most intimidating predators more likely to occur on plants, and thus to disrupt herbivore feeding, when within diverse communities.

The cascading diversity effects we observed appear to have been initiated by a reduction in the degree of interference among predators in the diverse communities. Almost invariably, caterpillars remained on their host plant until confronted by predators (Fig. 2b), which generally forced predators to converge on the plants in order to find prey. Plant-visitation by predators, therefore, represented an important indicator of predator-predator interactions. Our finding that plant-visitation was greater in the high diversity treatment (Appendix 1e) suggests that interference was relaxed to some extent among predators in the multi-species communities. In

our low diversity arenas, predators interacted only with members of their own species, whereas in diverse arenas predators interacted only with members of other species. Thus, intraspecific competition among predators was entirely eliminated within the diverse communities. To the extent that intraspecific competition may exceed interspecific competition for some species, relief from intraspecific interactions may facilitate greater foraging efficiency. It was evident in our study that the predators *Diadegma* and *Hippodamia* were much more likely to forage for prey within cages housing heterospecific than conspecific competitors (Fig. 4a), suggesting that the release from intraspecific interference in diverse communities allowed for increased foraging rates. There is good evidence in the literature of strong intraspecific interference among arthropod predators (Sih *et al.* 1998). Both lady beetles and parasitoids have the ability to recognize and respond to cues left by earlier-arriving conspecific competitors (Rosenheim 1998; Hodek & Michaud 2008), which is thought to be adaptive because of the high risk of cannibalism for progeny oviposited where conspecifics already occur. In our experiments predator mortality was rare overall, and we saw no evidence that intraguild predation exceeded mortality (if any) due to cannibalism (Fig. 4b). This strengthens our argument that predator-predator interactions were primarily behavioral. At least one other study has provided theoretical evidence that relief from negative intraspecific competition can generate positive diversity effects (Weis *et al.* 2007); the mechanism in that case was the reduction of intraspecific resource competition at higher diversity levels, rather than the avoidance of cannibalism.

Nonetheless, we were curious whether higher plant-visitation rates by *Diadegma* and *Hippodamia* could entirely explain the behaviorally-mediated diversity cascade that we observed. We can examine this quantitatively by calculating an “expected” number of displacements for each polyculture replicate, under the simplifying assumption that a plant visit

by a predator incites the same level of intimidation regardless of diversity treatment. We first calculate the number of caterpillar displacements per predator, per plant-visit for each species in monoculture (of course, displacements cannot be attributed to particular predator species in the polycultures). Then, multiplying this value by the observed plant visitation rate of each predator species in a given polyculture, and summing these values across all species in that polyculture, yields the expected total number of caterpillar displacements for that replicate. Intriguingly, observed caterpillar displacements in the polycultures were greater than predicted by this calculation ($t = 2.41$, $df = 19$, $P = 0.026$; Fig. 5). Thus, accounting for higher per-capita visitation rates by the two most intimidating predator species does not entirely explain the greater nonconsumptive effects of diverse predator assemblages, suggesting that another factor was also at work. One possibility is that predator consumptive and nonconsumptive effects scale differently with predator density. This could occur, for example, because a predator can simultaneously intimidate many prey individuals but kill only one at a time (Peacor & Werner 2001). Behaviorally-mediated effects could remain relatively intense as the density of particular predator species declined, as happens at higher diversity levels within substitutive designs. Consistent with this explanation, in our experiments nonconsumptive effects consistently equaled those of the single most intimidating predator species, whereas consumptive effects reflected the average predation rates across species (Fig. 3). A relative insensitivity of nonconsumptive effects to predator density would allow these effects to remain consistently intense in diverse communities, even when greater diversity dilutes densities of the more intimidating predator species. This points to the possibility that the “scariest” species can effectively saturate a system with fear.

More generally, similarity in hunting domain and foraging mode increases the probability of predator-predator encounters and thereby may heighten interference (Björkman & Liman 2005; Schmitz 2005; Preisser *et al.* 2007). Interference can be direct, manifesting as aggressive or despotic behavior among predators (Shaffer & Robinson 1996; Switalski 2003), or indirect, with intraguild prey assessing their surroundings for evidence of intraguild predators and actively avoiding encounters (Soluk & Collins 1988; Siddon & Witman 2004; Vance-Chalcraft & Soluk 2005; Berger *et al.* 2008). Given that conspecific predators would be expected to be more similar than heterospecifics, intraspecific competition should be greater than interspecific competition (MacArthur 1958). Hence, if foraging in a more diverse predator community can spare an actively hunting species from frequent encounters with conspecifics (e.g., Björkman & Liman 2005), then such predators should be more efficient in polyculture. Our results are consistent with this expectation, yet our data also represent a departure from studies isolating the cascading effects of diversity. Predator diversity effects have been shown to cascade via lethal predator-prey interactions (Bruno & Cardinale 2008), as well as by lethal predator-predator interactions (Finke & Denno 2004). Our data suggest that cascading diversity effects can also be initiated by non-lethal interactions among predators, which dovetails well with recent work investigating how the effects of complementary predator-prey relationships may cascade via non-lethal channels (Byrnes *et al.* 2006).

To our knowledge, no previous studies have isolated nonconsumptive from consumptive effects while also manipulating consumer diversity, *per se*. Theoretical and empirical evidence indicates that species richness may commonly influence the nature and magnitude of nontrophic interactions among species (Sih *et al.* 1998; Borer *et al.* 2002; Byrnes *et al.* 2006; Goudard & Loreau 2008). It is striking how often behavioral interactions underlie emergent diversity effects

for predators and other animal consumers. For example, inherent differences in foraging behavior and/or prey choice can lead to a positive relationship between predator diversity and herbivore exploitation (Preisser *et al.* 2007; Finke & Snyder 2008). Similarly, prey species may exhibit predator-specific antipredator behavior, modulated to reflect the differing risks posed by different predator species (Shaffer & Robinson 1996; Byrnes *et al.* 2006; Castellanos & Barbosa 2006). This suggests that behavioral interactions may mediate diversity effects to the same degree that lethal interactions may, as has been found when looking at simpler subsets of consumer communities (Preisser *et al.* 2005).

Our results bring us to several conclusions. First, behaviorally-mediated diversity effects can cascade across multiple trophic levels, as do density-mediated effects (Ives *et al.* 2005; Duffy *et al.* 2007). This reinforces suggestions that nonconsumptive interactions warrant greater attention in ecological research (Brown *et al.* 1999), and that it may be unwise to rely entirely on abundance or biomass metrics when quantifying diversity effects (Byrnes *et al.* 2006; Goudard & Loreau 2008). Second, consumer effects operating via nonconsumptive channels might act quite differently than, and in our study were independent from, those due to resource consumption. This raises the possibility that diversity effects attributed to consumptive interactions may instead be partially, or entirely, due to nonconsumptive interactions. It appears that many cases of consumptive diversity effects in animal communities have complex behavioral underpinnings (Bruno & Cardinale 2008), and only a more consistent consideration of how behavior influences diversity effects will allow incorporation of these effects into theory (Borer *et al.* 2002; Goudard & Loreau 2008).

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FIGURE LEGENDS

Figure 1 Factorial structure of the experiment: predator diversity (low, high) × consumptive effects (lethal+, lethal-) × nonconsumptive effects (fear+, fear-) × temporal block (1, 2). All factors were fully-crossed, achieving a complete $2 \times 2 \times 2 \times 2$ factorial design.

Figure 2 (a) Final plant biomass and (b) Total number of caterpillars observed off of plants, for three factors: predator diversity (low, high), predator nonconsumptive effects (fear+, fear-), and predator consumptive effects (lethal+, lethal-). Data are shown averaged across the two temporal blocks; the data are presented separately by block in Appendix 2. Data are means \pm 1 SE.

Figure 3 Two measures of overyielding, D_T and D_{max} , for predator diversity effects on (a) plant biomass and (b) herbivore density (Lethal predator effect) and behavioral responses (Fear effect). Predator diversity effects could be transmitted through two channels, either through the death of herbivores (lethal) or the initiation of herbivore antipredator behavior (fear). Data are means \pm 95% CI.

Figure 4 Occurrences, per-capita, of each predator species (a) observed foraging on plants, and (b) dying during the course of the experiment in high and low diversity compositions. Total number of caterpillars (c) displaced and (d) killed by each predator species when in monoculture. Note that caterpillars displaced or killed in predator polycultures could not be attributed to any particular predator species. Data are for the predator species *Hippodamia* (Hip), *Diadegma* (Dia), *Chrysopa* (Chr), *Nabis* (Nab), and *Geocoris* (Geo). Data are means \pm 1 SE.

Figure 5 Predicted and observed caterpillar displacements in high diversity species compositions. Predicted displacement for a given polyculture was calculated by summing the predicted displacements generated by each species constituting the polyculture: $\sum[(\text{observed displacements})_i / (\text{observed visits})_i] (\text{observed visits})_{ij}$, for the i^{th} species in monoculture, and the i^{th} species within the j^{th} polyculture. Data are means \pm 1 SE.

Figure 1

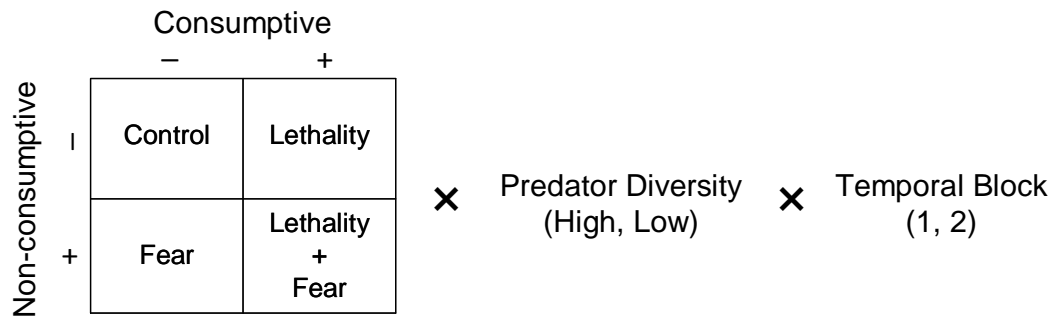


Figure 2

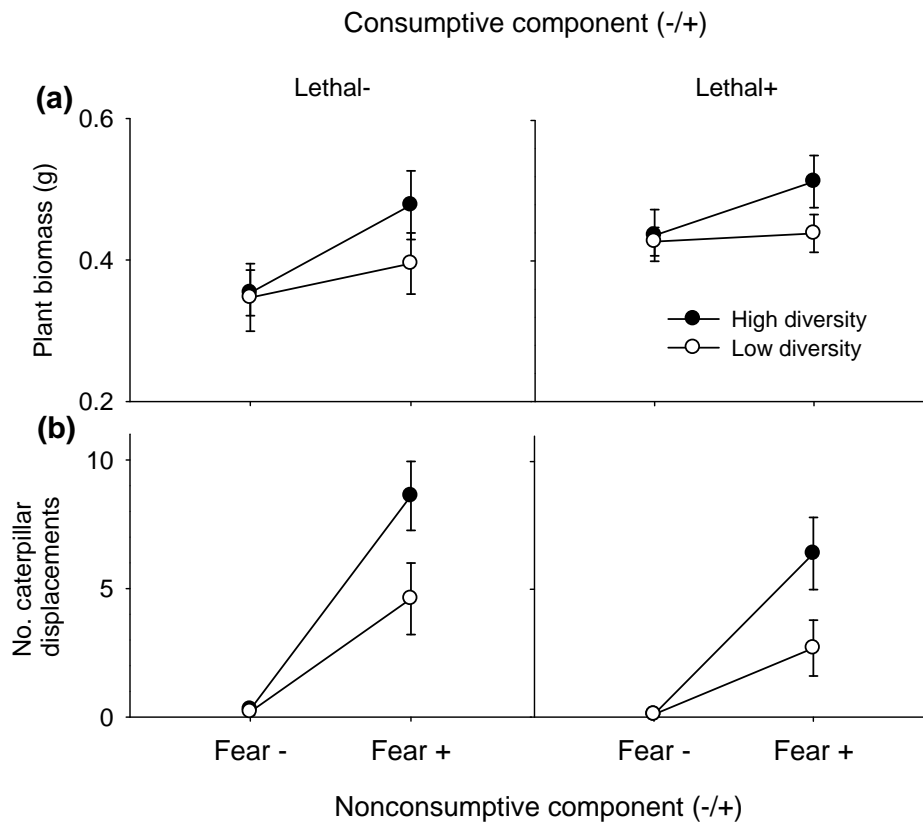


Figure 3

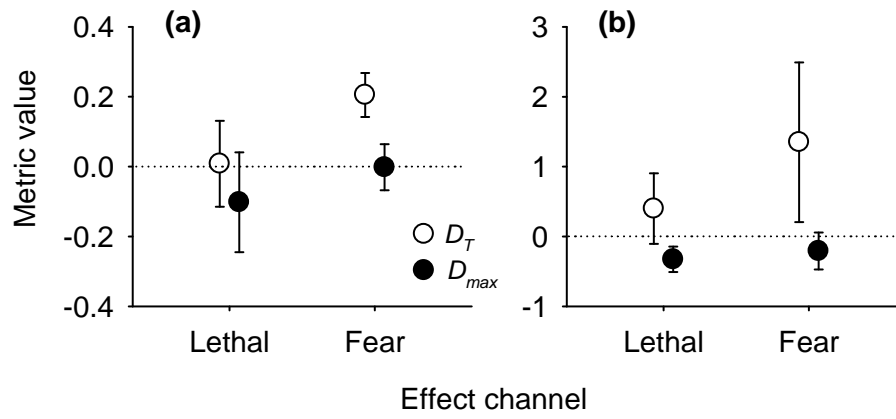


Figure 4

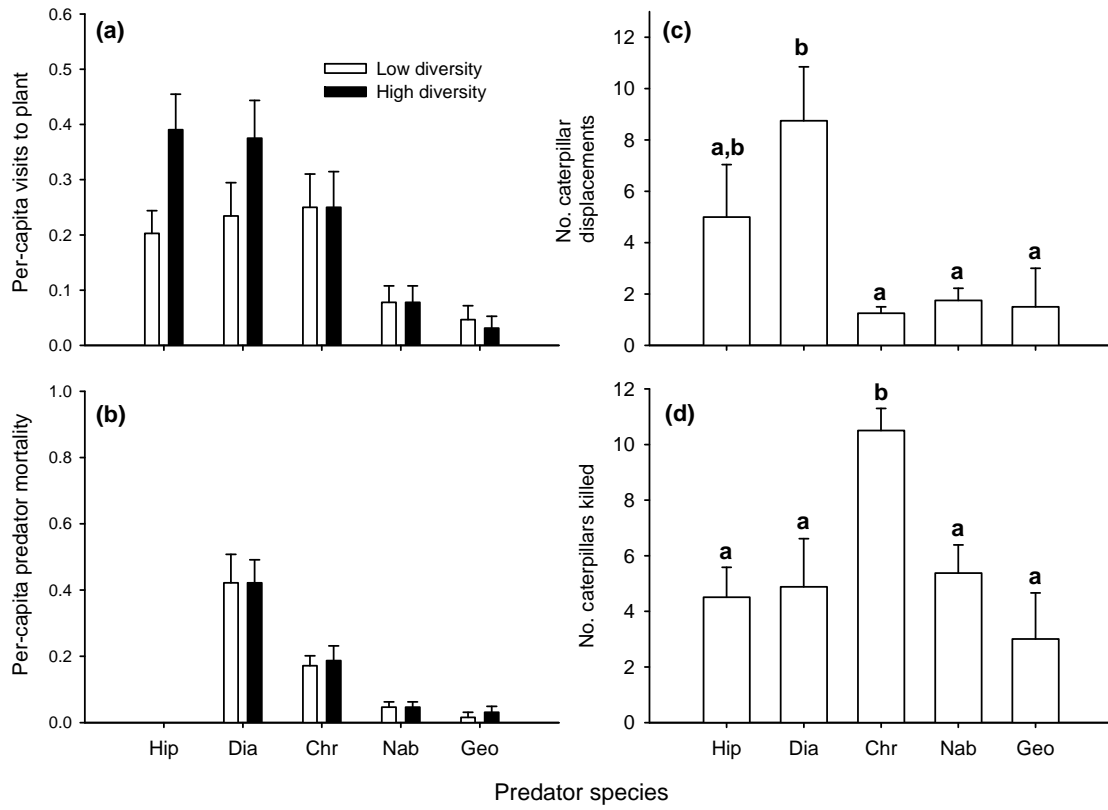
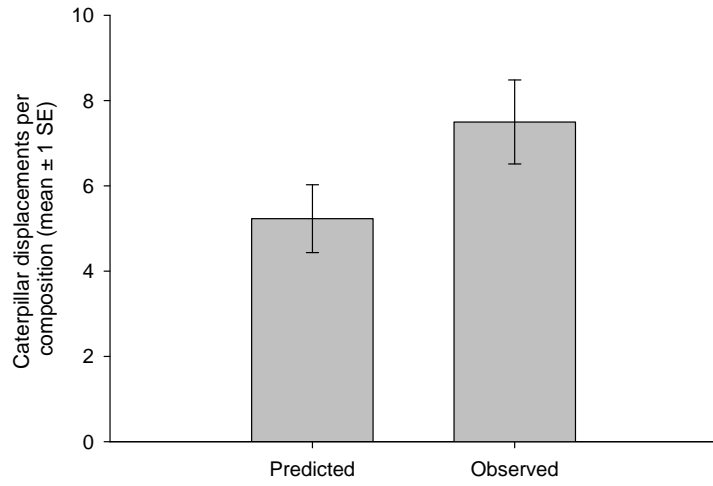


Figure 5



APPENDICES

Appendix 1: Statistical tables

Appendix 1a Four-way ANOVA of the effects on final plant biomass of an experimental manipulation of predator diversity, predator nonconsumptive effects, and predator consumptive effects. The experiment was conducted in two temporal blocks.

