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Experimental test of biotic resistance to an invasive herbivore provided by potential plant mutualists

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Abstract Understanding the influence of resident species on the success of invaders is a core objective in the study and management of biological invasions. We asked whether facultative food-for-protection mutualism between resident, nectar-feeding ants and extrafloral nectar-bearing plants confers biotic resistance to invasion by a specialist herbivore. Our research focused on the South American cactus-feeding moth *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) in the panhandle region of Florida. This species has been widely and intentionally redistributed as a biological control agent against weedy cacti (*Opuntia* spp.) but arrived unintentionally in the southeast US, where it attacks native, non-target cacti and is considered a noxious invader. The acquired host-plants of *C. cactorum* in Florida secrete extrafloral nectar, especially on young, vegetative structures, and this attracts ants. We conducted ant-exclusion experiments over 2 years (2008 and 2009) at two sites using

potted plants of two vulnerable host species (*O. stricta* and *O. ficus-indica*) to evaluate the influence of cactus-visiting ants (total of eight species) at multiple points in the moth life cycle (oviposition, egg survival, and larval survival). We found that the presence of ants often increased the mortality of lab-reared *C. cactorum* eggsticks (stacks of cohered eggs) and larvae that we introduced onto plants in the field, although these effects were variable across sites, years, host-plant species, ant species, and/or between old and young plant structures. In contrast to these “staged” encounters, we found that ants had little influence on the survival of cactus moths that occurred naturally at our field sites, or on moth damage and plant growth. In total, our experimental results suggest that the influence of cactus-visiting ants on *C. cactorum* invasion dynamics is weak and highly variable.

Keywords Biological invasion · Biotic resistance · *Cactoblastis cactorum* · Mutualism · *Opuntia*

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Introduction

Understanding the processes that determine the abundance and distribution of invasive exotic species is a central goal in the study of biological invasions. The hypothesis that interactions with resident species can reduce the establishment probability and/or population density of invaders—the biotic resistance hypothesis—has a long history in invasion biology

(Elton 1958) and has stimulated a large body of research. Patterns emerging from field experiments show that interactions with resident (native or established non-native) species can limit the performance or abundance of invaders, providing support for the biotic resistance hypothesis (reviewed in Levine et al. 2004; Maron and Vila 2001). Most studies of the role of biotic interactions in invasion dynamics have focused on competition, predation, herbivory, or disease (Levine et al. 2004). Less is known regarding the role of positive interactions in biological invasions, especially protection mutualisms.

Protection mutualisms involve offers of food and/or housing in exchange for defense against enemies. The interaction between ants and plants that secrete extrafloral nectar (EFN) is a widespread form of protection mutualism wherein ants provide body-guard services against plant enemies (e.g., herbivorous insects) in exchange for EFN, an energy-rich resource (Bronstein 1998; Rico-Gray and Oliveira 2007). Effective mutualism therefore includes at least three species: two partners (one offering a reward, the other providing protection) and an enemy of the rewarding partner. Protection mutualisms, such as those between ants and EFN-plants, can influence the dynamics of biological invasions in one of two contrasting ways, depending upon which side of the interaction the invader falls. First, invaders may engage as partners with resident species, as when invasive ants tend resident EFN-plants (reviewed in Holway et al. 2002; Ness and Bronstein 2004) or when invasive EFN-plants recruit resident ants. In these interactions, benefits provided by resident species could potentially enhance the establishment success or abundance of the invaders. Alternatively, if invaders are plant enemies, they may face biotic resistance in the form of mutualism between resident ants and plants. The increasing global traffic of herbivorous insects (Center et al. 1995; McCullough et al. 2006), combined with the ubiquity of EFN-mediated ant-plant interactions (Bentley 1977; Bronstein 1998; Rico-Gray and Oliveira 2007), suggests that protection mutualism between ants and plants may be an important pathway of biotic resistance to invasion by exotic herbivores. Research in agricultural systems has shown that visitation by native ants to EFN-bearing crop plants can reduce damage by invasive pest insects (e.g., Matthews et al. 2007). Similar studies in natural systems are currently

lacking, yet necessary in order to broaden the conceptual framework of biotic resistance and to identify services provided by native species that could contribute to the management and prevention of biological invasions.

The frequency and outcome of ant-plant protection mutualism—and the potential for mutualist-mediated biotic resistance to invasion—are often context-dependent and subject to multiple sources of variation (Bronstein 1998; Chamberlain and Holland 2009; Rosumek et al. 2009). For example, EFN secretion and therefore ant activity are often concentrated near vulnerable plant organs such as young leaves or flower buds (Heil et al. 2000). Consequently, within a plant, certain structures may be fiercely defended by ants while others remain vulnerable to herbivores (Palmer and Brody 2007). Ant partner identity is another potential source of variability, as EFN-plants typically associate with multiple species of ants that may vary in the quality of protection they provide (e.g., Frederickson 2005; Miller 2007; Ness et al. 2006). Finally, the outcomes of ant-plant interactions can vary across sites or years due to spatio-temporal variation in the abiotic or biotic environment (e.g., Barton 1986; Inouye and Taylor 1979; Rudgers and Strauss 2004), which can include factors such as the species composition of the ant community. All of these factors might influence the effectiveness of ant-plant protection mutualism as a mechanism of biotic resistance to invasion by exotic herbivores.

The cactus-feeding moth *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) Berg is among the most notorious exotic herbivores in North America. This species is native to South America and, during the mid twentieth century, was intentionally released throughout Africa, Asia, and Australia as a biological control agent against weedy and invasive cacti in the genus *Opuntia* (Petty 1948; Zimmerman et al. 2001). *Cactoblastis* was unexpectedly discovered in the Florida Keys in 1989, its arrival there possibly a result of island-hopping from release sites in the Caribbean (Habeck and Bennett 1990). Since its arrival, *Cactoblastis* has steadily expanded its range north and west along the Atlantic and Gulf coasts (Hight et al. 2002; Johnson and Stiling 1998). With a wide host range within the genus *Opuntia*, this “escaped” biological control agent currently attacks native, non-target cacti in the Southeast US and

threatens the global persistence of certain species (Stiling et al. 2004). It is feared that, without intervention, the moth will continue spreading westward along the Gulf coast and towards the cactus-rich ecosystems of the Southwest US and Northern Mexico, where the environmental and economic consequences of *Cactoblastis* invasion could be severe (Mahr 2001; Simonson et al. 2005; Zimmerman et al. 2004). Information on factors that could limit *Cactoblastis* population growth and impacts on native host-plants is urgently needed.

