Effects of plant age, experimental nutrient addition and ant occupancy on herbivory in a neotropical myrmecophyte

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Summary

1 Variation in antiherbivore defences among individuals within a plant species may be influenced by intrinsic characteristics of the plant, environmental conditions and the interactions between these factors.

2 We used a long-term field experiment and a laboratory palatability trial to elucidate the effects of plant age, nutrient availability and ant occupancy on herbivore damage sustained by *Cordia alliodora*, a common neotropical myrmecophyte.

3 Herbivory in this system was influenced by both plant characteristics and environmental conditions, and the relative importance of these variables changed with plant age.

4 Five-year-old plants had a higher frequency of ant occupation and more workers per domatium than 1-year-old plants.

5 Plant age did not significantly affect herbivory in either field studies or laboratory palatability trials conducted with a specialist herbivore. In the field, however, 1-year-old plants tended to have higher levels of herbivory than 5-year-old plants.

6 There was a trend towards lower herbivory in trees that had been fertilized. Additionally, leaves from fertilized trees were significantly less palatable to specialist herbivores.

7 In 1-year-old plants, the abundance of ants in domatia had no effect on herbivory of nearby leaves. In contrast, ant abundance and herbivory were negatively correlated for 5-year-old trees, demonstrating that ants provide effective defence only for older plants.

8 These results suggest that understanding ant-plant-herbivore relationships requires examining how biotic and abiotic factors and their interactions change with plant ontogeny. Although our work focused on one particular ant-plant system, the importance of ontogenetic variation in antiherbivore defence is increasingly recognized as a critical area of study in plant defence theory.

Key-words: ant-plant, *Azteca*, biotic defence, *Coptocycla*, *Crematogaster*, direct defence, herbivory, indirect defence, mutualism, plant defence

Introduction

Myrmecophytes, or ant-plants, have evolved a suite of biotic, chemical and physical mechanisms to reduce herbivory and are therefore excellent systems with which to study plant–herbivore interactions (Davidson & McKey 1993; Heil *et al*. 2002a; Heil & McKey 2003; Dyer *et al*. 2004). Myrmecophytes produce specialized

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structures called domatia in which ants nest, and they may also provide food bodies or extrafloral nectar for the occupying ants. In turn, the ants often provide protection for the host plants from herbivores and encroaching vegetation (reviewed in Bronstein 1998; Heil & McKey 2003). Most myrmecophytic species can be occupied by multiple ant species (Fonseca & Ganade 1996; Alonso 1998; Bruna *et al*. 2005) that range widely in their ability to defend the host plant against herbivorous insects (Janzen 1966, 1975; McKey 1984; Heil, Fiala, Maschwitz & Linsenmair 2001;

Lapola *et al.* 2003; Bruna *et al.* 2004; Frederickson 2005). Although numerous studies have examined the efficacy of ant defence, there has been less consideration of how other plant characteristics and environmental factors influence herbivory in these systems.

Because herbivore pressure and the relative cost of herbivore damage vary with plant age, selection should favour plant defence strategies that include a combination or succession of mechanisms to prevent herbivory or mitigate its negative consequences throughout plant ontogeny (Boege & Marquis 2005). The young individuals of myrmecophytes often house only small, newly established ant colonies that provide limited antiherbivore defence (Feldhaar *et al.* 2003; Dejean *et al.* 2004; but see Schupp 1986; Itino & Itioka 2001). The absence of ant defenders during this critical stage of plant growth has led to the suggestion that young plants may be more reliant upon chemical or physical defences than older ones protected by mutualist ant colonies (Heil & McKey 2003). However, studies investigating the antiherbivore defences of young myrmecophytes have varied widely in their support for this hypothesis. Nomura *et al.* (2001) found evidence that *Macaranga* trees had elevated chemical defences prior to ant colonization, whereas Del Val & Dirzo (2003) found no such pattern in *Cecropia*. Del Val & Dirzo (2003) suggested that tolerance to herbivory (*sensu* Strauss & Agrawal 1999) is an important alternative to chemical or biotic defences during early growth of ant-plants. This explanation is supported by the general finding of increased ability of plants to defend against herbivores as they grow from the sapling to mature stage, a pattern that may extend to indirect defences that require resource allocation, such as mutualist ants, as well as direct defences such as deterrent chemicals (Boege & Marquis 2005).