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Diversity	1	0.037	10.23	0.002
Consumptive	1	0.073	20.44	< 0.001
Nonconsumptive	1	0.085	23.54	< 0.001
Block	1	0.725	201.84	< 0.001
Diversity × Consumptive	1	0.0001	0.015	0.904
Diversity × Nonconsumptive	1	0.025	6.88	0.011
Diversity × Block	1	0.006	1.62	0.208
Consumptive × Nonconsumptive	1	0.009	2.42	0.125
Consumptive × Block	1	0.036	10.12	0.002
Nonconsumptive × Block	1	0.002	0.695	0.408
Diversity × Consumptive × Nonconsumptive	1	0.0002	0.041	0.840
Diversity × Consumptive × Block	1	0.013	3.48	0.067
Diversity × Nonconsumptive × Block	1	0.005	1.35	0.250
Consumptive × Nonconsumptive × Block	1	0.003	0.72	0.399
Diversity × Consumptive × Nonconsumptive × Block	1	0.008	2.11	0.151
Error	64	0.0036		

Appendix 1b Four-way ANOVA of the effects on herbivore displacement from plants of an experimental manipulation of predator diversity, predator nonconsumptive effects, and predator consumptive effects. The experiment was conducted in two temporal blocks.

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Diversity	1	76.05	8.96	0.004
Consumptive	1	24.20	2.85	0.096
Nonconsumptive	1	583.20	68.71	< 0.001
Block	1	36.45	4.29	0.042
Diversity × Consumptive	1	0.20	0.024	0.878
Diversity × Nonconsumptive	1	72.20	8.51	0.005
Diversity × Block	1	2.45	0.29	0.593
Consumptive × Nonconsumptive	1	18.05	2.13	0.150
Consumptive × Block	1	< 0.001	< 0.001	0.999
Nonconsumptive × Block	1	45.00	5.30	0.025
Diversity × Consumptive × Nonconsumptive	1	0.05	0.006	0.939
Diversity × Consumptive × Block	1	0.20	0.024	0.878
Diversity × Nonconsumptive × Block	1	1.80	0.212	0.647
Consumptive × Nonconsumptive × Block	1	0.45	0.053	0.818
Diversity × Consumptive × Nonconsumptive × Block	1	1.25	0.147	0.702
Error	64	8.49		

Appendix 1c Repeated measures MANOVA comparison of the effects on caterpillar densities of predators being present (low and high predator diversity treatments pooled) versus absent.

Between-Subjects:

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Predation	1	1670.56	89.97	< 0.001
Block	1	154.06	8.30	0.007
Predation × Block	1	97.66	5.26	0.028
Error	36	18.57		

Within-Subjects:

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Time	3	127.99	54.07	< 0.001
Time × Predation	3	70.17	29.65	< 0.001
Time × Block	3	22.87	9.66	< 0.001
Time × Predation × Block	3	6.17	2.61	0.055
Error	108	2.37		

Appendix 1d Repeated measures MANOVA comparison of the effects on caterpillar densities of predators at high versus low diversity.

Between-Subjects:

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Diversity	1	40.61	1.12	0.305
Block	1	248.51	6.88	0.018
Diversity × Block	1	13.61	0.38	0.548
Error	16	36.12		

Within-Subjects:

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Time	3	193.08	44.47	< 0.001
Time × Diversity	3	3.25	0.75	0.529
Time × Block	3	26.21	6.04	0.001
Time × Diversity × Block	3	2.91	0.67	0.574
Error	48	4.34		

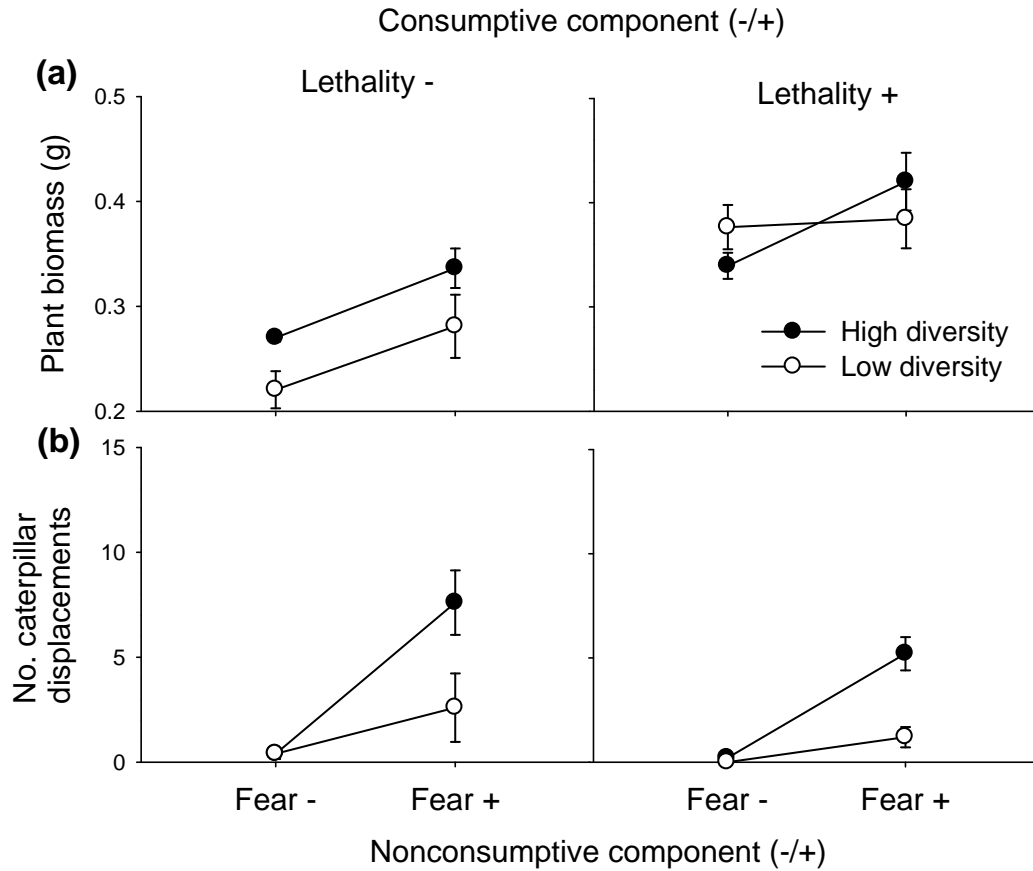
Appendix 1e Three-way ANOVA of predator plant-visitation frequency. The effects of Diversity (1 versus 4 species), Species Identity, and Block (2 temporal blocks) are fully crossed.

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Diversity	1	10.00	8.33	0.009
Species Identity	4	32.46	27.05	< 0.001
Block	1	0.900	0.750	0.397
Diversity × Species Identity	4	4.56	3.80	0.019
Diversity × Block	1	0.100	0.083	0.776
Species Identity × Block	4	1.59	1.32	0.296
Diversity × Species Identity × Block	4	1.04	0.865	0.502
Error	20	1.20		

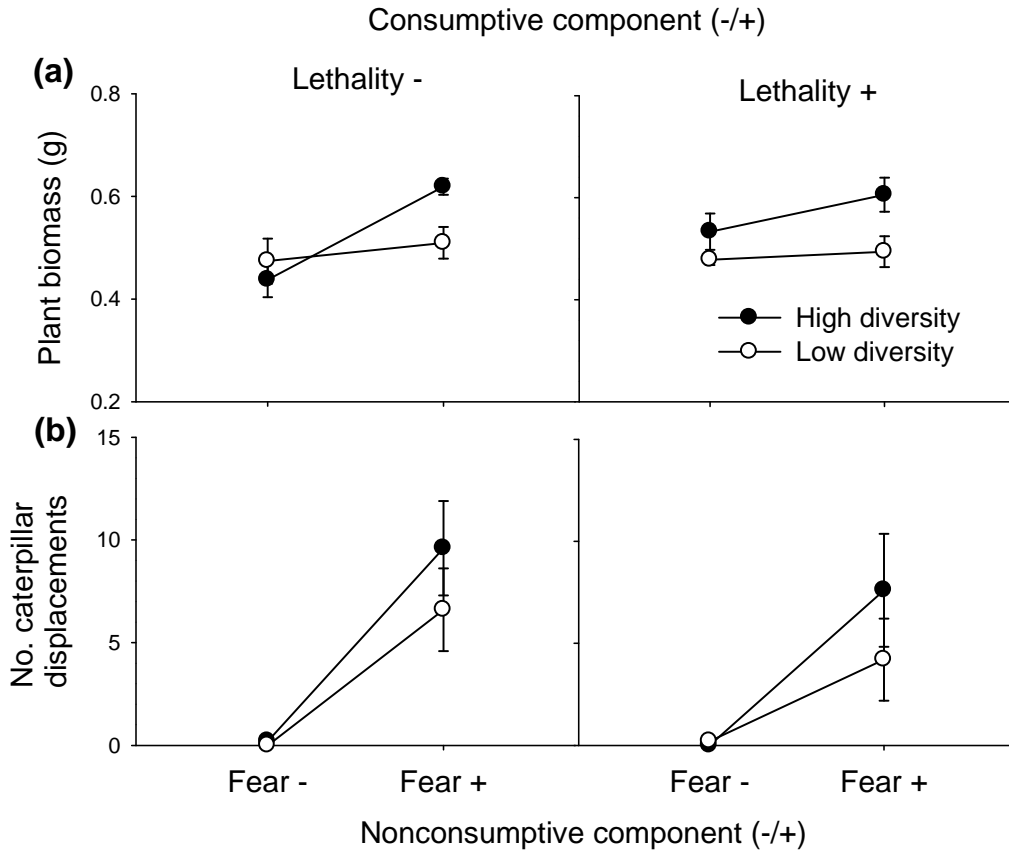
Appendix 1f Three-way ANOVA of predator mortality. Model terms as in Table 1e.

Source	d.f.	MS	<i>F-ratio</i>	<i>P</i>
Diversity	1	0.400	0.276	0.605
Species Identity	4	10.35	7.138	0.001
Block	1	0.400	0.276	0.605
Diversity × Species Identity	4	2.78	1.914	0.147
Diversity × Block	1	1.60	1.103	0.306
Species Identity × Block	4	2.78	1.914	0.147
Diversity × Species Identity × Block	4	3.35	2.310	0.093
Error	20	1.45		

Appendix 2a For temporal block 1, (a) Final plant biomass and (b) Total number of caterpillars observed off of plants, for three factors: predator diversity (Low, High), predator nonconsumptive effects (Fear+, Fear-), and predator consumptive effects (Lethality+, Lethality-). Data are means \pm 1 SE.



Appendix 2b For temporal block 2, (a) Final plant biomass and (b) Total number of caterpillars observed off of plants, for three factors: predator diversity (Low, High), predator nonconsumptive effects (Fear+, Fear-), and predator consumptive effects (Lethality+, Lethality-). Data are means \pm 1 SE.



Appendix 3 Diagnostic variables for the assessment of predator performance in monoculture and polyculture are presented. Performances are categorized as consumptive and nonconsumptive (prey intimidation), then further divided by direct and indirect effects. Mean prey consumption ($N = 4$) is quantified as the difference between the number of DBM surviving in the Controls (averaged across the four sampling dates) and the number of DBM surviving in a given treatment (averaged across the four repeated sampling dates). Prey intimidation is measured as the total number of DBM displaced ($N = 4$) in a given treatment over all sample dates. Indirect effects are measured in terms of plant protection (final plant biomass, g).

A. Trial 1

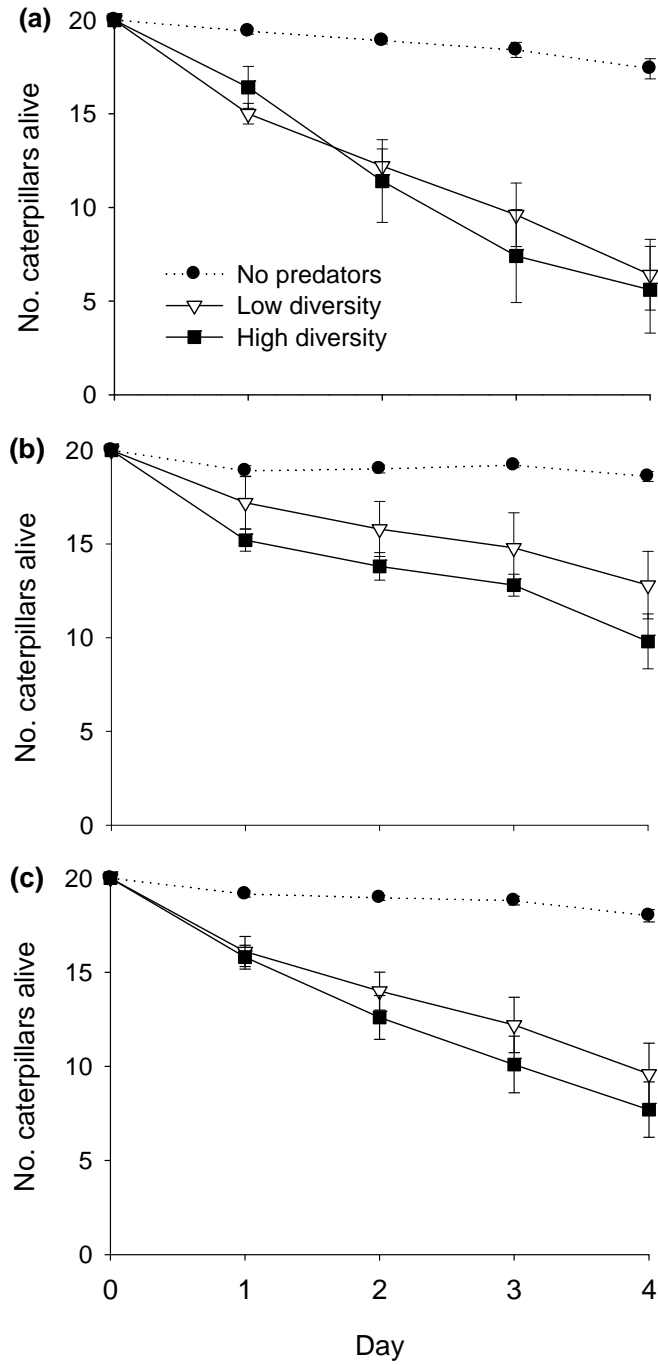
Monocultures	M_i	ε_i	Polycultures	O_j	E_j	D_{Tj}	D_{max}
Prey consumption:							
A) Direct effects (DBM consumed)							
<i>Hip, Hip, Hip, Hip</i>	6.28	1.57	<i>Dia, Chr, Nab, Geo</i>	13.27	8.09	0.641	0.127
<i>Dia, Dia, Dia, Dia</i>	7.78	1.94	<i>Hip, Chr, Nab, Geo</i>	11.27	7.71	0.462	-0.042
<i>Chr, Chr, Chr, Chr</i>	11.78	2.94	<i>Dia, Hip, Nab, Geo</i>	8.52	6.71	0.270	-0.276
<i>Nab, Nab, Nab, Nab</i>	7.03	1.76	<i>Dia, Hip, Chr, Geo</i>	5.03	7.90	-0.364	-0.573
<i>Geo, Geo, Geo, Geo</i>	5.78	1.44	<i>Dia, Hip, Chr, Nab</i>	3.25	8.21	-0.571	-0.701
B) Indirect effects (plant biomass)							
<i>Hip, Hip, Hip, Hip</i>	0.38	0.094	<i>Dia, Chr, Nab, Geo</i>	0.34	0.377	-0.085	-0.246
<i>Dia, Dia, Dia, Dia</i>	0.37	0.091	<i>Hip, Chr, Nab, Geo</i>	0.31	0.379	-0.184	-0.322
<i>Chr, Chr, Chr, Chr</i>	0.34	0.085	<i>Dia, Hip, Nab, Geo</i>	0.38	0.386	-0.005	-0.159
<i>Nab, Nab, Nab, Nab</i>	0.35	0.086	<i>Dia, Hip, Chr, Geo</i>	0.33	0.384	-0.141	-0.277
<i>Geo, Geo, Geo, Geo</i>	0.46	0.114	<i>Dia, Hip, Chr, Nab</i>	0.33	0.357	-0.079	-0.281
Prey intimidation:							
A) Direct effects (displacements)							
<i>Hip, Hip, Hip, Hip</i>	2.00	0.500	<i>Dia, Chr, Nab, Geo</i>	5.00	2.75	0.818	-0.444
<i>Dia, Dia, Dia, Dia</i>	9.00	2.250	<i>Hip, Chr, Nab, Geo</i>	6.00	1.00	5.000	-0.333
<i>Chr, Chr, Chr, Chr</i>	1.00	0.250	<i>Dia, Hip, Nab, Geo</i>	5.00	3.00	0.667	-0.444
<i>Nab, Nab, Nab, Nab</i>	1.00	0.250	<i>Dia, Hip, Chr, Geo</i>	9.00	3.00	2.000	0.000
<i>Geo, Geo, Geo, Geo</i>	0.00	0.000	<i>Dia, Hip, Chr, Nab</i>	13.00	3.25	3.000	0.444
B) Indirect effects (plant biomass)							
<i>Hip, Hip, Hip, Hip</i>	0.32	0.080	<i>Dia, Chr, Nab, Geo</i>	0.29	0.27	0.055	-0.160
<i>Dia, Dia, Dia, Dia</i>	0.32	0.079	<i>Hip, Chr, Nab, Geo</i>	0.34	0.27	0.251	0.000
<i>Chr, Chr, Chr, Chr</i>	0.25	0.063	<i>Dia, Hip, Nab, Geo</i>	0.35	0.29	0.212	0.024
<i>Nab, Nab, Nab, Nab</i>	0.34	0.085	<i>Dia, Hip, Chr, Geo</i>	0.31	0.27	0.161	-0.093
<i>Geo, Geo, Geo, Geo</i>	0.18	0.044	<i>Dia, Hip, Chr, Nab</i>	0.40	0.31	0.291	0.165