Extrafloral nectar production and visitation by nectar-feeding ants are common in the Cactaceae. Numerous studies have shown that ants can benefit cacti by reducing herbivore damage (e.g., Chamberlain and Holland 2008; Miller 2007; Oliveira et al. 1999; Pickett and Clark 1979). These studies of native cactus-herbivore interactions suggest potential for biotic resistance to *Cactoblastis* invasion via ant-cactus protection mutualism. Interestingly, the apparent failure of *Cactoblastis* as an effective biological control agent in South Africa has been partially attributed to predation of *Cactoblastis* eggs by ants attracted to cactus EFN (Robertson 1984, 1988). Yet, no studies have examined ant-cactus mutualism in the North American range of *Cactoblastis*, or quantified any effects of cactus-visiting ants on the invasive moth. Because *C. cactorum* is a strict cactus specialist, ant protection of native cacti could directly influence its population dynamics.

We conducted field experiments to test the central hypothesis that ant-plant protection mutualism confers biotic resistance to invasion by *Cactoblastis cactorum* in the panhandle region of Florida, USA. Since the moth is already established in *Opuntia* populations throughout Florida, we hypothesized that ants have negative effects on components of *Cactoblastis* population dynamics, not establishment success; this view of biotic resistance as a post-establishment process is consistent with most previous studies (Levine et al. 2004). We documented ant visitation (abundance and identity) to *Cactoblastis* host-plants and experimentally evaluated the effects of ants at three points in the cactus moth life cycle (female oviposition, survival of eggs, and survival of newly hatched larvae). We also quantified the net effects of ant visitation on plant damage and growth. We asked whether the frequency or outcome of ant-moth interactions varied: (1) across sites and/or years

of study, (2) between plant structures of different developmental stage, (3) between two host-plant species, and (4) with ant species identity. Our primary objectives were to advance understanding of the role of protection mutualism in invasion dynamics and to simultaneously identify services provided by native species that might aid in efforts to control a particularly threatening invasive herbivore.

Methods

Study sites and focal species

This study was conducted during spring of 2008 and 2009 at two coastal sites in the panhandle region of Florida, USA: Bottoms Road (30° 0'59.19"N, 84°22'3.27"W) and Picnic Lake (30° 5'0.25"N, 84°10'5.47"W). Both sites are located within the St. Mark's National Wildlife Refuge (Wakulla County, FL), separated by approximately 25 km. The Bottoms Road site is a sandy outcrop of coastal scrub vegetation surrounded by salt marsh. The Picnic Lake site is situated on a levee road where soils are sandy but higher in organic content than those at Bottoms Road.

Natural populations of *Opuntia stricta* (Haw.) Haw. occurred at both study sites. This native cactus is distributed throughout coastal regions of Florida and the Gulf coast states. Like other members of the genus, *O. stricta* secretes EFN from nectaries near the bases of the spines (e.g., Miller 2007; Oliveira et al. 1999). EFN production by *O. stricta* is restricted to young, actively expanding cladodes, or photosynthetic stem segments (*pers. obs.*). Young cladodes are easily distinguished by their size, color, and presence of true leaves. In our region, *O. stricta* initiates new cladodes and reproductive structures in a pulse during April–May, and many plants continue to produce new cladodes until September–October. A previous study showed that ant traffic was greater on young cladodes versus old cladodes (Robbins and Miller 2009). Because young cladodes are the primary EFN sources, these data suggest that ant activity on cacti is motivated by plant rewards (i.e., ants are not transient). We have also directly observed ants collecting EFN from young cladodes.

Opuntia stricta is the primary host-plant for *C. cactorum* in North America and *O. stricta*–*C. cactorum* interactions have been studied elsewhere

in Florida (Baker and Stiling 2009; Johnson and Stiling 1996). In the panhandle region, where this study was conducted, there are three *C. cactorum* generations per year (Hight and Carpenter 2009; Legaspi et al. 2009b). Female moths deposit “eggsticks” (stacks of 50–90 cohered eggs) on cactus spines or on the surfaces of cladodes (Legaspi et al. 2009a). Upon hatching, larvae burrow into the plant and move among adjacent cladodes. During the course of larval development (4–16 weeks, depending on temperature: Legaspi and Legaspi 2007), injury to the host-plant (cladode mortality and plant size reduction) can be severe. Late-instar larvae emerge from the host-plant and pupate among debris near the base of the plant. Based on the life cycle, we hypothesized that ant activity on cacti could deter ovipositing females, reduce the survival of eggsticks, and/or reduce the survival of newly hatched (neonate larvae), and so we focused on these demographic transitions. In contrast, there is little opportunity for effects of ants on pupae or emerging adults.

The native cactus-feeding moth *Melitara prodenialis* Walker (Lepidoptera: Pyralidae) also occurred at our study sites (Results). This species has a similar life cycle to *C. cactorum* but has two generations per year (Legaspi et al. 2008). Eggsticks of the two moth species can be distinguished by length and thickness (Legaspi et al. 2008). The spring period of *M. prodenialis* oviposition and larval development coincided with that of *C. cactorum*, providing the opportunity to compare effects of ants on the invasive and native cactus moths.

Potted cactus experiments

Testing hypotheses about protection mutualism and ant-mediated biotic resistance required comparisons between ant-tended plants and plants from which ants were experimentally excluded. Excluding ants from naturally occurring cacti is extremely difficult due to their often erratic growth form (a single plant can have many contact points with the substrate and surrounding vegetation). We therefore used potted cacti that were transported to the field sites. To broaden our inferences regarding biotic resistance to *C. cactorum* invasion, our potted plants included two cactus species: *O. stricta* (which naturally occurred at our field sites) and *O. ficus-indica* (L.) Mill., a cultivated variety that is considered a target weed of

C. cactorum in South Africa and is planted as an ornamental throughout the Southeast US (Griffith 2004). The *O. stricta* and *O. ficus-indica* plants used in this study were vegetatively propagated at the USDA-ARS Center for Biological Control (Tallahassee, FL) in 1-gallon pots from plants collected at nearby sites in the Florida panhandle. At the time of the experiments, potted cacti consisted of both old and young cladodes.