Nutrient availability directly affects the ability of plants to grow, defend and tolerate herbivory, and is therefore among the most important environmental factors influencing plant–herbivore interactions (Coley *et al.* 1985; Bryant *et al.* 1992; Wilkens *et al.* 1996; Stamp 2003). In addition to allocation to growth and direct defences, myrmecophytes must also invest energy and nutrients in costly ant-specific features such as domatia and food bodies (Folgarait & Davidson 1995; Heil *et al.* 1997; Brouat & McKey 2000; Brouat & McKey 2001; Heil, Hilpert, Fiala & Linsenmair 2001; Heil *et al.* 2002b). Although several studies have experimentally investigated how nutrient availability influences food body or extrafloral nectar production (Folgarait & Davidson 1995; Dyer *et al.* 2001, 2004; Heil *et al.* 2002b), to our knowledge no studies have evaluated the effects of nutrients in ant-plant systems that do not provide direct food rewards. In systems where no direct nutritional rewards are offered to ants, changes in nutrient availability may influence ant–plant–herbivore interactions by altering leaf chemistry and physical defences, mediating relationships between ant occupants and honeydew-producing hemipterans, increasing the production of domatia or influencing plant tolerance to herbivory.

We used a large-scale and long-term nutrient-addition experiment to examine the effects of plant age, nutrient availability and ant occupancy on patterns of herbivory in a neotropical myrmecophyte, *Cordia alliodora* Ruiz (Pavon and Oken) (Boraginaceae). We also conducted laboratory palatability trials to investigate how nutrient availability and plant age influence the palatability of *C. alliodora* to a specialist herbivore in the absence of ants. As our experimental trees were 1 and 5 years old, our study is among the few to evaluate how environmental conditions and variation in ant occupancy influence ant–plant–herbivore interactions at ecologically meaningful temporal scales.

Materials and methods

STUDY SYSTEM

Cordia alliodora is commonly found in pastures and secondary forests throughout Central America and northern South America, including La Selva Biological Station, where this study was conducted (Costa Rica, Heredia Province, 10°26′ N, 83°59′ W). The domatia of *C. alliodora* are naturally hollow cauline swellings produced at most branch nodes. In Costa Rica, these domatia are most commonly occupied by the specialist ants *Azteca pittieri* Forel and *Cephalotes setulifer* Emery, as well as several generalist live-stem inhabiting ants (Wheeler 1942; Longino 1996; Tillberg 2004). Stable isotope and behavioural studies suggest that *A. pittieri* and *Crematogaster carinata* Mayr, the two most abundant ant species occupying *C. alliodora* in our study plots, patrol the plant regularly and consume insect prey (Möser 2000; Tillberg 2004). In contrast, the isotopic profile of *Ce. setulifer* indicates that this species subsists primarily on honeydew secreted from hemipterans (Coccidae and Pseudococcidae) that cohabit the domatia (Tillberg 2004).

The leaves of *C. alliodora* are eaten by a number of generalist and specialist insect herbivores (Wheeler 1942; Flowers & Janzen 1997; Möser 2000; Rojas *et al.* 2001; Tillberg 2004). Wheeler (1942) suggested that the high levels of leaf damage on many *C. alliodora* plants indicated that the ant occupants did not benefit their host through antiherbivore defence. Among the most abundant and most damaging insect herbivores present at our sites was the tortoise beetle *Coptocycla leprosa* Boheman (Chrysomelidae: Cassidini). This beetle appears to be a specialist on the genus *Cordia* (Flowers & Janzen 1997), and at high population densities can nearly defoliate plants (Wheeler 1942; M. D. Trager, personal observations).