B. Trial 2

Monocultures	M_i	ε_i	Polycultures	O_j	E_j	D_{Tj}	D_{max}
Prey consumption:							
A) Direct effects (DBM consumed)							
<i>Hip, Hip, Hip, Hip</i>	2.93	0.731	<i>Dia, Chr, Nab, Geo</i>	4.18	3.99	0.047	-0.557
<i>Dia, Dia, Dia, Dia</i>	2.18	0.544	<i>Hip, Chr, Nab, Geo</i>	6.42	4.18	0.539	-0.318
<i>Chr, Chr, Chr, Chr</i>	9.43	2.356	<i>Dia, Hip, Nab, Geo</i>	7.18	2.36	2.037	-0.239
<i>Nab, Nab, Nab, Nab</i>	3.93	0.981	<i>Dia, Hip, Chr, Geo</i>	4.92	3.74	0.318	-0.477
<i>Geo, Geo, Geo, Geo</i>	0.43	0.106	<i>Dia, Hip, Chr, Nab</i>	7.42	4.61	0.610	-0.212
B) Indirect effects (plant biomass)							
<i>Hip, Hip, Hip, Hip</i>	0.45	0.112	<i>Dia, Chr, Nab, Geo</i>	0.45	0.486	-0.067	-0.103
<i>Dia, Dia, Dia, Dia</i>	0.50	0.124	<i>Hip, Chr, Nab, Geo</i>	0.55	0.474	0.158	0.085
<i>Chr, Chr, Chr, Chr</i>	0.51	0.127	<i>Dia, Hip, Nab, Geo</i>	0.66	0.472	0.391	0.297
<i>Nab, Nab, Nab, Nab</i>	0.48	0.119	<i>Dia, Hip, Chr, Geo</i>	0.54	0.479	0.116	0.057
<i>Geo, Geo, Geo, Geo</i>	0.47	0.117	<i>Dia, Hip, Chr, Nab</i>	0.47	0.482	-0.019	-0.066
Prey intimidation:							
A) Direct effects (displacements)							
<i>Hip, Hip, Hip, Hip</i>	9.000	2.250	<i>Dia, Chr, Nab, Geo</i>	8.00	6.00	0.333	-0.385
<i>Dia, Dia, Dia, Dia</i>	13.00	3.250	<i>Hip, Chr, Nab, Geo</i>	5.00	5.00	0.000	-0.615
<i>Chr, Chr, Chr, Chr</i>	2.000	0.500	<i>Dia, Hip, Nab, Geo</i>	16.00	7.75	1.065	0.231
<i>Nab, Nab, Nab, Nab</i>	3.000	0.750	<i>Dia, Hip, Chr, Geo</i>	14.00	7.50	0.867	0.077
<i>Geo, Geo, Geo, Geo</i>	6.000	1.500	<i>Dia, Hip, Chr, Nab</i>	5.00	6.75	-0.259	-0.615
B) Indirect effects (plant biomass)							
<i>Hip, Hip, Hip, Hip</i>	0.54	0.135	<i>Dia, Chr, Nab, Geo</i>	0.65	0.502	0.303	0.066
<i>Dia, Dia, Dia, Dia</i>	0.44	0.109	<i>Hip, Chr, Nab, Geo</i>	0.59	0.528	0.111	-0.044
<i>Chr, Chr, Chr, Chr</i>	0.47	0.117	<i>Dia, Hip, Nab, Geo</i>	0.58	0.520	0.122	-0.049
<i>Nab, Nab, Nab, Nab</i>	0.61	0.153	<i>Dia, Hip, Chr, Geo</i>	0.62	0.484	0.272	0.002
<i>Geo, Geo, Geo, Geo</i>	0.49	0.123	<i>Dia, Hip, Chr, Nab</i>	0.66	0.514	0.275	0.069

M_i = effect of species i in monoculture; $\varepsilon_i = p_i M_i$ = average per-capita performance of predators in the i^{th} monoculture (= *expected* per-capita performance of species i in polyculture), where p_i = proportion of M_i represented by one individual (= 0.25); O_j = observed effect of the j^{th} polyculture; E_j = expected effect of the j^{th} polyculture (= summed ε_i for the constituent species of the j^{th} polyculture); $D_{Tj} = (O_j - E_j) / E_j$ = proportional deviation of O_j from E_j . D_{Tj} quantifies the magnitude of the diversity effect relative to what would be expected based on the constituent species' performances in monoculture. $D_{max} = (O_j - M_{max}) / M_{max}$, where M_{max} was the single best performance of predators in monoculture. All M_{max} species and respective performances are in bold.

Appendix 4 Caterpillar densities through time for (a) temporal block 1, (b) temporal block 2, and (c) averaged across both blocks, by predator diversity treatment. Data are means \pm 1 SE.



CHAPTER THREE

DENSITY-DEPENDENCE IN CASCADING FEAR EFFECTS

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Abstract

1. Nonlethal interactions between predators and their prey are often important elements of community-scale predator effects. As herbivorous prey trade optimal feeding rates for immediate safety, the indirect effects of predators may be transmitted to the host-plants of the prey. Thus, the predator-plant relationship should be governed, to some degree, by the number of prey feeding on the plant.

2. We hypothesized that the magnitude of cascading fear effects is a function of prey density. To test this hypothesis, we investigated how indirect, nonconsumptive predator effects varied relative to prey density in a tri-trophic community module. Four densities of caterpillars were established on their host plant and then exposed to a non-lethal enemy. At each prey density, we measured enemy foraging behavior, caterpillar biomass, and plant biomass.

3. As the density of herbivorous prey increased, plant biomass decreased consistently, but only in the absence of the nonlethal enemy. Where a nonlethal enemy was allowed to forage freely within the community, plant biomass remained relatively unchanged over the full range of prey densities. Thus, with increasing prey density, the amount of plant biomass protected by enemy activities also increased.

4. The mechanism underlying this result was increased enemy foraging effort with increasing prey density, which induced suboptimal feeding rates in the prey. Prey exposed to the enemy weighed much less than those feeding in the absence of a predation threat.
5. There was strong evidence of a positive, linear relationship between prey density and the parasitoid-plant interaction strength, suggesting that cascading, nonlethal enemy effects were sensitive to the density of the prey.

Keywords

Fear ecology, predator-prey, trophic cascade, *Diadegma*, *Plutella xylostella*, non-lethal, trait-mediated interaction

INTRODUCTION

Predators shape community structure not only by consuming prey (Schmitz *et al.*, 2000), but also by evoking antipredator and other vigilance responses in the prey population (see reviews by Lima & Dill, 1990; Sih *et al.*, 1998; Werner & Peacor, 2003; Schmitz *et al.*, 2004). The nonlethal effects of predators often cascade to significantly affect the resource base on which their prey feed (Ripple & Beschta, 2004; Preisser *et al.*, 2005), and although community-level trophic cascades tend to be uncommon (Polis *et al.*, 2000), there is evidence to suggest that linear, species-level cascades within subsets of terrestrial communities may be relatively frequent phenomena (Schmitz *et al.*, 2000). Thus, even where predators do not substantially reduce prey populations, plant species within particular community modules may benefit if predators compel their prey to feed less in favor of vigilance against a perceived predation threat. To the extent that predators indirectly protect plants in this way, predators maintain significant indirect relationships with plants.

Fear of predators has been shown to linger over relatively long temporal scales (Valeix *et al.*, 2009), as well as to effectively saturate areas of a victim species' "fear landscape" (van der Merwe & Brown, 2008). In cases where a predator exerts a significant nonlethal effect on its prey, the cascading effect on the prey's resource may be, to some extent, a function of prey density. For example, consider a hypothetical predator-prey relationship between a mountain lion, *Puma concolor*, and its prey, a herd of mule deer, *Odocoileus hemionus* (an elaboration of an example originally discussed in Brown *et al.*, [1999]): the mule deer herd is stalked by the mountain lion, and until the moment the herd is attacked, the mule deer feed voraciously on the grasses and forbs within a meadow. In this example, the mule deer ultimately evade their enemy, fleeing far from the meadow, and although the predator failed to catch any deer, the meadow

flora benefitted from the relief from herbivory. Given that the entire herd ceased feeding and fled the meadow, the magnitude of the relief from herbivory should relate directly to the size of the herd. Thus, the magnitude of the indirect enemy effect should scale with the size of the herd being flushed from the meadow. More broadly, if a predator species imposes a significant nonconsumptive effect on its prey, and if this effect is effectively transmitted along a linear cascade, inhibiting consumption of the prey's food base, then the magnitude of the trophic cascade will increase linearly with the density of the prey.

To explore whether this hypothesis had empirical support, we examined data from a field experiment designed originally to explore predator-predator interactions (see Materials and Methods; Fig. 1A, B). These preliminary data showed that over a wide range of potential herbivory pressure, plant biomass remained remarkably unchanged when a particular enemy species was present—the actively foraging parasitoid, *Diadegma insulare* (Cresson) (Fig. 1B). This parasitoid had been rendered nonlethal by shortening and blunting its ovipositor. In the absence of *D. insulare*, plant biomass decreased with increasing herbivory pressure (Fig. 1A), as would be expected in the absence of any intervening factors. This linear relationship should also hold in the presence of a predator, in which case reductions in prey density due to predation would allow proportional increases in plant biomass. Our data suggested that *D. insulare*—a nonlethal parasitoid—increasingly deflected the effects of herbivory as herbivory pressure increased. The result may have arisen because the parasitoid modulated its foraging effort relative to prey density, as has been shown with a congener of *Diadegma* (Waage, 1983; Wang & Keller, 2002). Diamondback moth caterpillars are known to deploy a distinct antipredator behavior—dropping from leaf undersides by a silk, and hanging until the perceived threat is gone (Wang & Keller, 2002). This defensive behavior provides a mid-air refuge from enemies but

likely reduces feeding efficiency. While the exact mechanisms by which the nonlethal enemy effect cascaded in our study are unclear, the magnitude of the cascading effect on the plants appeared to increase with prey density. Because the field study (Fig. 1A, B) did not explicitly control the density of the prey (diamondback moth caterpillars, *Plutella xylostella* [L.]), we decided to investigate in a controlled greenhouse environment how the direct and indirect nonconsumptive effects of *D. insulare* scaled with prey density. To illuminate the mechanisms underlying the relationship, we measured response variables at each trophic level of a community module: enemy foraging time, caterpillar biomass, and plant biomass.

MATERIALS AND METHODS

Preliminary field study

The field study that motivated our greenhouse experiments was conducted at the Washington State University (WSU) R.B. Tukey Horticulture Orchard in Pullman (46°44' N, 117°10' W). In this study, the experimental units were 60 × 60 × 60-cm field cages (BugDorm-3120, MegaView Science Education Services Co., Taiwan), enclosed on three sides by polyester netting and on the fourth with a clear plastic panel that allowed easy viewing of and access to the interior of the cage. Within each cage one collard plant was established, and 8 third-instar *P. xylostella* larvae were released onto the plant. A fully-crossed multi-factorial design was employed in which the effect of a nonlethal parasitic wasp, *D. insulare* (present/absent), was crossed with that of a lethal predator, *Nabis alternatus* Parshley (present/absent), yielding a 2 × 2 factorial structure with four unique predator treatments. The parasitic wasps were rendered nonlethal by shortening and blunting their ovipositors (ovipositors of female wasps were clipped with a small blade, following immobilization of the wasps via brief cooling to a near-freezing temperature). There

were ten replications of the factorial structure, requiring 40 replicate cages. The experiment was initiated on 30 June, 2006, and terminated on 11 July, 2006. Every second day, the number of living *P. xylostella* larvae was counted. On the final day of the trial, the number of surviving larvae in each cage was counted and the plants removed for subsequent dry weight measurements. A “larva-day” unit was calculated for each replicate by multiplying the number of larvae in a cage by the number of days since the previous larval count, then summing across the experiment’s duration. Accrued larva-days represented an estimate of potential herbivory pressure for a given replicate (though the larva-days metric was likely an underestimate of true herbivory pressure).

Greenhouse experiment

In a controlled greenhouse environment (16:8 h photoperiod; 22°:16° C temperature regime) in the spring of 2007, two trials were conducted to discern whether prey density mediated the indirect, nonlethal effects of enemies on plants. We assembled model tri-trophic communities within BugDorms, in which collard plants, *Brassica oleracea* L., were fed upon by diamondback moth caterpillars, while nonlethal enemies (female *D. insulare* wasps with clipped ovipositors) foraged for and attacked the caterpillars. There were two collard plants in each cage, and caterpillar densities were manipulated such that there were 0, 4, 8, or 16 caterpillars per cage (0, 2, 4, and 8/plant). Half the cages received the parasitoid treatment (a clipped *D. insulare* wasp). The experiment was designed as a multi-factorial replicated regression (Cottingham *et al.*, 2005), thereby allowing for analyses of the independent and interactive effects of prey density and enemy presence on plant biomass, as well as assessments of the relationship between prey density and plant biomass. The factorial structure was $2 \times 4 \times 2$, in which *D. insulare*

(present/absent) was crossed with prey density (0, 4, 8, 16), then crossed with trial (1, 2). In both trials, plant biomass was the response variable measured; in the second trial, enemy foraging time (sec) and caterpillar biomass (dry weight, mg) were also measured. Enemy foraging time (secs) was repeatedly measured in two 5-min observation periods (morning and afternoon) per replicate, on each of the 5 days of the trial. On each date, enemy foraging time was quantified as the total number of seconds that a *D. insulare* female in a given cage was observed on a plant. Per capita caterpillar biomass was quantified (mg) by freezing, drying, and weighing individually all caterpillars at the conclusion of the experiment.

Analyses

The indirect effect of *D. insulare* on plant biomass was analyzed using a three-way ANOVA with *Diadegma*, prey density, and trial as the main effects. Assessments of a linear relationship between prey density and indirect fear effects (plant biomass) were conducted with least squares linear regression. Repeated measures MANOVA was used to analyze the independent and interactive effects of prey density and sampling date on enemy foraging behavior. Two-way ANOVA (with *D. insulare* and prey density as main effects) was used to assess treatment effects on caterpillar biomass; where there was a significant statistical interaction between the four levels of prey density and the two levels of *D. insulare*, the presence/absence of *D. insulare* was assessed at each prey density using two-sample *t*-tests. The per capita interaction strength was used as a metric of the indirect interaction between parasitoids and plants (after Paine, 1980; Wootton, 1997). Interaction strength was calculated as $\log_e(N_{\text{control}}/N_{\text{Diadegma}})$, where N_{control} represents the mean plant biomass of a given trial, and N_{Diadegma} represents the measurement of plant biomass in each replicate of that trial. Linear regression was used to determine whether the

parasitoid-plant interaction strength was linearly related to prey density. All analyses were conducted using SYSTAT version 11 (Systat Software, Richmond, CA, USA) (SPSS 1999). Test criteria were systematically verified to conform to assumptions of normality and homogeneity of variances.