We randomly assigned each potted cactus to a field site. In the field, we sunk all pots 20–25 cm into the ground for stability. At Bottoms Road, there were 28 *O. stricta* in both years and 33 *O. ficus-indica* in 2008 and 30 in 2009 (3 died). At Picnic Lake, there were 28 *O. stricta* and 30 *O. ficus-indica* in both years. Pots of both species were regularly spaced (~2 m apart) among the naturally occurring *O. stricta* at each site. In each year, we randomly assigned pots within sites to an ant treatment (control or exclusion). We used the same plants in both years and therefore assumed that there were no carry-over effects in 2009 of treatments applied in 2008; plants were re-randomized to ant treatments in 2009. Sample sizes of control and exclusion treatments were equal for each species within each site ($n = 14$ or 15) except for *O. ficus-indica* at Bottoms Road in 2008 (control: $n = 17$; exclusion: $n = 16$). For plants assigned to the exclusion treatment, we applied a layer of Tree Tanglefoot™, a sticky barrier, in a ring around the pot to prevent ant access. Control pots were not manipulated in 2008, the pilot year of the study, because our experiments were initially focused on the interactions between ants and lab-reared cactus moth eggs and larvae. However, we found that naturally-occurring cactus moths frequently oviposited on potted cacti during the 2008 experiments. In order to take full advantage of these “natural experiments” and strengthen inferences regarding the effects of ants on moth attack, we applied a sham treatment to control pots in 2009 (ant barrier around half of the pot). In 2009, we also switched to a clear and odorless ant barrier because field and laboratory data from 2008 suggested that adult *C. cactorum* females may have been deterred by Tree Tanglefoot (unpubl. data). The initiation and duration of the ant exclusion experiments coincided with the spring oviposition period of invasive and native cactus moths (late April) and most of the larval development period (May–June).

Ant visitation and species composition

We collected data to determine the abundances and identities of ants on potted cacti, and to determine the effectiveness of the ant exclusion treatment. We also collected similar data from naturally occurring, “resident” *O. stricta* to determine if ant activity on potted plants was representative of the natural populations. In 2008, we conducted seven censuses at Bottoms Road from 18 April to 20 May and five censuses at Picnic Lake from 29 April to 22 May. In 2009, we conducted six censuses at Bottoms Road from 27 April to 27 May and six censuses at Picnic Lake from 27 April to 26 May. Census data, including ant worker identity and abundance, were recorded early in the day (0800 to 1100); ants were counted during an observation period of approximately 1 min. At each census, ant exclusion treatments were maintained by removing debris from the sticky barrier and removing ant “bridges” provided by surrounding vegetation. We examined the effects of ant exclusion treatment on cumulative (across-census) worker abundances using two-way analyses of variance (ANOVA) with plant species (*O. stricta* or *O. ficus-indica*), ant treatment (exclusion or control), and their interaction as fixed factors; cumulative ant counts were square root-transformed, which stabilized variances. We conducted separate analyses for each site-year combination because the number of censuses varied among these combinations. For each site in each year, we also calculated the relative abundances for each of the ant species recorded on resident *O. stricta*, potted *O. stricta*, and potted *O. ficus-indica*, pooling all workers from each plant group.

Effects of ants on moth oviposition

We recorded oviposition on potted cacti by naturally occurring *C. cactorum* and *M. prodenialis* females during each of the ant surveys described above. Each plant was scored for the presence or absence of any *C. cactorum* or *M. prodenialis* eggsticks over the duration of the census period for each site in each year. We hypothesized that ant exclusion plants would have a greater probability of moth oviposition than control plants, and we used logistic regression to test this hypothesis (PROC LOGISTIC, SAS 9.2). The statistical models included effects of site, plant species, ant treatment, and their interactions on the probability of

oviposition. We inferred that a model factor was significant if its fitted slope differed significantly from zero ($\alpha = 0.05$); the test statistic for this comparison follows a χ^2 distribution. Because the frequencies of oviposition by each moth species were low (leading to problems with parameter convergence), we pooled *C. cactorum* and *M. prodenialis* eggsticks for analysis, though we present the data partitioned by species. Due to the unintended negative effect of Tree Tanglefoot on natural oviposition in 2008, we analyzed and present data from 2009 only, when we used a clear and odorless ant barrier and applied a sham treatment to control plants. Given the frequency of our surveys, we assumed that any effects of ants reflected deterrence of ovipositing females and not post-oviposition egg predation; the latter is considered below. While some plants received multiple eggsticks, we used a binomial response variable because it is likely that multiple eggsticks were laid by a single female and were therefore not independent. Analysis of eggstick counts yielded qualitatively similar results (not shown).

Effects of ants on eggstick survival

We conducted experiments to determine if ants influence the survival of *Cactoblastis* eggsticks. These experiments were conducted at both field sites in both years, and included three factors in a split-plot design. Plant species (*O. stricta*/*O. ficus-indica*) and ant access (control/exclusion) were whole-plot (plant-level) factors and cladode age class (young/old) was the split-plot (within-plant) factor. Because EFN production is concentrated near actively expanding tissues, we hypothesized that ant protection would be greater on young cladodes than on old ones.

We collected eggsticks from a colony of *C. cactorum* maintained at the USDA-ARS Center for Biological Control (Tallahassee, FL) and counted the numbers of eggs in each. We then transported the eggsticks to the field sites and added two eggsticks to each potted plant, one on a young cladode and one on an old cladode. Cladodes for eggstick addition were chosen haphazardly within age class, though we matched the heights of eggsticks on young and old cladodes. We secured eggsticks on cladodes by slipping each through a slit in a small piece of paper and pinning the paper to the cladode. This method proved reliable in preliminary experiments, more so than adhering eggsticks with glue (J.C.L., *unpubl.*

data). We censused eggstick survival (presence/absence) 8 days after addition. On day 8, we terminated the experiment and returned all remaining eggsticks to the lab, where the numbers of eggs in each was re-counted. We terminated the experiment short of the mean egg development time to minimize risk of moths from the lab colony escaping into the field. Naturally-deposited eggsticks would be exposed to ants for a longer period of time, and so our test is relatively conservative.

Due to the nested experimental design and binomially distributed response variable, we analyzed the probability of eggstick survival using a generalized linear mixed model (GLMM) with a logit link function (PROC GLIMMIX in SAS 9.2). The models included effects of host-plant species, ant treatment, cladode stage, and their interactions. We were unable to estimate model parameters for Picnic Lake in 2008 due in part to zero eggstick mortality for some treatment combinations. We therefore fit separate GLMM's to all other site-year combinations. We assessed factor significance using *F* statistics. For surviving eggsticks, we also compared numbers of individual eggs at the beginning and end of the field experiment. However, we found no changes in egg number and so we restrict our discussion to the survival of whole eggsticks.