STUDY SITE

Cordia alliodora was one of the focal species used in the Huertos Project, a long-term ecological study

established at La Selva in 1991. Haggar & Ewel (1995) provide a thorough description of the Huertos Project's objectives and experimental design. Briefly, the experiment design comprised three blocks, each of which contained one 80 m × 40 m stand of each of three hardwood tree species native to Costa Rica. These large plots were further divided into polyculture and monoculture plots, and the monoculture plots were subdivided into stands that were cleared and replanted at 1-, 4- and 16-year intervals. The focal tree species were planted in rows with 2 m spacing between individuals and 1.73 m spacing between rows, producing at an initial density of 2887 trees ha^{-1}. Trees were from a commercial nursery and were of the same provenance but not the same genotype. All stands were periodically thinned and regularly weeded to prevent recruitment of trees and large shrubs.

In our study we used a subset of the Huertos Project experiment in which 1-year-old and 5-year-old *C. alliodora* were grown in monospecific stands (one stand of each age in each of the three blocks). We conducted our work from June to August 2004. There was large variation in plant size and number of domatia within ages, but much greater differences between cohorts. The 1-year-old trees were subdivided evenly into fertilized and unfertilized treatments in each block; the 5-year-old trees were subdivided into fertilized and unfertilized treatments in two blocks, whereas in the other block all 5-year-old trees received fertilizer. Nutrient supplementation was conducted through biweekly addition of a liquid fertilizer containing urea and NO$_3$- (31% N volume:volume; 320 kg ha^{-1} year^{-1}; Silver *et al*. 2005). Given the high frequency and volume of precipitation at the site (approximately 4 m year^{-1}), this regime was deemed appropriate to elicit a fertilization effect if one existed (J. J. Ewel, personal communication). At the time we conducted our field surveys and experiments, all the fertilized plots included in this study had been supplemented for at least 1 year.

EFFECTS OF AGE, FERTILIZATION AND ANT OCCUPANCY ON HERBIVORY

To determine the effects of plant age, experimental nutrient addition and ant occupancy on herbivory, we collected one subterminal domatium from a single haphazardly selected branch on 115 *Cordia alliodora* plants (*n* = 8–10 individuals from each plant age × fertilization × block combination). We identified the ant species occupying each domatium, counted the number of workers present and collected all fully expanded leaves within 10 cm of the focal domatium. These leaves were digitized with a flatbed scanner and the proportion of leaf area missing was measured with Scion Image v. 4.02 (Scion Corporation) following the protocol described by O'Neal *et al*. (2002). Although measuring the leaf area once provides only a static view of herbivore damage rather than an estimate of the rate at which herbivores remove leaf tissue, it is nevertheless,

a robust means of comparing herbivory among conspecific individuals in the same location (Brown & Allen 1989) or that were planted at the same time with the same levels of initial herbivory. However, differences in leaf turnover rate due to fertilization or plant age could affect the amount of time leaves are exposed to herbivores.

We used the following linear mixed model procedure to test for the effects of plot-level variation (the block × plant age × fertilization combinations) and subplot-level (i.e. plant-level) variation (the number of worker ants in each domatium) on the proportion of leaf area missing: $y_{ijkl} = \mu + b_i + \alpha_j + \beta_k + \varepsilon_{ijk} + \gamma x_{ijkl} + \varepsilon_{ijkl}$. In this model, y_{ijkl} indicates the measure of herbivory, b_i is the random block effect, α_j is the fertilization effect, β_k is the plant age effect, ε_{ijk} is the whole-plot error, γx_{ijkl} is the effect of the covariate ant number within individual plants and ε_{ijkl} is the subplot (plant-level) error. In this analysis, individual plants were nested within the plots, which were defined as plant age × fertilization × block combinations. We used a similar, but simpler, approach to test for the effects of fertilization and plant age on the number of ant occupants per domatium.