RESULTS

Plant biomass

Significant differences in plant biomass were found among the four prey densities ($F_{3,48} = 3.730$, $P = 0.017$; Table 1). Respective means at 0, 4, 8, and 16 caterpillars/replicate were 2.39 ± 0.27 (± 1 SE), 2.27 ± 0.24 , 2.03 ± 0.18 , and 2.07 ± 0.25 g. The effect of *D. insulare* (present/absent) on plant biomass was not significant ($F_{1,48} = 0.527$, $P = 0.471$); mean plant biomass (± 1 SE) in the presence of *D. insulare* was 2.16 ± 0.16 ($n = 32$), and in the absence of *D. insulare*, 2.22 ± 0.17 ($n = 32$). Trial was a significant factor in the analysis ($F_{1,48} = 86.028$, $P < 0.001$), with mean plant biomass in trial 2 (3.04 ± 0.08 g) greater than that in trial 1 (1.34 ± 0.04 g). There were no statistically significant interactions between the three main effects (Table 1).

In trial 1, where caterpillars could feed without predation risk, plant biomass declined linearly as prey density increased ($R^2 = 0.276$; $P = 0.037$; Table 2; Fig. 2A); in trial 2, evidence of a linear relationship between plant biomass and prey density was weaker but did approach statistical significance ($R^2 = 0.238$; $P = 0.055$; Fig. 2A). Conversely, in the presence of *D. insulare*, there was no evidence to suggest that plant biomass was related to the density of herbivores in either trial (trial 1: $R^2 = 0.040$, $P = 0.458$; trial 2: $R^2 = 0.041$, $P = 0.452$; Table 2; Fig. 2B). In trial 1, plants patrolled by *D. insulare* averaged 1.35 ± 0.04 g (sum of both plants in

a given cage; mean \pm 1 SE), while in trial 2, plants patrolled by *D. insulare* averaged 2.97 ± 0.12 g. In the absence of *D. insulare*, mean plant biomass in trial 1 was 1.32 ± 0.07 g, and in trial 2, mean plant biomass was 3.12 ± 0.12 g.

Parasitoid foraging

Prey density was a significant predictor of the amount of time *D. insulare* spent foraging on collard plants ($F_{3,12} = 6.837$; $P = 0.006$; Table 3; Fig. 3), and this density-mediated effect was consistent over time (Sampling date \times Prey density interaction, $F_{12,48} = 1.747$; $P = 0.086$).

Diadegma spent significantly more time foraging on plants when prey were present than when absent (Fig. 3); further, *Diadegma* tended to devote increasingly greater amounts of time to foraging as the density of prey increased (linear regression: foraging time vs. prey density, $R^2 = 0.447$, $P = 0.005$).

Caterpillar biomass

Exposure to *Diadegma* strongly reduced caterpillar biomass, but this effect manifested only at low and intermediate caterpillar densities (*Diadegma* \times Prey Density interaction, $F_{2,18} = 3.765$; $P = 0.043$; Fig. 4A). At the lowest density (4 caterpillars per plant), mean per capita body mass for caterpillars feeding under predation risk, 0.89 ± 0.16 mg (\pm 1 SE), was significantly less than that of caterpillars feeding without predation risk, 1.86 ± 0.30 mg ($t = -2.811$, $df = 6$, $P = 0.031$). At the intermediate density (8 caterpillars/plant), caterpillar body mass was again strongly reduced in the presence of the parasitoid (*Diadegma*: 0.79 ± 0.13 ; no *Diadegma*: 2.27 ± 0.26 ; $t = -5.072$, $df = 6$, $P = 0.002$), whereas at the highest density (16 caterpillars), the effects of *Diadegma* were non-significant (*Diadegma*: 1.26 ± 0.24 ; no-*Diadegma*: 1.58 ± 0.09 ; $t = -1.243$, $df = 6$, $P =$

0.260). Overall, individual caterpillars exposed to *Diadegma* weighed 0.98 ± 0.11 mg, a significantly lighter body mass than caterpillars feeding in the absence of predation risk, 1.91 ± 0.15 mg ($F_{1,18} = 28.396$, $P < 0.001$; Fig. 4B). As a main effect, prey density did not have a significant impact on per capita body mass ($F_{2,18} = 0.271$; $P = 0.766$).

Parasitoid-plant interaction strength

As prey density increased, the interaction strength between *Diadegma* and plant biomass increased (Fig. 5); the respective interaction strengths (± 1 SE) for 0, 4, 8, and 16 prey/plant were -0.101 ± 0.064 , -0.0480 ± 0.051 , 0.004 ± 0.044 , and 0.062 ± 0.060 . The magnitudes of the cascading effects were a positive, linear function of prey density (linear regression, $R^2 = 0.974$, $P = 0.013$), indicating that the relationship between *Diadegma* and plant biomass was mediated by the density of herbivores feeding on the plant.

DISCUSSION

Our findings indicate that cascading, nonlethal enemy effects were governed, to a large extent, by the densities of prey. The magnitudes of the indirect enemy effects increased linearly with prey density, providing evidence that the parasitoid-plant interaction was mediated by the number of prey transmitting the enemy effects to the plant (Fig. 5). This relationship emerges from the fact that in the presence of the natural enemy (Fig. 2B), plant biomass tended to remain constant over the full range of the caterpillar densities, while in the absence of the enemy (Fig. 2A), plant biomass declined linearly; as a result, the disparity between the enemy-present and enemy-absent treatments increased with increasing prey density (Fig. 5). These findings mirrored the original field data (Fig. 1A, B), suggesting that the factors we isolated in our greenhouse

experiment—*Diadegma* and prey density—mediated plant biomass much as they had under field conditions.

The weakening of the relationship between plant biomass and prey density, as imposed by the presence of *D. insulare*, suggests that the wasps increasingly reduced per capita caterpillar herbivory with increasing prey density. For this to have occurred, wasps would have had to spend more time foraging as caterpillar density increased. Indeed, this was the case (Fig. 3), which corresponds with previous findings indicating that *Diadegma* parasitoids not only respond strongly to caterpillar-damaged plants (Ohara *et al.*, 2003) but also increase the frequency of plant-visitation with increasing diamondback moth density (Waage, 1983; Wang & Keller, 2002). There are two likely mechanisms by which an enemy's nonlethal effect might be sensitive to the density of its prey: 1) among the prey individuals in a localized group, the prey rapidly share information on a perceived threat, allowing a single cue to induce an enduring antipredator response in the entire group (Lima, 1995), or 2) the predator is an active forager, capable of rapidly locating prey individuals in a localized area and evoking an enduring antipredator response. *Diadegma* wasps are exceedingly active foragers (Waage, 1983; Wang & Keller, 2002), and its prey, diamondback moth caterpillars, do not drop from the plant en masse (S.A. Steffan, *personal observation*). Thus, the *D. insulare* females in our experiment likely drove their prey from the plant individually, thereby reducing herbivory via frequent encounters with prey.

The increased tendency of *D. insulare* wasps to forage on herbivore-damaged plants allowed these parasitoids to exert significant negative effects on caterpillar biomass, even as the number of caterpillars markedly increased (Fig. 4B). Given that diamondback moth caterpillars are known to drop down on silk threads in order to avert enemy encounters (Wang & Keller,

2002), their antipredator behavior forces them to forego feeding whenever confronted with a significant threat. Sacrificing feeding opportunities is a common means by which prey trade long-term fitness for short-term survival (Sih, 1987; Lima & Dill, 1990; Soluk, 1993; Peckarsky & McIntosh, 1998; Sih *et al.*, 1998; Werner & Peacor, 2003). The negative effects of the dropping behavior may be exacerbated by the fact that *D. insulare* is known to wait at the site of the caterpillar's silk attachment (Wang & Keller, 2002), potentially inducing the caterpillar to drop repeatedly. Our finding that caterpillar biomass decreased significantly in the presence of the wasp, but only at the low and intermediate densities (Fig. 4A), suggests that the parasitoid was able to intimidate most or all caterpillars at these two lower densities; however, at the highest density, there may have been a degree of "safety in numbers." In other words, there may have been a limit to the efficiency with which the parasitoid could physically confront all caterpillars in a cage. Nevertheless, the effect of *D. insulare* on plant biomass was significant at the highest density level (Fig. 5), and this result could have arisen as the per capita effect of the wasp was reduced to a lesser degree than the density was increased (i.e., even though the per capita effect of the wasp was small, the effect on the plant was discernible because there were so many caterpillars feeding).

Cascading fear effects are well-documented elements of tri-trophic systems (Beckerman *et al.* 1997, Schmitz *et al.*, 1997; Peckarsky & McIntosh, 1998; Griffin & Thaler, 2006; Trussell *et al.*, 2006; Preisser *et al.*, 2009). Further, the primacy of fear and other nontrophic interactions within animal communities is increasingly recognized as a driver of community dynamics and structure (Soluk, 1993; Sih *et al.*, 1998; Schmitz *et al.*, 2004; Preisser *et al.*, 2005; Byrnes *et al.*, 2006; Berger *et al.*, 2008). Given the potential of fear to effectively saturate a system, even when the predator is absent (Ripple & Beschta, 2004; Valeix *et al.*, 2009), the nature of fear effects

may allow immense effect sizes that closely correspond to the density of the species experiencing the fear. Our data provide empirical support for this expectation, and may be generalized to other animal communities.

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Table 1. Results of ANOVA comparing mean plant biomass across three factors: *D. insulare* (present, absent), prey density (0, 4, 8, 16), and trial (1, 2).

Source	df	MS	F-ratio	P
<i>D. insulare</i>	1	0.064	0.527	0.471
Prey density	3	0.452	3.730	0.017
Trial	1	46.752	386.028	< 0.001
<i>D. insulare</i> × Prey density	3	0.082	0.677	0.571
<i>D. insulare</i> × Trial	1	0.114	0.944	0.336
Prey density × Trial	3	0.209	1.724	0.175
<i>D. insulare</i> × Prey density × Trial	3	0.058	0.477	0.700
Error	48	0.121		

Table 2. . Simple linear regression results for the relationships between plant biomass and prey density, under various treatment combinations of *D. insulare* (present, absent) and trial (1, 2).

Independent variable	Slope	Intercept	R^2	P
<i>No D. insulare</i>				
Prey density—Trial 1	-0.023	1.483	0.276	0.037
Prey density—Trial 2	-0.037	3.379	0.238	0.055
<i>D. insulare</i> present				
Prey density—Trial 1	-0.006	1.386	0.040	0.458
Prey density—Trial 2	-0.016	3.082	0.041	0.452

Table 3. Repeated measures MANOVA, analyzing *Diadegma* foraging duration relative to prey density over repeated observation events. Response variable: total time (sec) *Diadegma* was observed on a plant in a given day (summed over two 5-minute observation periods).

Between subjects:

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Prey density	3	207115.93	6.837	0.006
Error	12	30293.87		

Within subjects:

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Sampling date	4	19230.31	1.091	0.360
Date × Prey density	12	30802.58	1.747	0.086
Error	48	17630.80		

FIGURE CAPTIONS

Figure 1. Least squares regressions of plant biomass versus herbivory potential (larva-days), based on data from an experiment designed to assess the indirect effects of natural enemies on plant biomass in the field. The “larva-day” unit was calculated for each replicate by multiplying the number of larvae in a mesocosm by the number of days since the previous larval count, then summing across the experiment’s duration. A) In the absence of *Diadegma* (○), a simple linear regression of plant biomass versus larva-days shows a significant, negative relationship (slope = -0.021 ± 0.004 , $P < 0.001$; intercept = 2.305 ± 0.193 , $P < 0.001$). B) In the presence of *Diadegma* (●), a linear regression of plant biomass versus larva-days indicates the slope of the regression line is not different from zero and that the y-intercept is a precise measure of the sample mean (slope = -0.0006 ± 0.005 , $P = 0.972$; intercept = 1.340 ± 0.226 , $P < 0.001$).

Figure 2. Least squares linear regressions of plant biomass versus prey density within each trial, for replicates that A) exclude and B) include *Diadegma* wasps. Data are means ± 1 SE ($n = 4$), separated into trials 1 (●) and 2 (○).

Figure 3. Time (secs) that the parasitoid, *Diadegma*, spent foraging on collard plants, relative to prey density. Data are means ± 1 SE ($n = 4$). Means with differing letters indicate significant differences (Tukey HSD multiple comparisons, $P > 0.05$)

Figure 4. A) Per capita biomass of diamondback moth larvae relative to prey density and the presence (●) or absence (○) of the parasitoid, *Diadegma*. Data are means ± 1 SE ($n = 4$). B) Overall effect of *Diadegma* on caterpillar mass (mg). Data are means ± 1 SE ($n = 12$).

Figure 5. Mean interaction strength ($\log_e[\text{control}/\text{treatment}]$) of *Diadegma* on plant biomass, calculated at each prey density. Data are means ± 1 SE ($n = 16$). Interaction strength was a positive, linear function of prey density (linear regression: $R^2 = 0.974$, $P = 0.013$).