We also tracked the survival of naturally deposited eggsticks, which were uniquely tagged upon detection. At each census, we recorded whether each eggstick that was present on a potted cactus at the previous census was still present, was no longer present, or whether larvae from the eggstick had hatched and entered the plant. Any eggsticks scored as "no longer present" were considered mortality events. For comparison with our "staged" eggstick addition experiment, we analyzed the probability of natural eggstick survival in relation to host-plant species and ant exclusion treatment. There were insufficient numbers of eggsticks to partition the analysis by moth species or to test for differences between cladode age classes. There were often multiple, naturally deposited eggsticks on an individual plant. Because survival probabilities of eggsticks on a single plant were not independent, we treated plant as a random, blocking factor and used a GLMM to analyze the probability of "success" (eggstick survival) given the total number of eggsticks ("trials") deposited on each plant. Due to small

sample sizes and zero mortality for some treatment combinations, we were unable to estimate model parameters for the Picnic Lake site in 2009. We therefore fit separate GLMM's to all other site-year combinations. We included 2008 data in these analyses because the deterring effect of the ant barrier in this year should have only influenced the numbers of eggsticks laid on ant exclusion plants and not their subsequent survival.

Effects of ants on larval survival

Next, we conducted experiments to determine if ants influence the survival of *Cactoblastis* larvae. We obtained neonate (<48 h old) larvae from the *Cactoblastis* colony and transported larvae to our field sites. We used both naturally occurring and potted cacti for these experiments (among the potted plants, we used only the ant access treatment). Each plant was used for a single, 2 min trial. For each trial, we introduced two larvae onto a plant, one on a young cladode and one on an old cladode, and recorded whether or not each larva survived on each cladode type. Larvae that were bitten, stung, and/or carried off the plant by ants were scored equivalently as mortality events; we observed that bitten or stung larvae ultimately died, although this often took more than 2 min. While a single *Cactoblastis* eggstick may contain up to 90 eggs, the larvae hatch asynchronously; therefore, interactions of ant workers with one or a few neonate larvae is a realistic scenario.

These experiments were conducted at both sites during mid-May in 2008. Because most plants were visited by only one ant species at a given time, we were able to compare the responses of different ant species to larva addition. However, due to variation in ant species relative abundances, sample sizes were highly unbalanced and some ant species were not available at the time of the experiments. We also made an effort to avoid using a single ant colony for multiple trials, which further limited sample sizes. We pooled trials across dates and sites to maximize sample sizes per species. The ant species tested and number of trials for each were: *D. bureni* ($n = 35$), *B. patagonicus* ($n = 25$), *P. dentata* ($n = 5$), *P. floridana* ($n = 3$), and *F. pruinosus* ($n = 3$). We conducted statistical analyses only for species with adequate sample sizes (*D. bureni* and *B.*

patagonicus). Because these experiments included two scales of replication (plants and cladodes within plants), we used a GLMM to examine the effects of ant species (whole-plot factor) and cladode age class (split-plot factor) on *C. cactorum* larval survival, a binomial response variable.

Effects of ants on moth infestation and plant growth

Finally, to evaluate the net effects of ants on cacti, we recorded damage by naturally occurring native and invasive cactus moths and quantified changes in the size of potted cacti over the duration of the experiment. These analyses were restricted to 2009 data. The numbers of live (green) cladodes on each plant were counted in April. We revisited the potted plants in mid-June and counted the number of live cladodes and the number with evidence of past or current moth infestation. Damage by *C. cactorum* and *M. prodenialis* could not be visually differentiated and so both species contributed to our damage estimates. We calculated the proportion of live pads that were infested and used analysis of variance (ANOVA) with arcsine-square root-transformed data to test for effects of site, host-plant species, ant exclusion treatment, and their interactions. To determine how moth damage translates to plant growth, we calculated the proportional change in plant size as $(\# \text{initial cladodes} - \# \text{final cladodes}) / \# \text{initial cladodes}$ and used ANOVA to test for effects of site, host-plant species, ant exclusion treatment, and their interactions; no data transformation was necessary. By mid-June, the first generation of cactus moth larvae had begun to pupate. Cladode infestation data therefore reflected cumulative damage over the larval development period, and changes in plant size reflected growth minus cladode loss due to larval damage. Because we removed lab-reared eggsticks before they hatched, most of the plant damage was caused by naturally occurring moths. While the ant exclusion treatment was not maintained through the June size and damage census, the exclusion period overlapped with the period of *C. cactorum* and *M. prodenialis* oviposition and initial larval infestation. Thus, we predicted that any protection by ants early in the moth life cycle (deterrence of ovipositing females, egg

predation, or larval predation) would have detectable effects on plant damage and size later in the season.

Results

Ant visitation and species composition

Four species of cactus-visiting ants occurred at both study sites: *Brachymyrmex patagonicus* Mayr, *Dorymyrmex bureni* (Trager), *Forelius pruinosus* Wheeler, and *Pheidole dentata* (Mayr). In addition, *Pheidole floridana* Emery occurred only at Bottoms Road, and *Crematogaster pilosa* Emery, *Monimorium viride* Brown, and *Camponotus floridanus* (Buckley) occurred only at Picnic Lake. All of these ant species are native to Florida with the exception of *B. patagonicus*, which is native to South America and is a recent invader in the Southeast US (MacGowan et al. 2007).

Ants visited potted *O. stricta* and *O. ficus-indica* at both field sites and ant abundances on potted cacti were similar to abundances on the resident *O. stricta* (Fig. 1). The exclusion treatment effectively reduced ant visitation though ambient ant densities per plant per observation period were low (<5 workers/plant). Ant exclusion led to significant reductions in cumulative worker abundance (with neither plant species nor species \times treatment effects) at Bottoms Road in both years and at Picnic Lake in 2008 (all $P < 0.002$; Fig. 1a–c, inset). However, ant activity was similarly low on control and ant exclusion plants for *O. ficus-indica* at Picnic Lake in 2009 (species \times treatment interaction: $F_{1,54} = 6.1$, $P < 0.016$; Fig. 1d, inset).