As the data were not balanced due to the incompletely crossed fertilization regime, we used maximum likelihood estimation methods to fit the models. The significance of the block effect, b_i, was determined using a likelihood ratio test and was treated as a random effect contributing to total variance in the final model rather than a fixed effect. Only the main effects of plant age and fertilization treatment were included in the final model following likelihood ratio tests showing that interaction effects did not improve the model fit. Due to substantial differences in ant occupancy and herbivory between 1- and 5-year-old plants, we also conducted a separate analysis, similar to the model above, for the two age groups. All mixed model analyses were conducted with the lme program in R 2.2.0 following the protocol of Pinheiro & Bates (2000). All *P*-values were calculated with marginal (Type III) tests for significance. Proportional data were logit transformed.

EFFECTS OF PLANT AGE, LEAF AGE AND FERTILIZATION ON LEAF PALATABILITY

To further explore the importance of plant ontogeny and nutrient availability for herbivory, we tested the palatability of *Cordia alliodora* leaves for adult *Coptocycla leprosa* beetles with a three-factor, fully crossed laboratory trial. The factors were plant age (1- or 5-year-old trees), leaf age (young or mature, which were easily distinguished based on leaf colour and texture) and fertilization treatment (unfertilized or fertilized). Beetles were collected in the field from 1-year-old plants and starved for approximately 24 hours in the laboratory. We then placed each of 48 randomly selected individuals in a perforated plastic bag for 24 hours with one freshly collected *C. alliodora* leaf. All leaves were collected from the same block to minimize the potential effects

of spatial variation in plant defences. We conducted three trials with identical treatments and experimental procedures; each trial included six replicates of the eight treatment combinations ($n = 144$ beetles tested). To quantify herbivory we measured the initial and final leaf area with a Licor 3100 area meter (Li-Cor Biosciences, Lincoln, Nebraska, USA). As the young leaves lost area over the course of the experiment due to reduced turgor pressure, we used a correction factor based on the initial area to calculate their change in area due to shrinkage (final leaf area = $0.9934 \times$ initial leaf area $- 0.7378$, $R^2 = 0.9988$, $P < 0.001$). We used ANOVA to test for effects of the treatments and their interactions on the amount of leaf material consumed, with each trial considered as a block to account for random temporal effects.

Results

EFFECTS OF AGE, FERTILIZATION AND ANT OCCUPANCY ON HERBIVORY

Most domatia (78.3%) contained ants, but the frequency of unoccupied domatia was approximately twice as high for 1-year-old plants as for 5-year-old plants (28.8%, vs. 14.3%, respectively). The most frequently recorded species in the occupied domatia was *Azteca pittieri* (43.3%), followed by *Crematogaster carinata* (33.3%), *Cephalotes setulifer* (14.4%) and *Pseudomyrmex fortis* Forel (8.9%). There was no effect of fertilization on worker ant number ($F_{1,6} = 0.17$, $P = 0.70$), but significantly more workers were present in domatia from 5-year-old plants than in those from 1-year-old plants ($F_{1,6} = 24.35$, $P = 0.0026$; Fig. 1a). The number of workers also varied according to which species occupied the plant (Fig. 1b). The two most frequent occupants, *A. pittieri* and *Cr. carinata*, also had the most workers per domatium in the 5-year-old plants (mean $\pm$ SD = 20.9 ± 10.7 and 34.1 ± 16.5, respectively). Additionally, there were frequently many *Cr. carinata* workers in the domatia of 1-year-old plants (mean $\pm$ SD = 17.2 ± 12.7, Fig. 1b), whereas none of the other species had more than four workers per domatium in these plants.