FIGURES

Figure 1

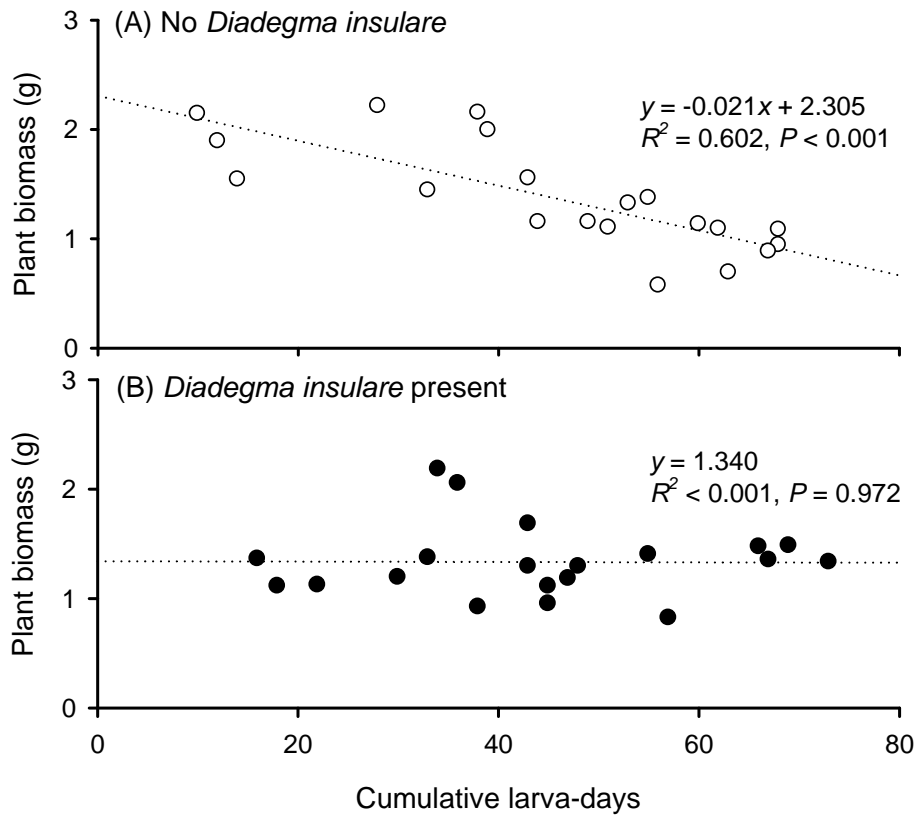


Figure 2

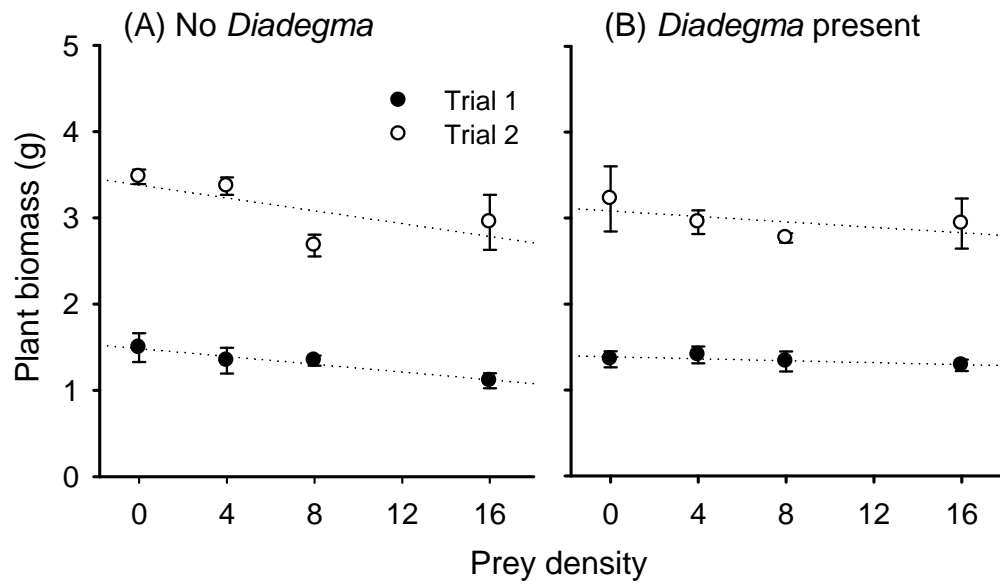


Figure 3

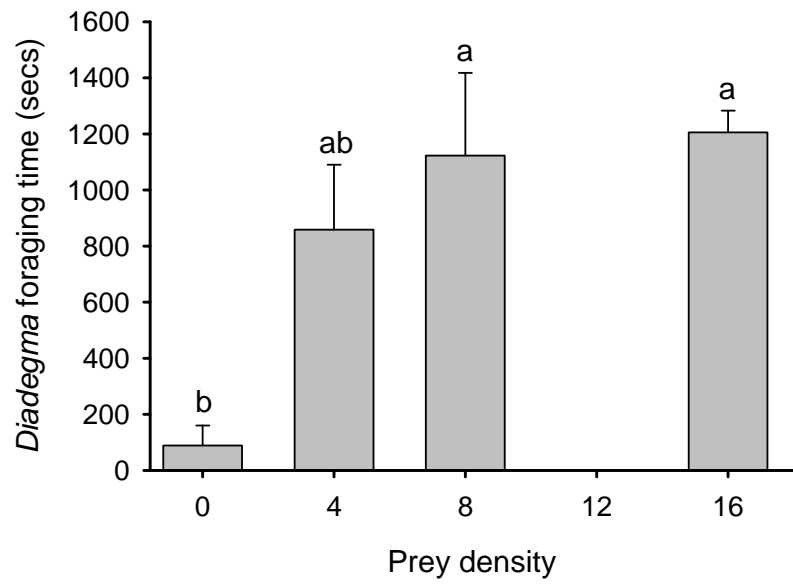


Figure 4

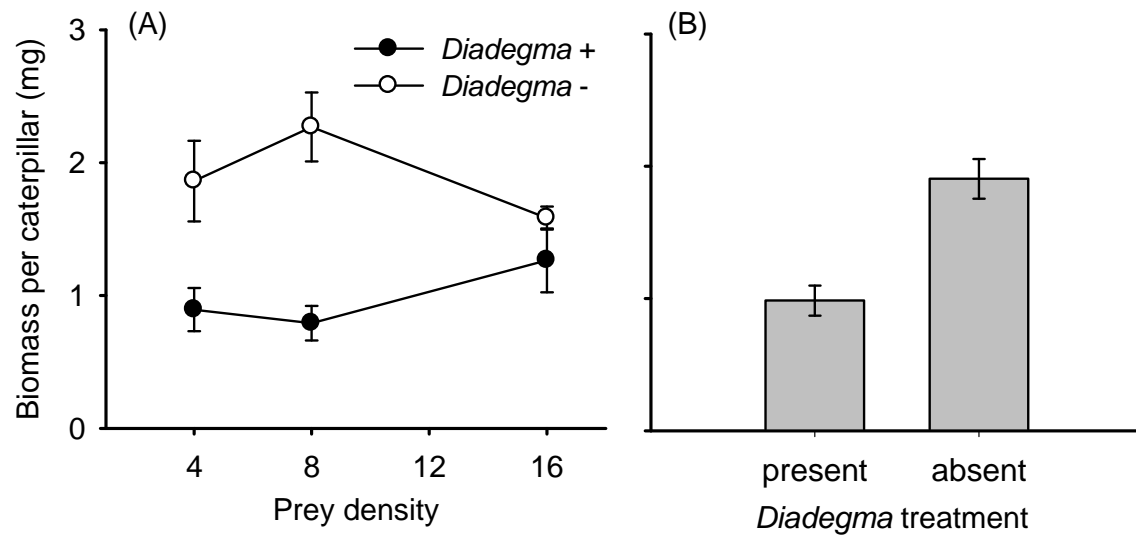
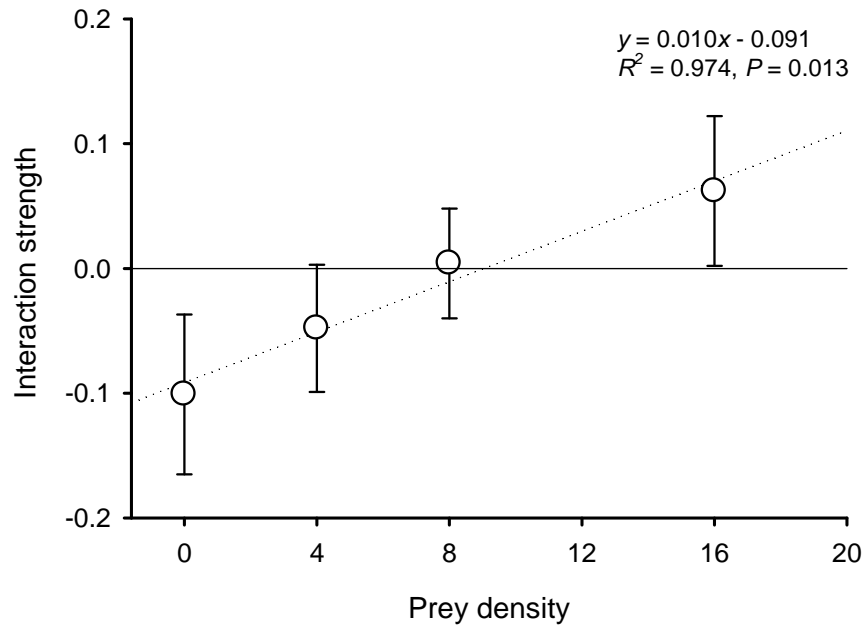


Figure 5



CHAPTER FOUR
NONTROPHIC INTERACTIONS AMONG SPECIES MODIFY TROPHIC AND
NONTROPHIC LINKS IN FOOD WEBS

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Abstract

We investigated the mechanisms by which nontrophic interactions among species can modify the trophic relationships in a community. Food web models that rely exclusively on trophic links tend to confine species interactions to consumptive effects, yet much evidence suggests nontrophic processes also shape community structure and dynamics. To facilitate parameterization of nontrophic links within food web models, empirical evidence relating nontrophic interactions to community structure and dynamics is needed. Our study focused on the nontrophic impacts of insect folivory, a ubiquitous and important ecological phenomenon in terrestrial ecosystems. In the collard agroecosystem, we explored the following hypotheses: the nontrophic effects of chewing folivores on collard plants will 1) indirectly alter predator foraging patterns, reducing the degree of enemy-free space in the collard canopy, and 2) tend to attenuate with each successive link along an interaction pathway. We quantified non-focal herbivore impacts (caterpillar folivory) on host plant architecture, predator foraging behavior, and focal prey (aphids) in open field plots and a controlled greenhouse setting. Our findings indicate that

folivory significantly altered host plant architecture, inducing predators to forage more often and more effectively, ultimately increasing aphid predation. Folivory also indirectly reduced aphid feeding rates, independent of the effects of predators. We present a metric for measuring nontrophic effects at each link of an interaction pathway, and discuss our findings in terms of food web ecology.

Keywords

apparent competition, food web, indirect interaction, inducible plant defenses, interspecific facilitation, predator-mediated

INTRODUCTION

In food webs, species are generally linked via trophic connectance within their respective communities, yet there is substantial evidence that nontrophic links among species also play a significant role in shaping community dynamics and structure (Soluk 1993, Lawton 1994, Abrams 1995, Sih et al. 1998, Borer et al. 2002, Schmitz et al. 2004, Preisser et al. 2005, van der Merwe and Brown 2008). Recent theoretical evidence suggests nontrophic interactions tend to increase connectance among species and can substantially modify trophic relationships within a food web (Goudard and Loreau 2008). Nontrophic links can be both direct and indirect, and may affect community dynamics via interspecific facilitation (Cardinale et al. 2002), behavioral interference (Soluk 1993), habitat modification (Lawton 1994), or apparent predation (Huang and Sih 1990), to name just a few potential manifestations. However, nontrophic interactions can present challenges for empirical measurement because they may be less apparent or more difficult to isolate and quantify (Berlow et al. 2004). Further, nontrophic processes such as abiotic disturbances, or interference competition between basal species, may have idiosyncratic, transient effects on the taxa in a community. Among higher-order consumers, nontrophic interactions have been shown to allow competing species to not only indirectly antagonize one another (Holt and Lawton 1994, Karban et al. 1994, Biere et al. 2002) but also confer benefits upon their competitors (White and Andow 2006). Such indirect effects often vary in symmetry, reciprocity, or mechanism, all of which are recognized as determinants of the net effects of indirect nontrophic interactions (see reviews by Chaneton and Bonsall 2000, Schmitz et al. 2004).

Theory predicts that the addition or loss of a species from a food web may fundamentally reconfigure the nature and magnitude of trophic and nontrophic interactions among species in the

community (Borer et al. 2002, Goudard and Loreau 2008). To assess the full impact of changes in species richness, ecologists need to develop a more refined mechanistic understanding of how various nontrophic impacts of species may penetrate and influence multi-species systems (Soluk 1993, Borer et al. 2002). Indeed, complexity among interacting species can arise as much from behavioral interactions as from trophic interactions (Soluk 1993), and thus if food web models are to accurately represent changes in species richness, models may need to account for nontrophic interactions and processes. Because nontrophic interactions effectively link species that otherwise may have no direct trophic relationship (Sih et al. 1998, Goudard and Loreau 2008), the presence of significant nontrophic links should increase the probability that multiple pathways exist by which species indirectly interact. Here, interaction pathways represent chains of direct links that indirectly connect two species (Holt and Lawton 1994, Wootton 1994). As nontrophic interactions create increasingly reticulate networks, interaction pathways may be lengthened, becoming increasingly circuitous and thereby allowing the impacts of species to influence a greater proportion of their respective communities. Therefore, the loss (or addition) of a species may have far-reaching trophic and nontrophic effects within a community. This recognition has been accompanied by calls for the inclusion of nontrophic interactions within ecological theory (Soluk 1993, Borer et al. 2002, Bruno et al. 2003, Berlow et al. 2004, Goudard and Loreau 2008). To this end, it has been suggested that empirical work focus on particularly common nontrophic interactions among certain taxa or categories of consumers (Soluk 1993), as well as to quantify the interaction strengths between species such that biologically relevant model coefficients can be derived from empirical data (Borer et al. 2002, Berlow et al. 2004).

To provide empirical measurements of common nontrophic interactions, we present data focusing on the direct and indirect nontrophic effects of one of the more ubiquitous ecological

phenomena in terrestrial systems: folivory by insects (Lawton and Strong 1981). Our experiments were designed to reveal how nontrophic interactions allow species to extend their impacts within a community, directly or indirectly modifying the trophic relationships among the resident species. Based on field observation and past research (Eigenbrode et al. 1996, Straub and Snyder 2008), we developed a simple interaction network to represent the potential interaction pathways through which functional groups in a collard agroecosystem interact (Fig. 1). In this conceptual model, chewing herbivores are represented by a caterpillar, actively hunting predators are represented by a ladybird beetle, and phloem-feeding species are represented by aphids. The caterpillar-plant, plant-beetle, beetle-aphid, and plant-aphid interactions are denoted respectively by links A, B, C, and D (Fig. 1). We used this simple framework to examine two primary questions: 1) what are the nontrophic impacts of arthropod folivory in a terrestrial community?, and 2) do the indirect effects of this folivory tend to amplify or attenuate with each successive link along a given interaction pathway?

Within a controlled greenhouse setting, we quantified the degree to which chewing folivores altered the canopy architecture of collard plants, and then, to track the nontrophic effects of canopy alteration on predators, we measured how predators allocated their time to foraging relative to different areas of the leaf. Next, we measured the abundance and distribution of another functional group of herbivores in the collard canopy—phloem-feeding aphids. Finally, we measured the effect of canopy folivory on the feeding rates of the aphids, as well as the total surface area of the collard leaves. This tri-trophic model system provided a means to map two distinct, multi-link interaction pathways between the herbivore species. We quantify the per capita interaction strength (Wootton 1997) of each direct link within the multi-link pathways, thereby tracking how nontrophic effects (and any attendant trophic effects) may propagate from

one species to another. Measures of interaction strength are key variables because they relate the dynamics of a community to its structure, and it is the particular arrangement of interaction strengths in a food web that mediates its functioning (Berlow et al 2004). Additionally, measuring the interaction strengths at each link should allow an assessment of whether the effects of these interactions amplify or attenuate. In our system, nontrophic interactions generated significant impacts on community structure and dynamics, and were not predicated on top-down predator-prey cascades. Ultimately, an empirically derived basis for parameterization of nontrophic effects should improve food web models and refine predictions of the effects of species loss and/or invasion.

METHODS

Study sites and natural history

Field observations were conducted at two sites in eastern Washington, USA: Washington State University (WSU) R.B. Tukey Horticulture Orchard in Pullman (46°44' N, 117°10' W), and the WSU Research Station in Othello (46°49' N, 119°7' W). At each site, we established and maintained fields of collard plants (*Brassica oleracea* L.). There was a single plot of collards in Pullman, and two plots in Othello (Othello plots were separated by > 30 m of weeded, open ground). In 2007 and 2008, aphids were the most abundant herbivores at these sites (S.A. Steffan, *unpublished data*), represented largely by the cabbage aphid, *Brevicoryne brassicae* (L.). These phloem-feeding insects produce copious amounts of honeydew (a sugar-rich, watery form of excrement), the volume of which has long been used as an estimate of aphid feeding rate (Mittler 1970). Collard plants at our study sites were also attacked by a complex of chewing folivores: three lepidopteran species (moths and butterflies) and adult flea beetles (*Phyllotreta*

sp.). The lepidopteran species were diamondback moths (*Plutella xylostella* [L.]), imported cabbageworms (*Artogeia rapae* [L.]), and cabbage loopers (*Trichoplusia ni* [Hübner]). These chewing folivores feed on the leaves, producing distinct folivory sites (i.e., irregularly-shaped holes) at the periphery and interior of the leaves.

A diversity of natural enemies forage for prey in the canopies of eastern Washington collard fields (Snyder et al. 2006). Coccinellid beetles, such as *Hippodamia convergens* and several species of *Coccinella*, are particularly common, as are damsel bugs (*Nabis* spp.) and various specialist parasitoids. Coccinellids and other generalist predators have been shown to patrol the edges of brassicaceous leaves much more often than leaf interior areas (Eigenbrode et al. 1996, Straub and Snyder 2008). This phenomenon derives from the difficulty that coccinellids have in traversing waxy leaf surfaces on leaf undersides (Eigenbrode et al. 1996). Leaf microsites near edges, therefore, may endure greater predation pressure.

Field observations

To assess the nontrophic impacts of chewing folivores in the collard system, we measured leaf area, extent of folivory, aphid abundance and distribution, and predator foraging behavior within three collard plots during July and August of 2008. Aphid abundance and predator behavior were measured relative to their spatial position on a given leaf. Given the tendency of larger enemy species to concentrate foraging efforts at leaf edges (Eigenbrode et al. 1996, Straub and Snyder 2008), we partitioned the leaf surface area into edge and non-edge areas. Based on previous studies of ladybird beetle morphology (Evans 2000), as well as observations of foraging behavior (S.A. Steffan, *personal observation*), it was estimated that a 5 mm-wide swath around a leaf edge represented the most readily accessible, highly trafficked

hunting domain for such predators. Lady beetles certainly are not entirely confined to a 5 mm-wide swath around leaf edges, but in defining edge areas as such, we could effectively partition leaves into two distinct zones: edge and non-edge zones. Our 5 mm estimate was purposefully held at a low, conservative level to avoid over-estimating the proportion of a leaf's area represented by edge zones (over-estimation would inflate measurements of the nontrophic effects of folivory, enhancing the likelihood of statistical significance).

We quantified total leaf area, as well as the edge area of each leaf, by photographing in the field randomly selected leaves (of intermediate age, and all bearing evidence of folivory). The relative edge and non-edge areas of these leaves were quantified using the image analysis software ImageJ version 1.41o (W.S. Rasband, National Institutes of Health, Bethesda, MD, U.S.A.). From the digital image, we also counted the number of folivory sites per leaf.