The relative abundances of cactus-visiting ant species differed strongly between field sites (Fig. 2). The non-native *B. patagonicus* was the most common visitor to cacti at Bottoms Road while the native *D. bureni* was the most common ant visitor at Picnic Lake. Within sites and years, species relative abundances were generally consistent between potted *O. stricta* and potted *O. ficus-indica*, and between potted and resident plants. Thus, our potted plants were generally representative of naturally occurring cacti with respect to the abundances and identities of visiting ants. There were some exceptions, however,

Fig. 1 Time series of mean (\pm SE) ant worker abundance at the Bottoms Road (a, c) and Picnic Lake (b, d) sites in 2008 (a, b) and 2009 (c, d). **Symbol key:** X's = naturally occurring *O. stricta*, circles = potted *O. stricta*, triangles = potted *O. ficus-indica*. Among potted plants, open shapes = ant access (control) and filled shapes = ant exclusion. **Insets** show mean (\pm SE) cumulative (across-census) worker abundance on control (open bars) and ant exclusion (filled bars) potted *O. stricta* and *O. ficus-indica*. Stars in insets indicate significant effects ($P < 0.05$) of ant exclusion

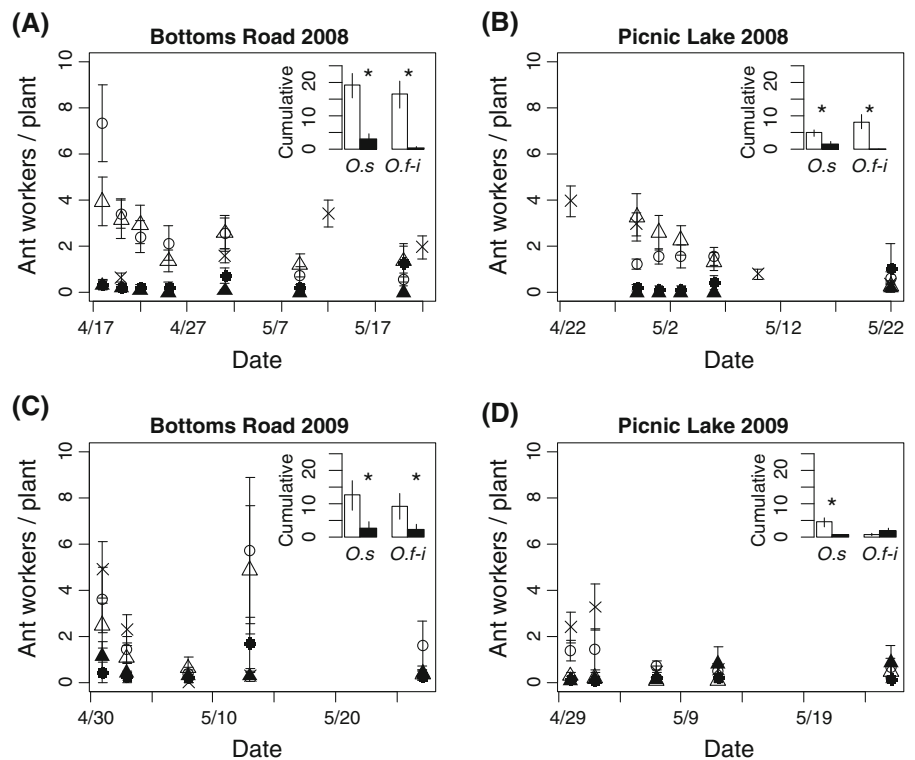
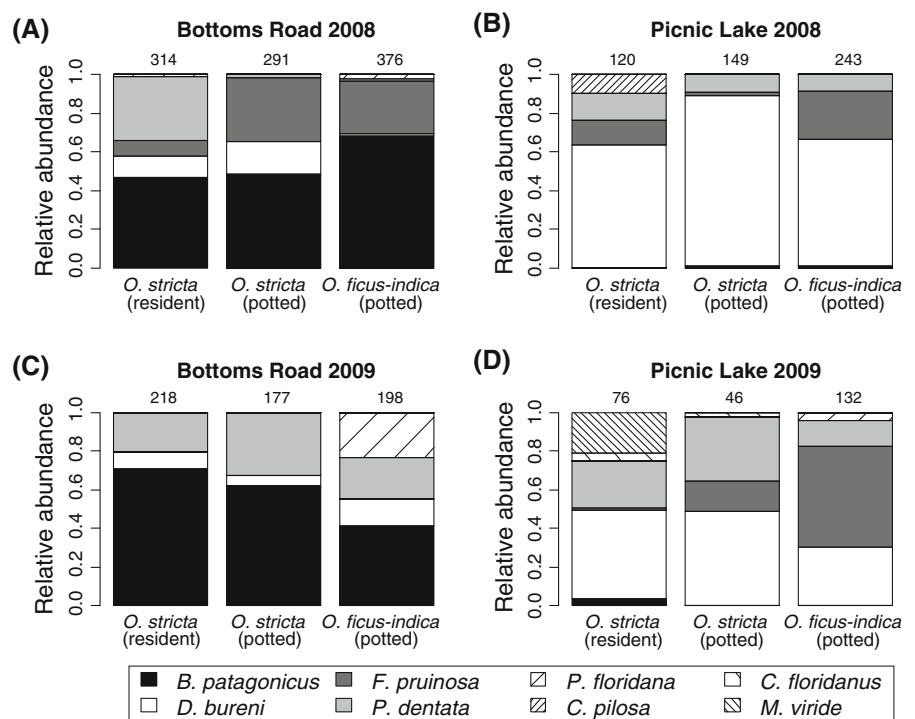


Fig. 2 Relative abundances of eight species of cactus-visiting ants on potted and resident cacti at the Bottoms Road (a, c) and Picnic Lake (b, d) sites in 2008 (a, b) and 2009 (c, d). Numbers above bars indicate sample sizes (total number of workers observed on each plant type). Species fill codes as in key



with certain ant species disproportionately represented across plant types. For example, *P. floridana* occurred only on *O. ficus-indica*, while *C. pilosa* and *M. viride* were found only on resident *O. stricta*.

Effects of ants on moth oviposition

The exclusion of ants from potted cacti provided no evidence that ants deterred ovipositing cactus moths. We found no main or interaction effects of ant treatment on the probability of oviposition in 2009 (Fig. 3a, b), though there was a strong effect of study site ($\chi^2 = 8.62$, $P = 0.003$), with more frequent oviposition at Bottoms Road than at Picnic Lake. This result was driven largely by the native *M. prodenialis*, which was rare at Picnic Lake (Fig. 3).