The proportion of leaf area missing from the leaves surrounding focal domatia ranged from 0.02 to 0.45 (mean $\pm$ SD = 0.12 ± 0.09). Although not statistically significant at the $\alpha = 0.05$ level, there was a trend towards a higher proportion of leaf area missing in 1-year-old plants than in 5-year-old plants ($P = 0.096$) and unfertilized plants tended to have more leaf area missing than fertilized plants ($P = 0.067$; Table 1, Fig. 2). When plants of the two ages were analysed separately, the effects of fertilization were not significant for either age class (Table 2). When the number of workers per domatium was included as a plant-level covariate, it had a significant negative effect on herbivory (Table 1). However, this effect varied with plant age: when 1- and 5-year-old trees were analysed separately, the negative effects of ant abundance on herbivory were significant only for 5-year-old trees (Table 2).
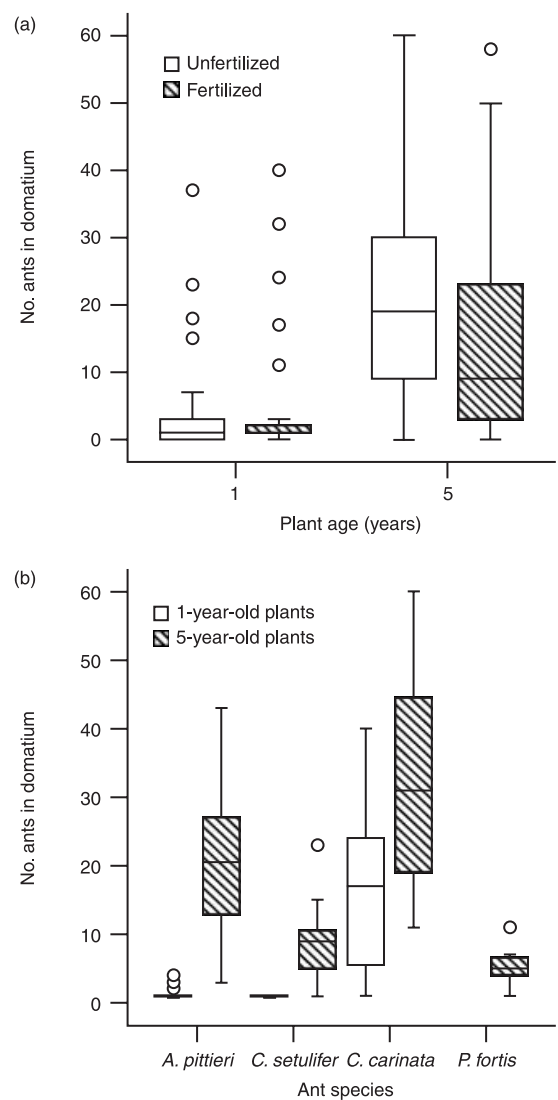
Figure 1 The number of worker ants per focal domatium varied according to: (a) plant age; and (b) the ant species present. Boxplots show interquartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles.

Table 1 Results of mixed model analysis testing the effects of plant age, fertilization and the number of worker ants on the proportion of leaf area missing. The number of ants was $\log_{10}$-transformed and the proportion of leaf area missing was logit-transformed to improve the distribution for this analysis

| Source of variation	Numerator d.f.	Denominator d.f.	F	P
Plant age	1	6	3.90	0.096
Fertilization	1	6	4.98	0.067
Number of ants	1	103	7.54	0.0071

EFFECTS OF PLANT AGE, LEAF AGE AND FERTILIZATION ON LEAF PALATABILITY

Beetles consumed nearly 60% more from the leaves of unfertilized plants than from fertilized ones ($P = 0.016$,

Table 2 Results of mixed model analyses testing the effects of fertilization and the number of worker ants present on herbivory for 1-year-old and 5-year-old plants. The number of ants was $\log_{10}$-transformed and the proportion of leaf area missing was logit-transformed to improve the distribution for this analysis. The analyses were conducted separately for the two ages