We measured aphid abundance relative to edge and non-edge areas on each leaf. Here, our sample unit was the collard plant, and the sample size in a given plot, on a given date, was 10 plants. There were three plots, each sampled on two dates, creating six independent spatio-temporal blocks (replicates). The plots in Othello were sampled on 30 July and 14 August, 2008; the plot in Pullman was sampled on 3 August and 21 August, 2008. Within each plant, we randomly selected two leaves to count (again, of intermediate age), with each leaf representing a subsample; thus, across the six spatio-temporal blocks, 60 plants (120 leaves) were examined. Three response variables were recorded on a given leaf: total aphid abundance, number of aphids within 5 mm of a leaf edge, and total number of folivory sites.

To assess the time a predator allocated to searching inside or outside an edge area, we set up digital video cameras (Sony Handycam® DCR-HC36) in the field. The use of video cameras allowed us to capture foraging behaviors without influencing the predators by observing them at

close proximity. Near the base of a plant, two video cameras were situated below the canopy, with the cameras focused on leaf undersides; recordings lasted 40 minutes per plant. Recordings were conducted in Pullman (24 July and 12 August, 2008) and Othello (31 August, 2008), and on each recording date, four plants were randomly selected for observation. Each plant represented an independent assessment of predator behavior, thus a plant represented our replicate, and a given leaf underside was a subsample. Over three recording events (four plants per event), we generated twelve independent assessments of predator foraging behavior (16 h total recording time, across 24 observed leaves). We quantified predator behavior in terms of time (seconds) allocated to foraging inside and outside the edge areas of a leaf.

Greenhouse experiment

To further examine the effects of folivory on the collard community, we set up a multi-factorial experiment in a controlled greenhouse environment (16:8 h photoperiod; 22°:16° C temperature regime) and isolated the effects of folivory and predation. This experiment involved the introduction of folivory damage by chewing herbivores (diamondback moth caterpillars *P. xylostella*) into a simple tri-trophic community comprised of collard plants (*B. oleracea*), phloem-feeders (cabbage aphids, *B. brassicae*) and predators (convergent lady beetles, *H. convergens*). Our experiment fully crossed three factors: folivory (present/absent), predators (present/absent), and a temporal blocking factor (trial-1/trial-2), yielding a $2 \times 2 \times 2$ factorial structure. The experimental unit was a 45-L glass aquarium with a fine mesh, removable lid. There were seven replications within a trial; hence, for each trial, 28 aquaria were used. In each aquarium, we placed a single potted collard plant (~6 weeks old) that had been propagated under greenhouse conditions. When the plants were ~5 weeks old, all but a single leaf had been

removed. To maintain the horizontal orientation of the remaining leaf, it was carefully affixed to a short supporting rod.

Three days later, 20 winged cabbage aphids (from a greenhouse culture) were gently applied to each leaf underside. The aphids were allowed to settle and reproduce for three days, at which point 8 third-instar diamondback moth caterpillars (from greenhouse cultures) were applied to each leaf and allowed to feed for three days. We then removed the caterpillars and waited another 24 h before adding the predators. Only adult female ladybird beetles that had been collected in an unsprayed alfalfa field in Pullman were used (beetles had been collected ~2 weeks prior and allowed to acclimate to greenhouse conditions). After counting initial aphid densities on all plants in the trial, 2 female beetles were released into the aquaria requiring predator treatments. At this point, we placed in each aquarium four water-sensitive cards (Novartis Corporation, East Hanover, NJ, 07936), arranged to form a contiguous 10.16×15.24 cm (4×6 in) platform. This platform was positioned underneath each leaf to measure the amount of honeydew being excreted by aphids. Wherever a droplet of honeydew fell to the surface of the platform, the area contacted by the droplet changed color irreversibly. The percent of a platform showing a color-change could subsequently be quantified. Given that honeydew production corresponds closely to the volume of plant fluids ingested by aphids (Mittler 1970), the percent coverage on the platform could be used as a relative estimate of aphid feeding.

At 1, 3, and 5 d following the commencement of a trial, we observed predator foraging behavior. During 10 min observation periods at each aquarium, the number of seconds a predator allocated to foraging in edge and non-edge areas of the leaf was recorded. At the conclusion of the last observation period on the fifth day of the trial, all predators were removed and final aphid densities counted. We then photographed all leaves and water-sensitive cards for

subsequent image analyses. Trials 1 and 2 of this experiment were initiated on 28 July and 8 August, respectively.

Analyses

To establish whether naturally-occurring levels of folivory in collard fields mediate the proportion of a leaf's surface area represented by edge area, we regressed the number of folivory sites against the proportion of a given leaf represented by edge-area. Aphid densities in edge-versus non-edge areas of collard leaves in the field were \log_{10} -transformed to meet assumptions of normality and homogeneity of variance, then analyzed using one-way ANOVA. Predator foraging time in edge- versus non-edge areas was analyzed with one-way ANOVA. To assess whether the edge proportion (of leaf surface area) in field-collected leaves significantly departed from 1) the proportion of aphids in edge areas or 2) the proportion of time allocated by enemies to foraging within edge areas, we compared the proportions using two-sample *t*-tests (pooled variance, 2-tailed). In the greenhouse experiment, linear regression was used to relate folivory damage to the proportion of a leaf's area represented by edge area. Three-way ANOVA (folivory, predation, and trial as main effects, and initial aphid density as a covariate) was used to analyze aphid density. To meet assumptions of normality for measurements of aphid proportions within edge areas, data were square root-arcsine transformed, then analyzed with a three-way ANOVA (again, with folivory, predation, and trial as main effects) using initial edge proportion as a covariate. Three-way ANOVA with average aphid density as a covariate was used to analyze honeydew coverage (%) on the water-sensitive cards. Two-way ANOVA (main effects: folivory and trial) was used to assess the interactive effects of folivory and block on predator foraging behavior. Between directly interacting species in the greenhouse experiment, we

quantified for each relevant replicate the per-capita impact that one species had on the other (e.g., the impact of caterpillar folivory on collard plants, the impact that collard plants exerted on beetle foraging). Referred to as per-capita interaction strength (Wootton 1997, Straub and Snyder 2008), this metric may be represented as $\ln[(N_{\text{control}} + 1)/(N_{\text{treatment}} + 1)]/D$, where N denotes the response variable of interest, and D denotes the density of the species initiating the interaction. The N_{control} value in this calculation was derived from the mean value of the control replicates within a given trial. All analyses were performed in SYSTAT version 11 (Systat Software, Richmond, CA, USA).

RESULTS

Field data

The edge area of a given leaf was positively correlated with the number of folivory sites in the leaf ($R^2 = 0.731$, $P = 0.003$; Appendix 1), showing that with increasing folivory, the proportion of edge area in a leaf tended to increase linearly. At our field sites during mid- to late-summer in 2008, the average number of folivory sites in collard leaves of intermediate age was 167.11 ± 39.02 ($\pm 1\text{SE}$). While aphids were not significantly less abundant in edge areas than in non-edge areas ($F_{1,10} = 4.365$, $P = 0.063$; Fig. 2a), the proportion of aphids in edge areas (0.135 ± 0.019) was significantly less than what would be expected based on the proportion of edge area on leaf surfaces (0.268 ± 0.035) ($t_{13} = -2.902$, $P = 0.012$; Fig. 2b). Predator foraging was commonly observed on the undersides of collard leaves (24.4% of the total observation time), and of this time, 81.7% was spent in edge areas. Most of the predators observed were coccinellid beetles (89.9%). When present, coccinellids patrolled leaf edges 92.0% of the time, a significantly

greater proportion of their time than would be predicted based on the average edge area available on leaf surfaces ($t_{13} = -10.061$, $P < 0.001$; Fig. 2b).

Greenhouse data

Caterpillar-plant interaction (link A). Image analyses of leaf surface area allowed for a precise measurement of the edge and non-edge areas of damaged and undamaged leaves. The relative proportion of a leaf's surface area represented by edge area (i.e., within 5 mm of an edge) increased significantly from $0.123 (\pm 0.003)$ in the absence of folivory to $0.374 (\pm 0.015)$ in the presence of folivory ($F_{1,47} = 248.97$, $P < 0.001$; Fig. 3a; Appendix 2a). There was a positive, linear relationship between the number of folivory sites in a given leaf and its edge area ($R^2 = 0.816$, $P < 0.001$; Fig. 3b; Appendix 2b), indicating that as the extent of folivory damage increased, so too did edge area. On average, greater edge area was not accompanied by a significant decrease in leaf surface area ($F_{1,47} = 2.28$, $P = 0.138$; Appendix 2c). Folivory, therefore, fundamentally altered leaf architecture without significantly reducing the size of a leaf.

Plant-beetle interaction (link B). When foraging over plants with folivory damage, ladybird beetles not only spent more time on the plants ($F_{1,23} = 6.39$, $P = 0.019$; Fig. 3c; Appendix 3a), but also significantly concentrated their foraging at leaf edges ($F_{1,23} = 9.869$, $P = 0.005$; Appendix 3b). On leaves with folivory damage, beetles allocated $32.00 \pm 0.045\%$ (mean ± 1 SE) of a given observation period to foraging within edge areas, compared to $14.30 \pm 0.039\%$ of the time when foraging on undamaged leaves. Further, there was a significant, positive correlation between proportion of edge area on a given leaf and the proportion of time that the beetles foraged at edge areas ($R^2 = 0.191$; $P = 0.023$; Fig. 3d; Appendix 3c). Thus, folivory damage

indirectly induced beetles to forage on the plants more often, and to increasingly concentrate their foraging time in edge areas as the level of leaf damage increased.

Beetle-aphid interaction (link C). Folivory and beetle predation interactively mediated aphid density—the beetles ate significantly more aphids where caterpillars had altered leaf architecture than where leaves were undamaged (folivory \times predation interaction, $F_{1,47} = 7.326$, $P = 0.010$; Fig. 3e). Interestingly, the beetles were not able to reduce aphid numbers below no-predator controls without folivory damage (Fig. 3e), suggesting that the non-edge areas represented functional refuges for aphids. As a main effect, the presence of folivory was significant ($F_{1,47} = 21.889$, $P < 0.001$; Appendix 4a), allowing for a reduction in aphid densities (folivory: 2.338 ± 0.081 ; no folivory: 2.540 ± 0.075). Predation, as a main effect, also significantly reduced aphid numbers (predation: 2.355 ± 0.082 ; no predation: 2.516 ± 0.077 ; $F_{1,47} = 23.809$, $P < 0.001$). There was a significant effect of trial on final aphid density ($F_{1,47} = 23.722$, $P < 0.001$), though this factor did not interact significantly with any other terms in the analysis (Appendix 4a). Initial aphid density (as a covariate) was a significant predictor of variability in the data.

In the absence of predation, the likelihood that aphids would be found within edge areas on a given leaf increased as the leaf's edge area increased ($R^2 = 0.785$, $P < 0.001$; Fig. 3f; Appendix 4b). Conversely, in the presence of predation, there was no significant relationship between the amount of edge area on a leaf and the proportion of aphids found in such edge areas ($R^2 = 0.109$, $P = 0.093$; Fig. 3f; Appendix 4c). Here, the lack of a significant relationship between leaf edge area and the proportion of aphids in edge areas is attributable to the effect of predators; the beetles reduced aphid numbers in edge areas either by eating them or inducing them to flee, such that even when the proportion of edge area in a given leaf was relatively high,

a small number of aphids remained. Caterpillar folivory, in the absence of any predators, increased the likelihood that aphids would be observed within edge areas, but this effect was strongly reduced in the presence of beetles (folivory \times predation interaction, $F_{1,46} = 11.006$, $P = 0.002$; Appendix 4d). As a main effect, predation was a significant factor influencing the proportion of aphids in edge areas ($F_{1,46} = 108.878$, $P < 0.001$), but folivory was not ($F_{1,46} = 0.194$, $P = 0.662$). Independent of the three main effects (folivory, predation, and trial), tests comparing the edge area proportion of a given leaf to the proportion of aphids in this edge area indicated that aphids were significantly rarer in these edge areas than would be predicted based solely on the leaf's edge proportion (paired t -test: $t_{26} = -9.248$, $P < 0.001$).

Plant-aphid interaction (link D). Honeydew coverage (%) under damaged leaves, $3.19 \pm 0.42\%$ (mean \pm 1 SE), was significantly less than that under undamaged leaves, $5.32 \pm 0.54\%$ ($F_{1,45} = 13.809$, $P = 0.001$; Fig. 3g), and this effect was stronger in trial 1 than trial 2 (folivory \times trial interaction, $F_{1,45} = 4.492$, $P = 0.040$; Appendix 5b). On average, $98.35 \pm 0.24\%$ of the aphid population in each aquarium existed on the leaf underside; therefore, honeydew deposition on the water-sensitive cards likely represented accurate estimates of aphid feeding rate. As expected, the percentage of aphids on leaf undersides varied relative to the interactive effects of predation and folivory (Appendix 5a). Thus, the percentage of aphids on leaf undersides was used as a covariate in the analysis of variability in the honeydew dataset. Across the range of aphid densities in this experiment, the effect of folivory on honeydew excretion tended to amplify with increasing aphid density (Fig. 3h; Appendix 5c, d). These results indicate that folivory indirectly induced aphids to feed less, particularly at higher aphid densities.

Per capita interaction strength. The sign and magnitude of the effect that a species directly imposed on another was quantified for each individual of the initiating species. There were two pathways by which caterpillar folivory affected aphids. The first pathway was a sequence of three links: caterpillar-plant, plant-beetle, and beetle-aphid (A-B-C, Fig. 1). The per capita interaction strength for each link in this pathway was significantly different from the other two links ($F_{2,52} = 51.05, P < 0.001$; Fig. 4a). Per capita interaction strength values were the following: for the caterpillar-plant interaction (link A), -0.137 ± 0.005 ; for the plant-beetle interaction (link B), -0.918 ± 0.179 ; for the beetle-aphid interaction (link C), 0.544 ± 0.113 . The second pathway was a two-link sequence (A-D, Fig. 1): caterpillar-plant and plant-aphid, with the per capita interaction strength of the plant-aphid interaction (link D) measured as 0.4690 ± 0.098 . Again, the interaction strength for each was significantly different from the other ($F_{1,53} = 39.254, P < 0.001$; Fig. 4b).

DISCUSSION

Our data show that nontrophic links may extend the impacts of a species within a community by modifying existing trophic and nontrophic relationships between species. It was evident that the chewing folivores attacking collard plants did not merely feed on their host plant, they significantly altered the leaf architecture (see images, Appendix 6). In so doing, they indirectly influenced predator foraging behavior in both field and greenhouse settings (Fig. 2b; Fig. 3c, d), which ultimately affected aphid density and distribution (Fig. 3e, f). Additionally, caterpillars indirectly suppressed aphid feeding rates by altering an aspect of host plant palatability (Fig. 3g, h). Together, these findings provide empirical evidence that nontrophic interactions can increase the capacity for significant interaction among species in a community.

By modulating the existing trophic and nontrophic relationships among species, the nontrophic impacts of caterpillars propagated along two distinct pathways (pathways A-B-C and A-D, Fig. 5), facilitating the indirect antagonism of aphids. The per capita interaction strength of each direct link in these pathways suggested that these direct interactions do not necessarily attenuate with each successive “downstream” link from the initiating species (Fig. 4a, b). Nontrophic interactions between species, therefore, are likely to affect adjacent links, but we did not find evidence of a trend in the magnitude or sign of these effects along a given pathway.

Folivory by insects is one of the most pervasive phenomena in terrestrial ecosystems (Lawton and Strong 1981), and thus the effects of herbivory by innumerable chewing folivores may represent non-trivial factors for the species sharing these plants. In collard fields, the nontrophic effects of chewing folivores appeared to have significant consequences for the many phloem-feeders that also exploit the collards. In this system, it was clear that as the number of folivory sites in a leaf increased, so too did the proportion of edge area in the leaf (Appendix 1). These edges were the most commonly searched leaf surfaces for a variety of predators. In particular, coccinellid beetles searched the edge areas of leaves far more than they searched non-edge areas, and this likely shaped the aphid distribution on leaves (Fig. 2b), if not the overall aphid abundance (Fig. 2a). We show that aphids on collard leaves were much less likely to be observed in edge areas than would be predicted based solely on the proportion of edge area in the leaves. To attribute this result to either folivory or predation, we separated these factors in a controlled greenhouse environment. In the greenhouse, our findings show that chewing folivores were indeed capable of dramatically altering leaf architecture by chewing many holes throughout the leaf surface (Fig. 3b; see image in Appendix 6c). This effectively created “interior edges” in

the leaves, increasing the proportion of edge area (Fig. 3a), and in so doing, caterpillars imposed a nontrophic effect on the plant. Thus, folivory produced both trophic and nontrophic effects.