Effects of ants on eggstick survival

Ant exclusion influenced the mortality of lab-reared *C. cactorum* eggsticks that we introduced onto host-plants, although this effect was highly variable across sites, years, cactus species, and cladode age classes (Fig. 4). At Bottoms Road in 2008, ant exclusion significantly reduced eggstick mortality for *O. ficus-indica* but not for *O. stricta* (species * treatment interaction: $F_{1,54} = 4.08$, $P = 0.048$) and there were no significant effects of cladode age class (Fig. 4a). We were unable to fit an appropriate statistical model to the 2008 data from Picnic Lake, but the data

suggest no consistent effects of ant exclusion (Fig. 4b). At Bottoms Road in 2009, there was a significant main effect of ant exclusion ($F_{1,53} = 8.23$, $P = 0.006$), with greater eggstick mortality on ant-access plants, and no other significant main or interaction effects (Fig. 4c). Results were more complex at Picnic Lake in 2009 (Fig. 4d), where we found a significant three-way interaction of cactus species, ant exclusion treatment, and cladode age class ($F_{1,54} = 4.37$, $P = 0.04$). For *O. stricta*, ant exclusion reduced the mortality of eggsticks on young cladodes but not on old cladodes. However, for *O. ficus-indica*, ant exclusion reduced mortality on old cladodes but not on young cladodes.

In contrast to the lab-reared, introduced eggsticks, we found no significant effects of ant exclusion on the survival of eggsticks laid by naturally occurring *C. cactorum* and *M. prodenialis* at Bottoms Road in either year nor at Picnic Lake in 2008 (Fig. 5a–c). We were unable to analyze natural eggstick data from Picnic Lake in 2009, though there was a trend of reduced mortality on ant exclusion plants of both cactus species (Fig. 5d). Patterns of natural eggstick survival should be interpreted cautiously, given the small sample sizes for many treatment combinations.

Effects of ants on larval survival

Ants frequently attacked the lab-reared *C. cactorum* larvae that we introduced, though larval mortality

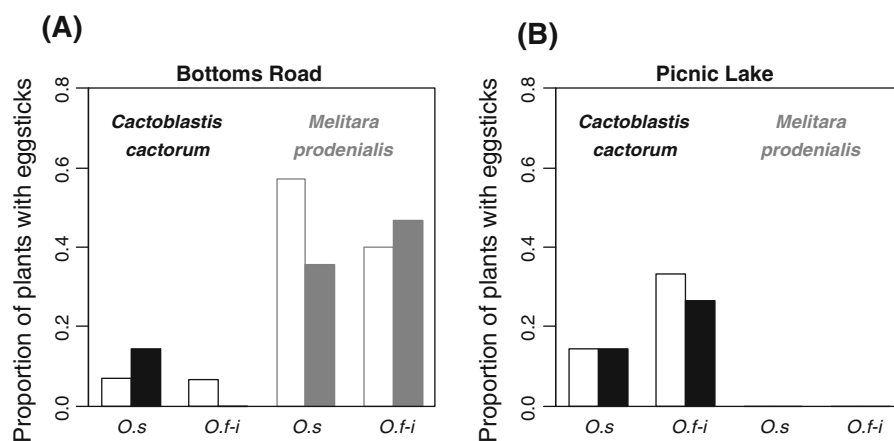


Fig. 3 Oviposition by naturally occurring *Cactoblastis cactorum* (black bars) and *Melitara prodenialis* (gray bars) on potted *O. stricta* and *O. ficus-indica* at the Bottoms Road (a)

and Picnic Lake (b) sites in 2009. Open bars = ant access plants, filled bars = ant exclusion plants. Bar height indicates the proportion of potted plants that received any eggsticks

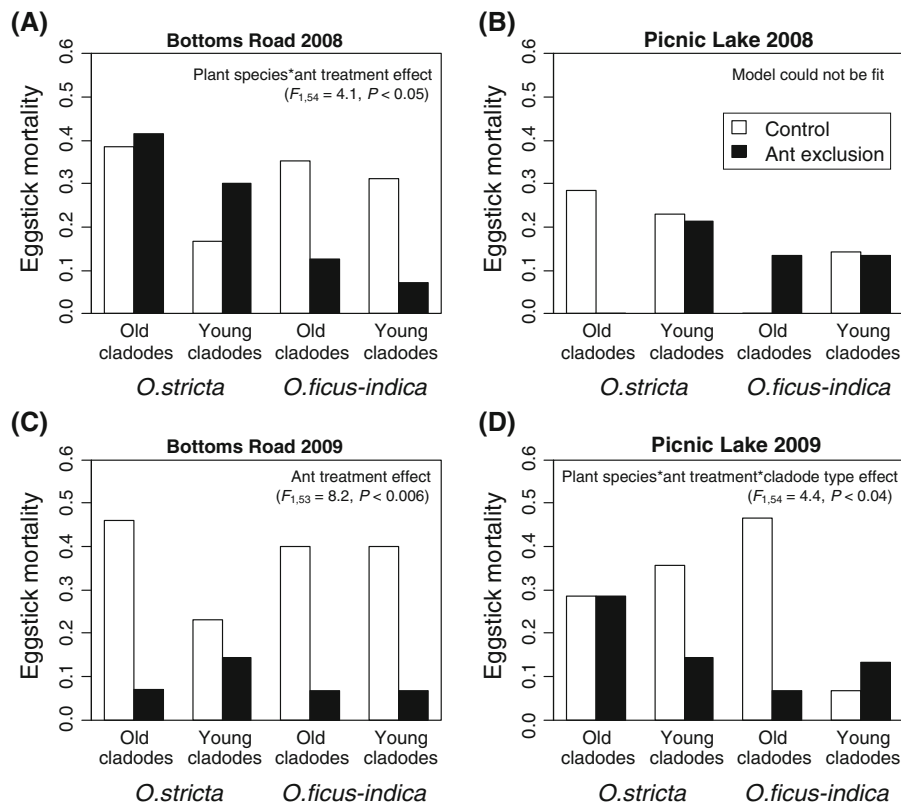


Fig. 4 Mortality of laboratory-reared *C. cactorum* eggsticks on potted *O. stricta* and *O. ficus-indica* at the Bottoms Road (a, c) and Picnic Lake (b, d) sites in 2008 (a, b) and 2009 (c, d). For each potted plant, one eggstick was added to an old cladode and one was added to a young cladode. *Open*