Plant age	Source of variation	Numerator d.f.	Denominator d.f.	F	P
1	Fertilization	1	2	4.78	0.16
	Number of ants	1	52	0.026	0.87
5	Fertilization	1	1	0.34	0.66
	Number of ants	1	50	6.53	0.014
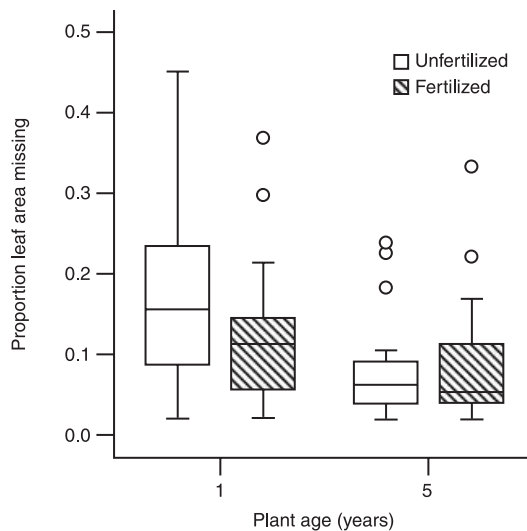
Figure 2 1-year-old plants experienced somewhat more proportional leaf damage than 5-year-old plants ($P = 0.097$), and fertilized plants experienced somewhat less herbivory than unfertilized plants ($P = 0.067$). There was no significant interaction between plant age and nutrient treatment. Boxplots show interquartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles.
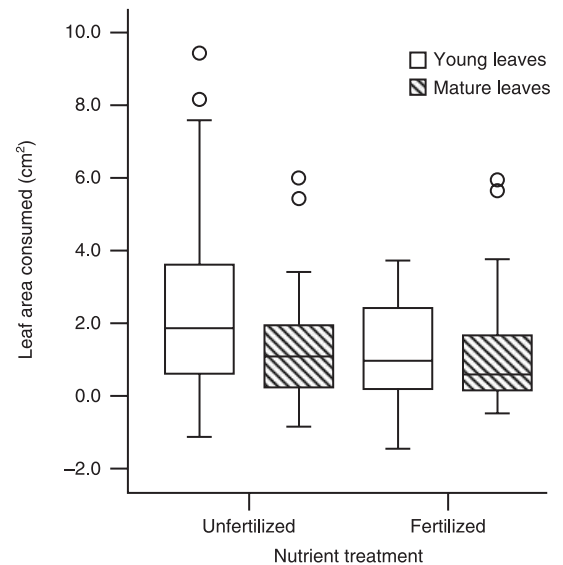
Figure 3 Fertilization significantly reduced the area consumed by individual *Coptocycla leprosa* beetles in the leaf palatability trial. This was particularly true for young leaves, as indicated by the marginally significant interaction effect between fertilization and leaf age (Table 3). Boxplots show interquartile ranges and expected minimum and maximum values, with values beyond the 95% CI indicated by open circles.

Fig. 3), and nearly 50% more material from young leaves than from mature leaves ($P = 0.061$; Table 3). There was no effect of plant age ($P = 0.16$). The main effects were due primarily to the high leaf area consumed from young, unfertilized leaves, as indicated by the marginally significant interaction effect between fertilization treatment and leaf age ($P = 0.056$). None of the other interactions among factors were statistically significant (Table 3).

Discussion

The relationships among the different antiherbivore defences of myrmecophytes are often complex and may vary as a function of plant ontogeny and resource availability, phylogeny and other factors (Folgarait & Davidson 1995; Nomura *et al*. 2001; Heil *et al*. 2002a; Del Val & Dirzo 2003; Dyer *et al*. 2004). However, many questions regarding the interactions among these defences have not been addressed, in part because of the logistical difficulties in conducting manipulative experiments with long-lived perennial plants at ecologically

Table 3 Results of ANOVA testing the effects of plant age, leaf age and fertilization treatment on the leaf area consumed by one *Coptocycla leprosa* beetle in 24 hours, with trial as a random block effect

Source of variation	d.f.	MS	F	P
Plant age	1	6.25	2.03	0.16
Leaf age	1	10.99	3.58	0.061
Fertilization	1	18.41	5.99	0.016
Plant age × leaf age	1	0.35	0.12	0.74
Plant age × fertilization	1	5.40	1.76	0.19
Leaf age × fertilization	1	11.38	3.70	0.056
Plant age × leaf age × fertilization	1	3.38	1.10	0.30
Trial	2	28.02	9.11	< 0.001
Error	134	3.08		

relevant temporal scales. The results of our study, in which we manipulated the resources available to 1- and 5-year-old trees, suggest that the patterns of herbivory vary as a function of tree age, nutrient availability, ant presence and their interaction. We suggest that the outcome of experiments investigating ant-plant mutualisms

may be context-dependent in ways that are important but rarely evaluated empirically.