In turn, the increased edge area of damaged leaves induced beetles to spend more time foraging on such leaves (Fig. 3b), as well as to concentrate their foraging at the edges (Fig. 3c). The beetles' strong preference for leaf edges may be explained by their difficulty in maintaining their hold on waxy leaf surfaces. Previous research showed that convergent ladybird beetles spent ~85% of their time crawling along the leaf veins and edges of cabbage leaves with normal wax blooms, whereas when allowed to crawl over plants with minimal wax, the beetles spent similar amounts of time on all areas of the leaf (Eigenbrode et al. 1996). Concentrating foraging effort along stems and leaf edges has been documented for the convergent ladybird beetle as well as the seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Straub and Snyder 2008).

Aphid abundance declined significantly under the effects of beetle predation, but only when aphids were on damaged leaves (Fig. 3e). In effect, the beetles were unable to further reduce aphid densities below no-predator controls without the advantages afforded by folivory. This suggests that the aphids exploited, to some extent, a refuge within the interior spaces of leaf undersides. We see further evidence of this explanation in the regressions of “proportion of aphids in edge area” versus “leaf edge-area proportion” (Fig. 3f). At the lower range of leaf edge area (0.10 to 0.15, representing the edge areas of undamaged leaves), there is little indication that the proportion of aphids in edge areas is influenced much by the presence of predators—here, the lower range represents edge areas of undamaged leaves, and since the proportion of edge area is so small, there are few aphids in these areas to begin with. However, at the mid- and upper end of the leaf edge area range, we see greater separation between the predator-absent and predator-present replicates. This is intuitive considering that as the amount of edge area increases, the

likelihood that an aphid would find itself near an edge would also increase—as long as predators were not patrolling that edge. Where predators were present, the proportion of aphids near edges was very low, even as leaf edge area increased (Fig. 3f), indicating that aphid densities were held low because folivory sites provided beetles with increased access to the aphids. In broader terms, folivory effectively reduced the amount of enemy-free space in the collard canopy via multi-link chains of trophic and nontrophic interactions (see pathway A-B-C, Fig. 5). This interaction pathway represents an example of interspecific facilitation, given that the effects of caterpillar folivory increased predation of aphids by facilitating access for coccinellid predators.

Using measurements of honeydew coverage on water-sensitive cards (see image of water-sensitive card, Appendix 7), we show that caterpillar folivory also indirectly influenced aphid feeding behavior (Fig. 3g). Reduced honeydew coverage under damaged leaves indicates that aphids consumed less phloem on these leaves. This effect operated independently of predators, and since caterpillars had damaged the leaves prior to the measurement of honeydew production, the effect of folivory on aphid feeding was not attributable to interference competition between caterpillars and aphids. The effect of folivory on aphid feeding, therefore, was mediated by the plant, allowing the caterpillars to indirectly antagonize the aphids by way of a second pathway (see pathway A-D, Fig. 5). Previous work has documented that inducible phytochemical defenses may reduce herbivore fitness (Agrawal and Karban 2004), and indeed, the concentrations of glucosinolates in the tissues of brassicaceous plants have been associated with the degree of feeding pressure by chewing herbivores, including diamondback moth larvae (Siemens and Mitchell-Olds 1996). In our system, an intrinsic plant response to caterpillar folivory seems to have compelled aphids to reduce their feeding (Fig. 3g); interestingly, this may partially explain our finding that leaves with folivory damage did not have significantly less

surface area than leaves without. Perhaps the reduced aphid feeding in the damaged plants allowed these plants to more fully compensate for the effects of folivory.

Given the two indirect pathways by which the caterpillars interacted with aphids, there were four direct links between species. Along the A-B-C pathway (Fig. 5), the caterpillar-plant link was both trophic and nontrophic in nature (i.e., caterpillars consumed plant biomass, but their feeding pattern also changed leaf architecture). The plant-beetle interaction, link B, was nontrophic in nature, and it was significantly influenced by the nontrophic impacts of the caterpillars on the leaves. Finally, while the beetle-aphid interaction (link C) was trophic in nature, it was modified significantly by the nontrophic plant-beetle interaction. In the second interaction pathway (see A-D, Fig. 5), the caterpillar-plant interaction not only affected leaf architecture but also induced a change in plant quality such that the aphids reduced their feeding rate. As a result, the trophic link between the aphids and the plants (link D) was significantly modified. These results suggest that nontrophic interactions among the species in this system allowed caterpillars to significantly antagonize their competitors, the aphids, via two indirect pathways. In each multi-link pathway, our evidence shows that nontrophic effects modified both the trophic and nontrophic links between species.

To assess the magnitude of the effect at each link in the two pathways, we quantified the per capita interaction strength of the initiating species. We show that the sign and magnitudes of the effects differ, and that the direct links within an interaction pathway can amplify or attenuate with each successive link from the initiating species (Fig. 4a, b). Note that link A (i.e., the caterpillar-plant interaction) has a relatively small, negative magnitude, but despite this low interaction strength, the altered plant architecture has a rather large effect on beetle foraging (link B) (Fig. 4a). By link C, the magnitude of the interaction strength attenuates (and becomes

positive), indicating that while enhanced foraging among beetles did increase aphid predation, the propagating effect of caterpillar folivory did not continue to amplify. Thus, within a chain of direct interactions, the strength of the interaction at any given link may have little bearing on the strength of an adjacent link. The implication of idiosyncratic interaction strengths is that for the purpose of food web modeling, the magnitude of a given link affords little predictive value for the magnitude of other links in the pathway.

Our study has explored the nontrophic effects of folivory in a subset of the broader collard community, showing empirically that folivory by insects has the capacity to transform the architecture of the plant canopy, effectively enhancing enemy access to prey, and altering the feeding rates of other herbivores in the plant canopy. The nontrophic effects of folivory generated community-scale consequences in the collard system, and these effects were not predicated on cascading predator-prey interactions; thus, our findings complement the growing body of literature documenting the cascading effects of trait-mediated interactions in a wide variety of ecosystems (e.g., Schmitz et al. 2004, Preisser et al. 2005). By quantifying the interaction strengths of the species in our system, we provide biological bases for parameterization of the per capita effects of caterpillar folivory on plants, on predator foraging, and on aphid density and feeding rate. Given that interaction strengths among species effectively relate food web structure to community dynamics (Berlow et al. 2004), the integration of nontrophic interaction strengths into food web models should permit such models to more accurately predict community dynamics from their structure (Borer et al. 2002, Berlow et al. 2004, Goudard and Loreau 2008).

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FIGURE LEGENDS

Fig. 1 Hypothetical interaction network among the species in a subset of the collard plant community. Primary production is represented by a collard plant, primary consumption is represented by chewing folivores (caterpillar silhouette) and phloem-feeders (aphid silhouettes), and secondary consumption is represented by predators (beetle silhouette). Link “A” denotes the interaction between chewing folivores and the plant; link “B” denotes the relationship between plants and predators; link “C” denotes the interaction between predators and phloem-feeders; link “D” denotes the interaction between the plant and phloem-feeders.

Fig. 2 a) Aphid density in edge and non-edge microsites of collard leaves in the field. b) Proportions of total leaf area, predator foraging time, and aphid density represented by edge and non-edge microsites. Data are means \pm 1 SE.

Fig. 3 a) Effect of folivory on the proportion of edge area in collard leaves; b) regression of edge area vs. number of folivory sites in collard leaves; c) effect of folivory on the total time predators spent foraging on leaves; d) regression of the proportion of time predators spent foraging within edge areas vs. leaf edge area proportion; e) the effects of folivory in the presence (●) or absence (○) of predators; f) regressions of proportion of aphids in edge areas vs. leaf edge area proportion, in the presence (●) or absence (○) of predators; g) effect of folivory on honeydew coverage (%); h) regressions of honeydew coverage vs. aphid density, in the presence (●) or absence (○) of folivory.

Fig. 4 Per capita interaction strengths of each link in two indirect pathways: a) caterpillar-plant, plant-beetle, and beetle aphid; b) caterpillar-plant and plant-aphid. Per capita interaction strength calculated as $\ln[(N_{\text{control}} + 1)/(N_{\text{treatment}} + 1)]/D$, where N denotes the relevant response variable, and D denotes the density of the species initiating the interaction.

Fig. 5 Hypothetical interaction network among the species in a subset of the collard plant community. Two indirect interaction pathways between chewing folivores and phloem-feeders are mapped. The overarching top arrow (pathway A-B-C) represents one multi-link chain of indirect interactions, and the lower arrow (pathway A-D) represents the second multi-link pathway.

FIGURES

Figure 1

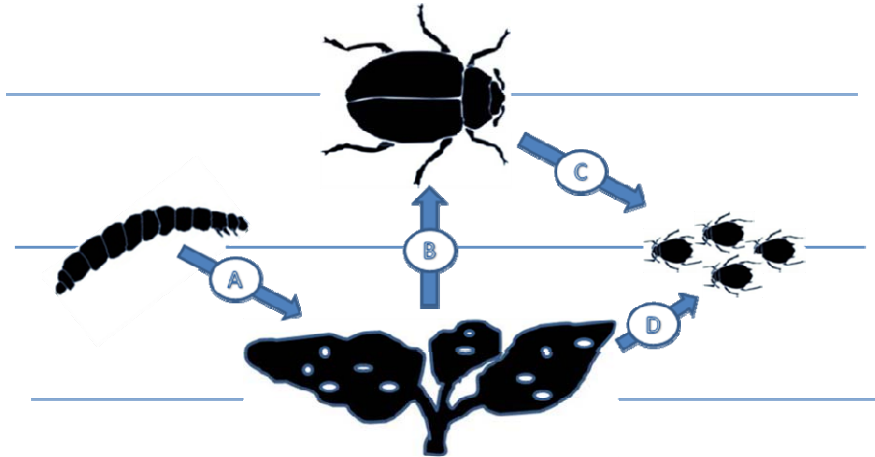


Figure 2

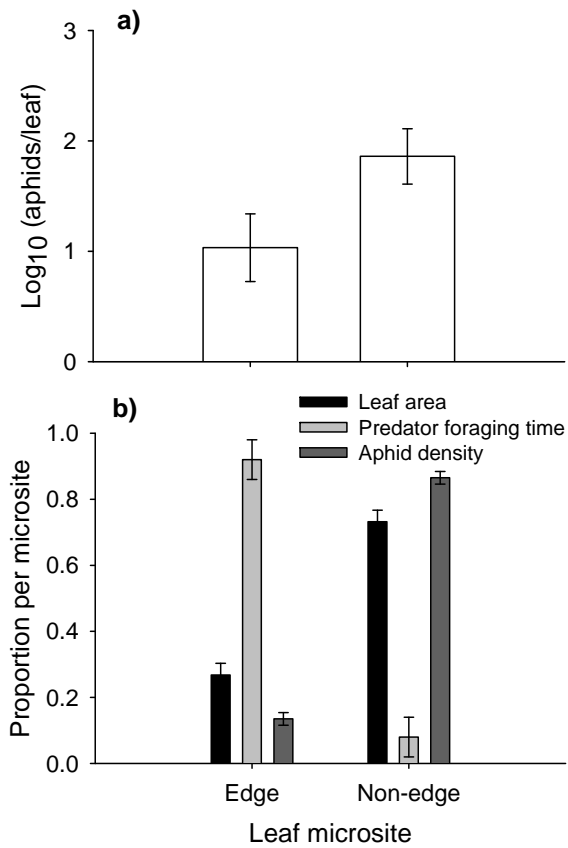


Figure 3

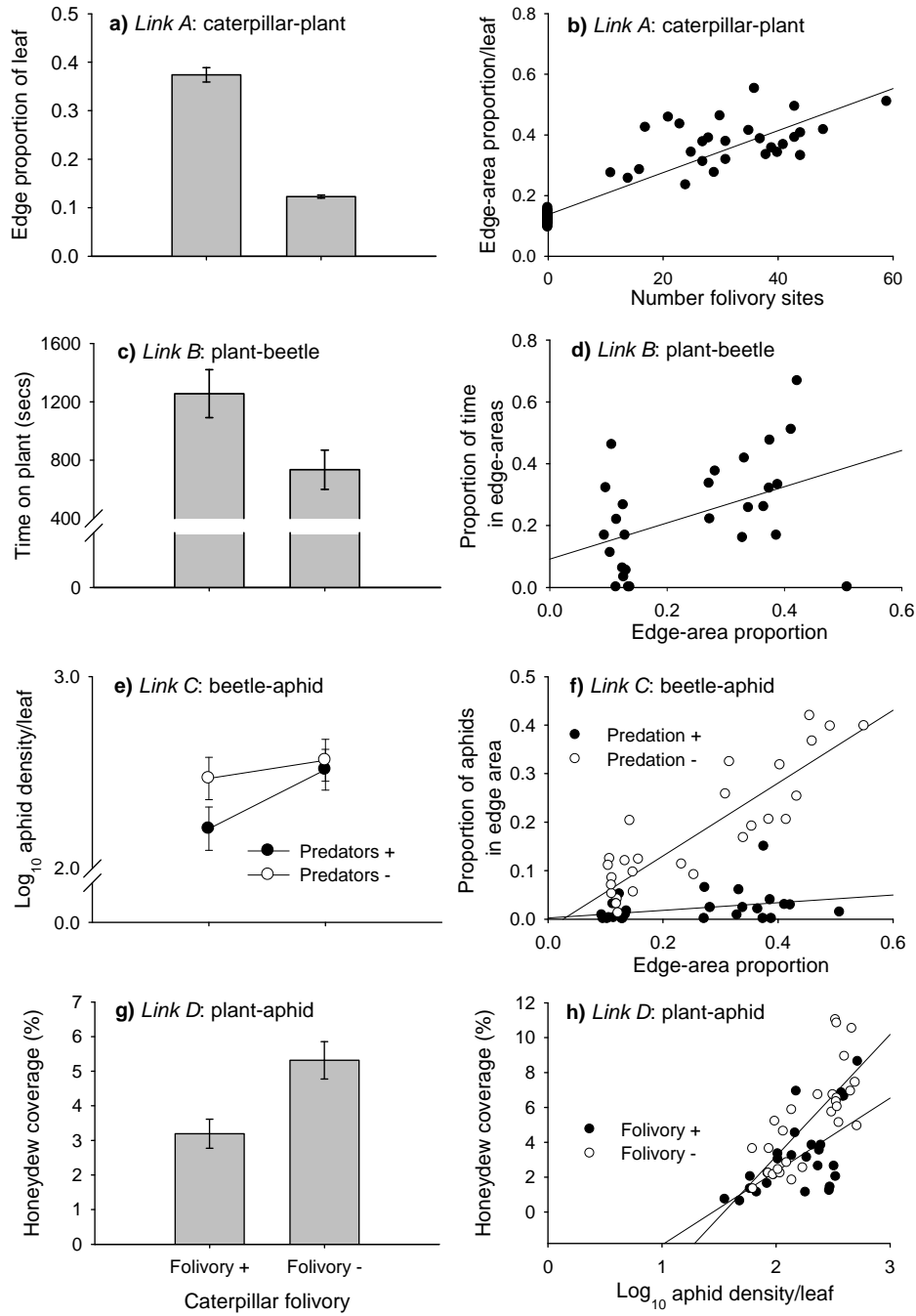


Figure 4

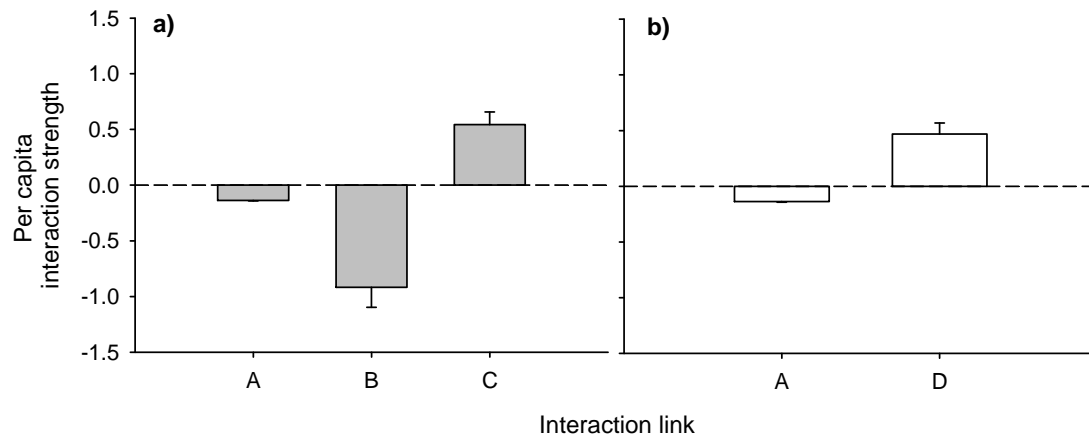
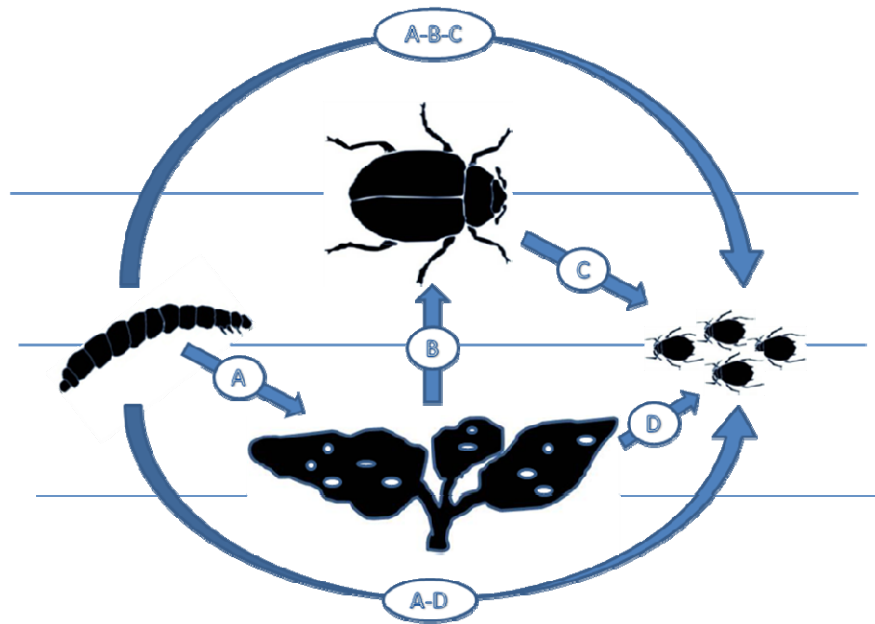