bars = ant access plants, *filled bars* = ant exclusion plants. *Bar* height indicates the proportion of eggsticks not recovered. Statistically significant effects are summarized for each site-year combination

varied across ant species and cladode age classes (Fig. 6). We had adequate sample sizes to test for differences between *Brachymyrmex patagonicus*, the non-native ant that was dominant at Bottoms Road, and *Dorymyrmex bureni*, the native ant dominant at Picnic Lake. We found that larval mortality was significantly greater on young cladodes vs. old ones ($F_{1,58} = 4.36, P = 0.04$) and that this difference was consistent between the two ant species (non-significant effects of species and species * cladode type). We did not have sufficient sample sizes to include *F. pruinosa*, *P. dentata*, or *P. floridana* in these analyses. However, it was apparent during the trials we were able to conduct that *F. pruinosa* workers were uninterested in cactus moth larvae on either cladode type (all larvae survived trials with this species) while *P. floridana* workers reacted very aggressively, especially when larvae were introduced onto young cladodes.

Effects of ants on moth damage and plant growth

Ant exclusion led to an increase in cumulative moth damage to *O. ficus-indica* at Bottoms Road (mean [\pm SE] proportional cladode infestation of control plants: 0.65 ± 0.21 ; ant exclusion plants: 0.34 ± 0.12) but had no effects on damage to *O. stricta* or to either species at Picnic Lake, as indicated by a marginally significant site * host species * ant treatment interaction effect: $F_{1,93} = 2.7, P < 0.1$. The effect of ant exclusion on damage did not influence plant growth over the larval development period ($F_{1,108} = 0.001, P < 0.96$; all interaction terms were non-significant).

Discussion

Our results confirm that ants visit cacti within the North American invasive range of *Cactoblastis*

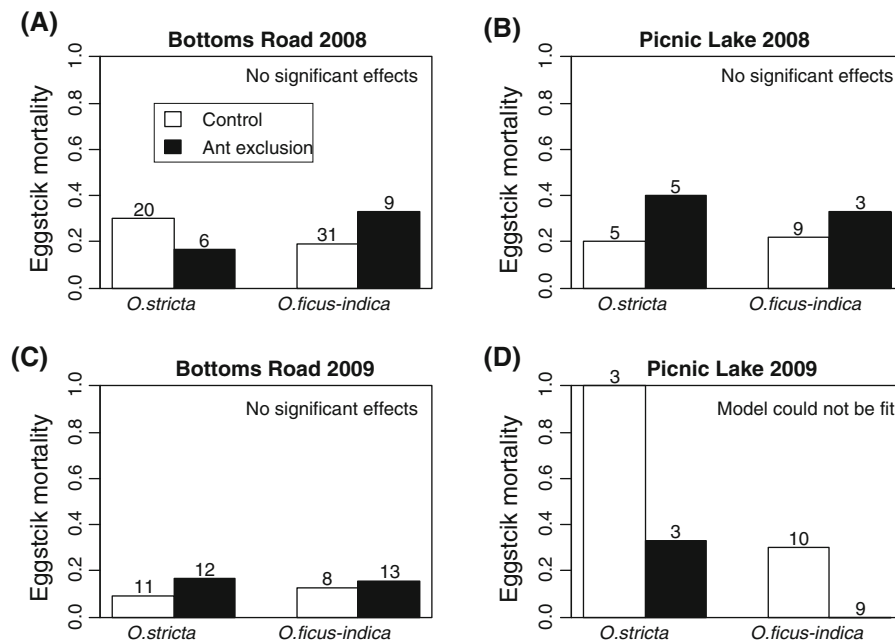


Fig. 5 Mortality of naturally deposited eggsticks (invasive *C. cactorum* and native *M. prodenialis*, pooled) on potted *O. stricta* and *O. ficus-indica* at the Bottoms Road (a, c) and Picnic Lake (b, d) sites in 2008 (a, b) and 2009 (c, d). Open bars = ant access plants, filled bars = ant exclusion plants.

Bar height indicates the proportion of eggsticks not recovered and numbers above bars indicate sample sizes (numbers of eggsticks). There were no significant effects of host species or ant exclusion for the site-year combinations that could be analyzed (a–c)

cactorum (Fig. 1) and provide new information about the species composition of the cactus-associated ant guild (Fig. 2). We used a combination of staged and natural experiments to test the central hypothesis that EFN-mediated protection mutualism between cacti and ants confers biotic resistance to *C. cactorum* invasion. Our experiments generated mixed results. On the one hand, introductions of lab-reared *C. cactorum* eggsticks (Fig. 4) and larvae (Fig. 6) indicated that ants can reduce the survival probabilities of both stages, providing support for the hypothesis of protection mutualism and therefore (because *C. cactorum* is a cactus specialist) mutualist-mediated biotic resistance. However, activity by naturally occurring cactus moths at our study sites was largely unaffected by ant exclusion. Ants did not deter ovipositing females (Fig. 3) nor was there any clear indication that they removed naturally deposited eggsticks (Fig. 5), as they did our lab-reared eggsticks. There was limited evidence that ant visitation reduced infestation of cactus cladodes but the reduction in damage had no detectable effect on plant growth. In total, our results suggest that effects of

cactus-visiting ants on *C. cactorum* invasion dynamics are weak and highly variable. Our results also highlight the importance of distinguishing between effects that *can* happen during controlled experiments and those that *do* happen under natural field conditions.

Evidence for effects of ants on the survival of lab-reared but not naturally deposited eggsticks is difficult to interpret, especially given the frequently low sample sizes of the latter (Fig. 5). However, the weak effects of ant exclusion on cladode infestation and plant growth support the notion that the often strong effects of ants on lab-reared eggsticks were not representative of ambient interactions. It is possible that our method of introduction rendered eggsticks more conspicuous or more vulnerable to ant removal than naturally deposited eggsticks, which are affixed to host-plants by an adhesive substance secreted by females. It is also curious that ants removed whole eggsticks but not individual eggs. The reverse is reported from South Africa (Robertson 1988), a discrepancy that might be attributable to different ant communities.