That young myrmecophytes may have increased chemical defences prior to ant colonization is a logical extension of current plant defence theories (Heil & McKey 2003; but see Boege & Marquis 2005), but evidence supporting this idea remains equivocal (Itino & Itioka 2001; Nomura *et al*. 2001; Del Val & Dirzo 2003). Support for this hypothesis in the *C. alliodora* system would require that ants effectively defend older plants but not younger plants, that *C. alliodora* produces defensive chemicals to deter herbivory and that production of such chemicals is relatively higher in young plants, resulting in decreased leaf palatability. There were substantially more ants in the domatia of 5-year-old plants than in 1-year-old trees (Fig. 1a) and, consequently, herbivore damage to leaves surrounding domatia of the 5-year-old plants was significantly lower (Table 1). However, in the palatability trial there was no difference in herbivory damage due to plant age, and in the field survey of herbivory leaves from 1-year-old plants, they actually had slightly more herbivore damage than leaves from 5-year-old plants even when the effect of ants was accounted for in the model. This suggests that plants probably make similar investments in direct defences regardless of age and that young *C. alliodora* trees are tolerant of herbivory, as has been shown in other fast-growing tropical plants (Del Val & Dirzo 2003; Coley *et al*. 2005). Rather than producing defensive chemicals, saplings of *C. alliodora* may instead increase direct available resources to the production of domatia that house colonies of defensive ants. Such a defensive strategy would entail substantial allocation of plant resources and physiological trade-offs (Brouat & McKey 2000, 2001) and it would be consistent with the pattern of increased ability to resist herbivores that generally accompanies the transition from sapling to mature plant (Boege & Marquis 2005).

The relationship between nutrient availability and plant defence is complex and varies substantially among systems (Coley *et al*. 1985; Stamp 2003). Work in other ant-plant mutualisms has found that nutrient addition enhances plant defence indirectly through the production of food bodies (Heil, Hilpert, Fiala & Linsenmair 2001; Heil *et al*. 2002b) and directly through the synthesis of deterrent chemicals (Dyer & Letourneau 1999; Dyer *et al*. 2004). Although we found that fertilization produced only a marginally significant reduction in leaf damage in the field component of our study, fertilization significantly reduced the palatability of *C. alliodora* leaves for a specialist herbivore. The effects of fertilization on herbivory that we observed could be driven by differences in allocation to physical (structural) defences by fertilized plants, but they are also consistent with the possibility that fertilized *C. alliodora* plants have increased levels of N-based chemical defences in their leaves. Unfortunately, relatively little is known about *C. alliodora* defensive chemistry, including whether the species produces N-based

antiherbivore defences. However, even if such defences are not present in this system, increased N availability can also increase the production of carbon-based defensive compounds (Mihaliak & Lincoln 1985; Wilkens *et al*. 1996). Several triterpenoid compounds that repelled leafcutter ants in experimental trials have been isolated from the leaves of *C. alliodora* (Chen *et al*. 1983). Additionally, a number of secondary metabolites with fungicidal or insecticidal properties have also been isolated from the bark and wood (Ioset *et al*. 2000).