Figure 5



APPENDICES

Appendix 1 Regression: Folivory vs. Edge area: field sites

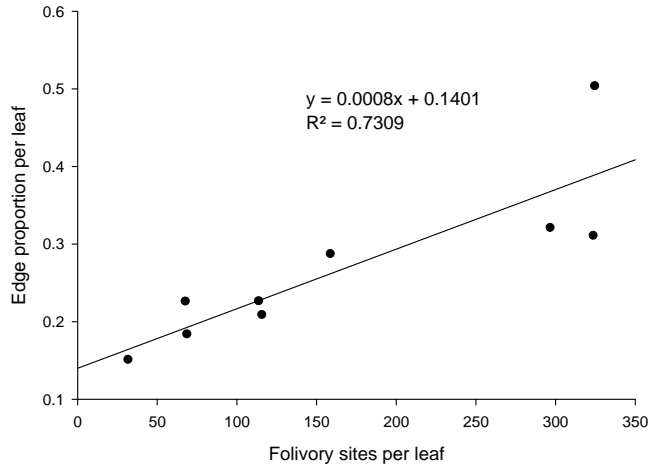


Figure (above) shows significant linear relationship between the number of folivory sites in a leaf and the proportion of the leaf's surface area represented by edge area.

Regression statistics: Folivory sites vs. Edge proportion

Model: $y = \beta_1x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	0.140	0.035	3.976	0.005
Folivory sites (β_1)	0.001	< 0.001	4.360	0.003

Regression ANOVA

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Regression	1	0.065	19.011	0.003
Residual	7	0.003		

Appendix 2a Caterpillar-plant interaction: ANOVA table

Response variable: proportion of leaf area represented by edge area

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	0.866	248.97	< 0.001
Enemies	1	0.003	0.949	0.335
Trial	1	0.001	0.281	0.598
Folivory × Enemies	1	0.001	0.303	0.585
Folivory × Trial	1	0.002	0.555	0.460
Enemies × Trial	1	< 0.001	0.063	0.803
Foliv × Enemies × Trial	1	< 0.001	0.012	0.913
Error	47	0.003		

N = 55; one replicate deleted because one *H. convergens* died.

Appendix 2b Caterpillar-plant interaction: regression statistics

Variables: Folivory sites vs. Edge proportion

Model: $y = \beta_1 x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	0.137	0.011	12.425	< 0.001
Folivory sites (β_1)	0.007	< 0.001	15.235	< 0.001

Regression ANOVA

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Regression	1	0.848	232.096	< 0.001
Residual	53	0.004		

N = 55; one replicate deleted because one *H. convergens* died.

Appendix 2c Caterpillar-plant interaction: ANOVA table

Response variable: Total leaf area

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	2763.78	2.28	0.138
Enemies	1	2987.04	2.45	0.124
Trial	1	54597.76	44.95	< 0.001
Folivory × Enemies	1	87.724	0.072	0.789
Folivory × Trial	1	123.23	0.101	0.751
Enemies × Trial	1	3307.41	2.723	0.106
Foliv × Enemies × Trial	1	33.64	0.028	0.869
Error	47	1214.55		

N = 55; one replicate deleted because a beetle had died.**Appendix 3a** Plant-beetle interaction: ANOVA table

Response variable: Total time (secs) foraging on leaves

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	1872330.4	6.39	0.019
Trial	1	202814.9	0.69	0.414
Folivory × Trial	1	774820.5	2.64	0.118
Error	23	293170.3		

N = 27 (one replicate removed because a beetle had died)

Appendix 3b Plant-beetle interaction

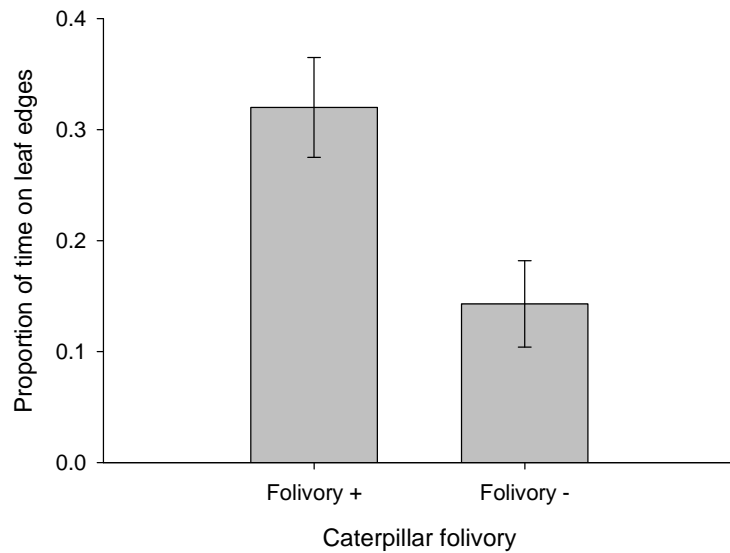


Figure (above) shows differing proportions of time spent in edge areas, relative to the presence/absence of caterpillar folivory (mean \pm 1 SE).

Plant-beetle interaction: ANOVA table

Response variable: Proportion of time spent in edge areas

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	0.221	9.869	0.005
Trial	1	0.002	0.092	0.764
Folivory \times Trial	1	0.086	3.860	0.062
Error	23	0.022		

N = 27 (one replicate removed because a beetle had died)

Appendix 3c Regression statistics: plant-beetle interaction

Variables: Leaf edge area proportion vs. Time spent foraging at edges

Model: $y = \beta_1x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	0.091	0.067	1.369	0.183
Leaf edge area prop. (β_1)	0.586	0.241	2.431	0.023

Regression ANOVA

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Regression	1	0.156	5.911	0.023
Residual	25	0.026		

Appendix 4a ANOVA table: beetle-aphid interaction

Response variable: Final aphid density

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	0.478	21.889	< 0.001
Enemies	1	0.520	23.809	< 0.001
Trial	1	0.518	23.722	< 0.001
Folivory × Enemies	1	0.160	7.326	0.010
Folivory × Trial	1	0.009	0.424	0.518
Enemies × Trial	1	0.001	0.046	0.830
Foliv × Enemies × Trial	1	< 0.001	0.008	0.928
Covar: Initial density	1	0.580	26.564	< 0.001
Error	47	0.022		

N = 55; one replicate deleted because a beetle had died.

Appendix 4b Regression statistics: beetle-aphid interaction (beetles absent)

Variables: Leaf edge area proportion (no preds) vs. Proportion of aphids in edge areas (no preds)

Model: $y = \beta_1x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	-0.019	0.023	-0.854	0.401
Leaf edge area prop (β_1)	0.750	0.077	9.751	< 0.001

Regression ANOVA

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Regression	1	0.328	95.083	< 0.001
Residual	26	0.003		

Appendix 4c Regression statistics: beetle-aphid interaction (beetles present)

Variables: Leaf edge area proportion vs. Proportion of aphids in edge areas

Model: $y = \beta_1x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	0.002	0.012	0.166	0.869
Leaf edge area prop. (β_1)	0.079	0.045	1.748	0.093

Regression ANOVA

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Regression	1	0.003	3.056	0.093
Residual	25	0.001		

Appendix 4d ANOVA table: beetle-aphid interaction

Response variable: Proportion of aphids in edge areas (square root-arcsine transformed)

Source	df	MS	F-ratio	P
Folivory	1	0.002	0.194	0.662
Enemies	1	0.858	108.878	< 0.001
Trial	1	0.010	1.242	0.271
Folivory × Enemies	1	0.087	11.006	0.002
Folivory × Trial	1	0.006	0.763	0.387
Enemies × Trial	1	0.001	0.081	0.778
Foliv × Enemies × Trial	1	0.008	0.976	0.328
Covar: Initial edge proportion	1	0.090	11.387	0.002
Error	46	0.008		

N = 55 (one replicate removed because a beetle had died)

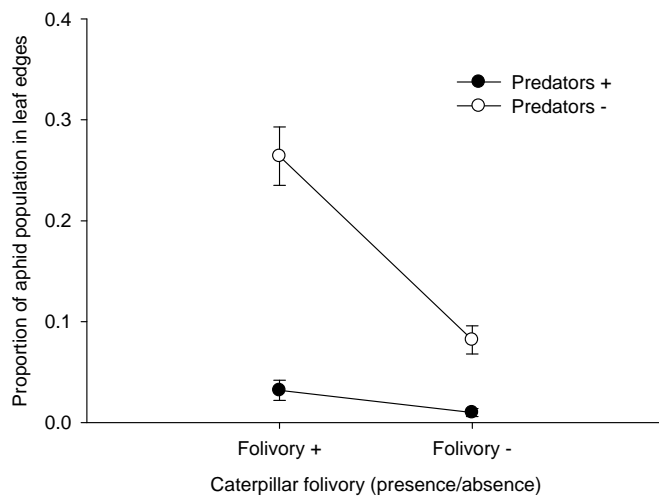


Figure (above) shows the interactive effects of Folivory and Predation (mean \pm 1 SE) on the proportion of aphids in edge areas.

Appendix 5a ANOVA table: aphid distribution on leaf undersides

Response variable: Percent of aphids on leaf undersides

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	0.365	0.195	0.661
Enemies	1	13.966	7.451	0.009
Trial	1	16.394	8.746	0.005
Folivory × Enemies	1	14.518	7.745	0.008
Folivory × Trial	1	13.698	7.308	0.010
Enemies × Trial	1	25.575	13.644	0.001
Foliv × Enemies × Trial	1	1.247	0.665	0.419
Error	47	1.874		

N = 55 (one replicate removed because of dead beetle)

Appendix 5b ANOVA table: plant-aphid interaction

Response variable: honeydew coverage (%) on water-sensitive cards

Source	df	MS	<i>F</i> -ratio	<i>P</i>
Folivory	1	55.739	13.809	0.001
Enemies	1	0.924	0.229	0.635
Trial	1	73.280	18.155	< 0.001
Folivory × Enemies	1	1.128	0.280	0.600
Folivory × Trial	1	18.129	4.492	0.040
Enemies × Trial	1	0.111	0.028	0.869
Foliv × Enemies × Trial	1	0.473	0.117	0.734
Covar: % on leaf underside	1	4.554	1.128	0.294
Error	45	4.036		

N = 54 (one replicate removed because of dead beetle, and one replicate missing among water-sensitive cards)

Appendix 5c Regression statistics: plant-aphid interaction (folivory present)

Variables: Aphid density vs. Honeydew coverage (%)

Model: $y = \beta_1x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	-6.096	2.384	-2.557	0.017
Aphid density (β_1)	4.212	1.070	3.935	0.001

$R^2 = 0.382$; *N* = 27 (one missing water-sensitive platform)

Appendix 5d Regression statistics: plant-aphid interaction (folivory absent)

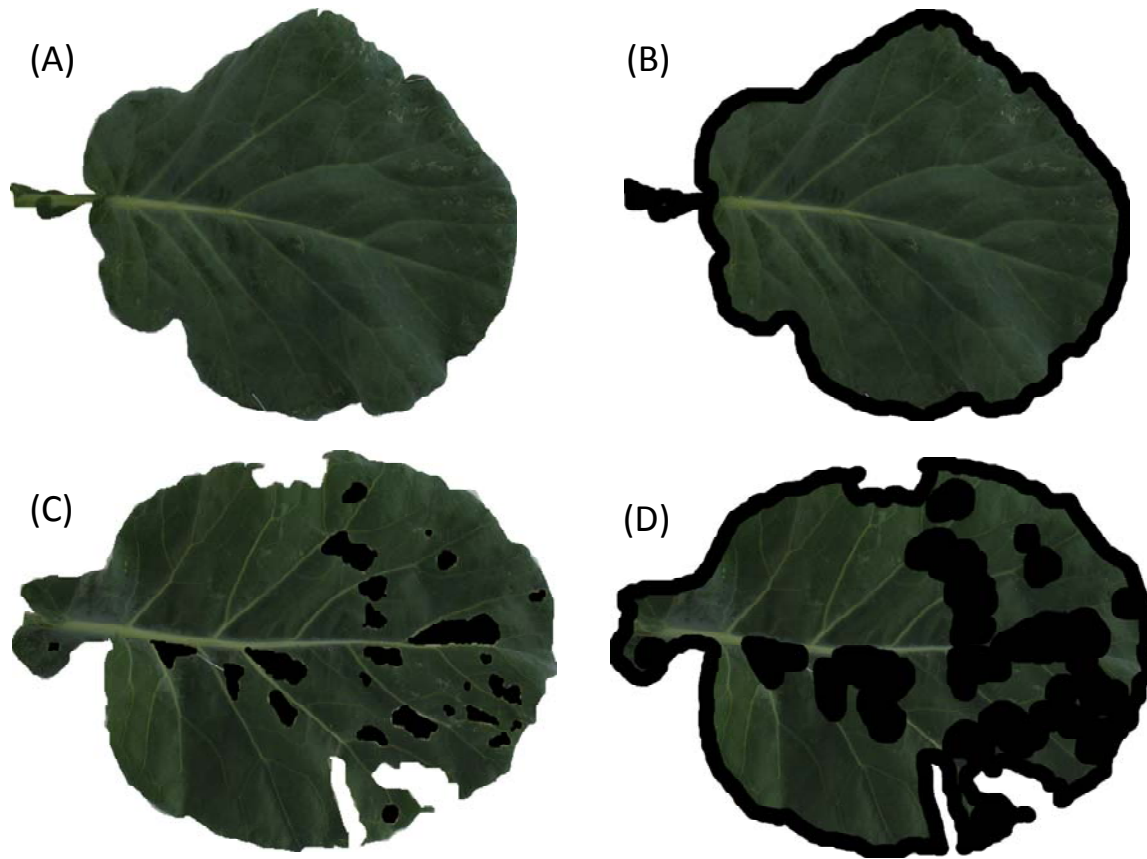
Variables: Aphid density vs. Honeydew coverage (%)

Model: $y = \beta_1 x + \beta_0$

Effect	Coefficient	Std. Error	<i>t</i>	<i>P</i> (2 tail)
Constant (β_0)	-10.788	2.840	-3.799	0.001
Aphid density (β_1)	6.992	1.223	5.717	< 0.001

$R^2 = 0.567$; $N = 27$ (one replicate with missing Hippo)

Appendix 6



Digital images of collard leaves: (A) an undamaged leaf; (B) the same undamaged leaf, with leaf edge areas (i.e., surfaces < 5 mm of edges) blackened; (C) a leaf damaged by folivory (folivory sites at the leaf interior have been blackened for emphasis); (D) the same damaged leaf, with leaf edge areas blackened. Note in panel B that the aphid refuge at the interior of the leaf remains intact, as opposed to panel D, where predators would have gained considerable access to the leaf interior (indicated by blackened areas along interior edges).

Appendix 7



An example of the water-sensitive platform used to measure aphid feeding rates. Each blue dot represents the deposition of honeydew, the watery and sugar-rich excrement of aphids. Honeydew coverage (%) was quantified using ImageJ software.