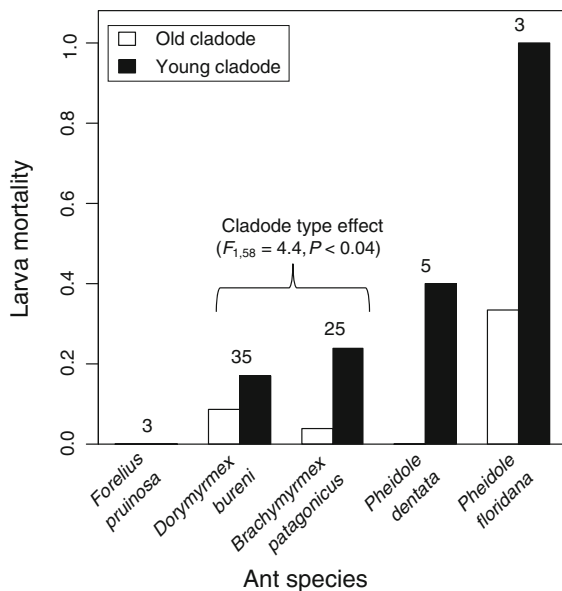


Fig. 6 Mortality of lab-reared, early-instar *C. cactorum* larvae placed on old (open bars) and young (filled bars) cactus cladodes. Plants were occupied by workers of one of five ant species. Bar heights indicate the proportion of larvae that were killed by ants (i.e., bitten, stung, and/or carried off the plant). Numbers above bars indicate the number of larvae introduced to each cladode type (trials were always paired). Only data for *D. bureni* and *B. patagonicus* were statistically analyzed

Our experiments incorporated numerous factors that could influence the frequencies and outcomes of ant-moth interactions, and hence potential for biotic resistance, including moth life stage, host-plant species, cladode age class, ant species composition, as well as unmeasured variables that differed between years and sites. We found significant variation in every dimension considered, and the effects of one factor were often conditional on another. For example, ant predation of larvae was generally greater on young cladodes than on old ones, consistent with predictions based on availability of EFN and levels of ant activity (Robbins and Miller 2009). However, the effects of cladode age class on the survival of introduced eggsticks were negligible at Bottoms Road and erratic at Picnic Lake. There were also differences between the native host-plant *Opuntia stricta* and the cultivated *O. ficus-indica*, and these too were highly variable. At Bottoms Road, ant exclusion led to a decrease in the mortality of introduced eggsticks only for *O. ficus-indica* in 2008 but for both host-plant species in 2009. Given the complex patterns of spatio-temporal variability and

factor interactions, no straightforward picture emerges regarding the conditions under which ant-cactus mutualism and ant-mediated resistance to *C. cactorum* invasion are likely to occur.

The only clear evidence for negative effects of cactus-visiting ants on cactus-feeding moths came from *O. ficus-indica* at Bottoms Road in 2009, where ant exclusion led to an increase in cladode infestation. While we cannot differentiate *C. cactorum* damage from native *M. prodenialis* damage, it is useful to ask what set of conditions might have contributed to this result. There is no evidence, for either moth species, that ants deterred ovipositing females (Fig. 3c) or removed naturally deposited eggsticks (Fig. 5c) from this plant group. We therefore suspect that ants inflicted mortality on cactus moth larvae, as they often did during larva addition experiments; because larvae feed internally, non-consumptive effects of ants are unlikely. The ant species composition data indicate that *Pheidole floridana* was unusually common on *O. ficus-indica* at Bottoms Road in 2009 (Fig. 2c). While our sample size was quite limited, we observed that this species was extremely aggressive toward introduced larvae, more so than other species tested (Fig. 6). We therefore hypothesize that this relatively rare species was responsible for the positive effects of ant visitation that we observed. It is unclear why this species occurred only on the cultivated *O. ficus-indica* but, given its potential as a plant mutualist and an agent of biotic resistance, further studies of this ant species and the factors limiting its abundance and host-plant distribution would be valuable.

Our study relied on experimental exclusion of ants at sites that were already invaded by *C. cactorum*, and was therefore not designed to test the effects of ants on cactus moth establishment. A complementary approach and potentially valuable future direction would be to compare ant abundance and species composition between invaded and un-invaded cactus populations or among populations that vary naturally in *C. cactorum* densities. Of course, testing hypothesized causal factors underlying the absence of an invader would require experimental introductions. The ethical concerns associated with such experiments impose constraints on knowledge about why invasions fail (Levine et al. 2004).

It is interesting to note that the cactus-visiting ant assemblage at one of our sites (Bottoms Road) was dominated by the exotic species *Brachymyrmex*

patagonicus (Fig. 2). There is much interest in the interactions between exotic species in their shared invasive range and the effects of established invaders on the success of those that arrive subsequently (e.g., Simberloff and Von Holle 1999). We found that *B. patagonicus* was statistically indistinguishable from *D. bureni*, the native ant dominant at Picnic Lake, in its interactions with introduced *C. cactorum* larvae. Also, there were no consistent differences in the effects of ant exclusion between the two study sites, which would be expected if the dominant ants differed in protective abilities. Thus, our data provide no clear indication that invasion by *B. patagonicus* has either strengthened or weakened biotic resistance against *C. cactorum*. However, we do not know what role, if any, competition plays in structuring patterns of ant relative abundances on cacti or in the environment. If the dominance of *B. patagonicus* limits access to cactus EFN by more effective plant guards then the invasive cactus moth may experience an indirect benefit from the invasive ant. *Brachymyrmex patagonicus* is a relatively recent invader, has spread rapidly, and is a known exudate-feeder (MacGowan et al. 2007), suggesting that it may become an increasingly common visitor to cacti throughout the invasive range of *C. cactorum*. Further studies of the cactus-visiting ant community, including additional data on protective abilities, determinants of relative abundance, and consequences of *B. patagonicus* invasion, are clearly needed.

Conclusions

While the occurrence of EFN-mediated ant-plant interactions is widespread in nature, the effects of these interactions on plant performance and herbivore dynamics are known to be highly variable and context-dependent (e.g., Barton 1986; Bronstein 1998; Chamberlain and Holland 2009; de la Fuente and Marquis 1999; Di Giusto et al. 2001; Mody and Linsenmair 2004; Rashbrook et al. 1992). Our rather idiosyncratic results support this notion and suggest that the efficacy of ant-plant protection mutualism as a form of invasion resistance may be similarly variable and context-dependent. However, ours is only among the first studies to test the effects of ant-plant protection mutualism on invasive herbivores (Matthews et al. 2007). More studies are needed to

determine if protection mutualisms are, in general, a weaker or more variable form of biotic resistance than predation, competition, and parasitism.

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