It is unknown how herbivores other than *C. leprosa*, including generalists, react to interplant variation in this system. Whereas many specialist herbivores are able to metabolize, excrete or sequester plant defensive chemicals, generalist herbivores are often ill equipped to deal with such substances and therefore perform poorly on plants that have high concentrations of chemical defences (Bowers & Puttick 1988; Dyer *et al*. 2004; but see Barrett & Agrawal 2004). Therefore, the negative effect of fertilization on leaf palatability for a specialist herbivore strongly suggests that defensive chemicals synthesized by *C. alliodora* also deter generalists. Identifying the defensive chemicals of *C. alliodora* and determining the factors that influence their production are needed to fully understand how plant and environmental factors influence the interactions among these plants, their ant occupants and their herbivores.

Although the results of our leaf palatability trial generally corroborate those of the field survey of herbivory, there were differences between the results of these approaches. Whereas there was no effect of plant age on leaf area consumed by beetles in the laboratory, 1-year-old plants in the field had proportionally greater leaf damage than 5-year-old plants. In addition, in the laboratory trial there was significantly less herbivory on leaves from fertilized plants, regardless of plant age, whereas fertilization only marginally significantly reduced herbivory in the field study (Table 3). The contrast between the results of the palatability trial and the field surveys suggests that *Coptocycla leprosa* beetles do not base their foraging choices solely on a preference for more palatable leaves. Despite the ability of both the adult beetles and larvae to defend themselves against ants, we hypothesize that *C. leprosa* selectively attacks young plants that do not house large colonies of ant defenders. Assessment of plant defences by herbivores, which may be accomplished through tactile, chemical or visual cues, has been observed in other ant-plant systems and may be important for mobile herbivores to select host plants (Freitas & Oliviera 1996).

Due to the non-random spatial distribution of the four ant species in this study, we were not able to compare statistically their effects on leaf damage. However, because the number of workers in domatia and herbivory were negatively correlated, differences in worker abundance may be indicative of interspecific differences in defensive ability (but see Bruna *et al*. 2004). The two

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most frequently occurring ant species in this study, *Azteca pittieri* and *Crematogaster carinata*, also had the most workers per domatium (Fig. 1a). Previous research has shown that *A. pittieri* attacks and consumes insect herbivores of *C. alliodora* (Möser 2000; Tillberg 2004), and *Azteca* species are the most abundant ants in *C. alliodora* throughout the plant's geographical range (Wheeler 1942; Longino 1996). Conversely, *Ce. setulifer*, which is also a specialist inhabitant of *Cordia*, was only present in small numbers and appears to be at best a passive defender of *C. alliodora* (Tillberg 2004). Interestingly, *Cr. carinata* is locally abundant in *C. alliodora* at our study site but is rarely found in *C. alliodora* throughout the remainder of the plant's range (Longino 1996). Therefore, it probably has an insubstantial impact on the *C. alliodora*-ant relationship at broader geographical scales. These results clearly support the conclusions of previous studies indicating that not all ant partners in ant-plant mutualisms are functionally equivalent as defenders (Lapola *et al*. 2003; Bruna *et al*. 2004; Frederickson 2005), and that considering mutualistic ant–plant interactions from a community-wide perspective is necessary to understand their ecology and evolution (Stanton 2003).

The benefits conferred to partner species in ant-plant mutualisms can vary both spatially and temporally (Horvitz & Schemske 1990; Alonso 1998; Bronstein 1998; Kersch & Fonseca 2005). Our results and those of others indicate that a full appreciation of these benefits requires a broad perspective in which one considers how plant age (Itino & Itioka 2001; Nomura *et al.* 2001; Del Val & Dirzo 2003), local environmental conditions (Folgarait & Davidson 1995; Heil *et al.* 2002b; Kersch & Fonseca 2005) and the identity and abundance of ant partners (McKey 1984; Heil, Fiala, Maschwitz & Linsenmair 2001; Bruna *et al.* 2004; Frederickson 2005) affect ant–plant–herbivore interactions. We further suggest that assessing the relative costs and benefits of direct and indirect defences, including the costs associated with domatia production, in relation to plant ontogeny is particularly important for understanding the evolution and ecology of ant-plant mutualisms and plant defence strategies.

Acknowledgements

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