

Indirect benefits of symbiotic coccoids for an ant-defended myrmecophytic tree

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Abstract. The net benefits of mutualism depend directly on the costs and effectiveness of mutualistic services and indirectly on the interactions that affect those services. We examined interactions among *Cordia alliodora* myrmecophytic trees, their symbiotic ants *Azteca pittieri*, coccoid hemipterans, and foliar herbivores in two Neotropical dry forests. The tree makes two investments in symbiotic ants: it supplies nesting space, as domatia, and it provides phloem to coccoids, which then produce honeydew that is consumed by ants. Although higher densities of coccoids should have higher direct costs for trees, we asked whether higher densities of coccoids can also have higher indirect benefits for trees by increasing the effectiveness of ant defense against foliar herbivores. We found that trees benefited from ant defense against herbivores. Ants defended trees effectively only when colonies reached high densities within trees, and ant and coccoid densities within trees were strongly positively correlated. The benefits of reduced foliar herbivory by larger ant colonies were therefore indirectly controlled by the number of coccoids. Coccoid honeydew supply also affected per capita ant aggression against tree herbivores. Ants experimentally fed a carbohydrate-rich diet, analogous to sugar obtained from coccoids, were more aggressive against caterpillars per capita than ants fed a carbohydrate-poor diet. Ant defense was more effective on more valuable and vulnerable young leaves than on older leaves. Young domatia, associated with young leaves, contained higher coccoid densities than older domatia, which suggests that coccoids may also drive spatially favorable ant defense of the tree. If higher investments by one mutualistic partner are tied to higher benefits received from the other, there may be positive feedback between partners that will stabilize the mutualism. These results suggest that higher investment by trees in coccoids leads to more effective defense by ants against the tree's foliar herbivores.

Key words: ant plant; Area de Conservación Guanacaste, Costa Rica; *Azteca pittieri*; Chamela-Cuixmala Biosphere Reserve, Mexico; Coccoidea; *Cordia alliodora*; herbivory; multispecies mutualism; Neotropics; positive feedback; seasonally dry tropical forests.

INTRODUCTION

Many pairwise mutualisms function only in the presence of an additional partner (Bronstein and Barbosa 2002), and the net effects of a mutualism for each partner depend on all of the species involved. Total costs of mutualisms may be diverse and difficult to quantify (Bronstein 2001a), but they include and are most easily measured as a partner's investment in services. Total benefits of mutualisms depend on the effectiveness of services, which depends on their quantity and quality (Ness et al. 2006), and on how closely the provisioning of services matches spatiotemporal variability in requirements for those services (Heil et al. 2004). In symbiotic ant-plant mutualisms, myrmecophytic plants invest in hollow nesting spaces, known as "domatia," and food for symbiotic plant ants (phytoecious ants). Production of stem domatia may be costly to the plant, particularly early in ontogeny, because hollow stems require more structural area than solid

stems to prevent mechanical failure (Brouat and McKey 2001). Provisioning of food is also costly to the plant because it diverts photosynthate and/or nutrients to ants that could otherwise be used for the plant's growth and reproduction. In return for these investments by the plant, ants typically defend the plant against herbivores (Heil and McKey 2003).

Theoretical models of ant-plant mutualisms have predicted that costs to the plant of its investments in ants increase linearly with the growth of the ant colony, whereas benefits to the plant of ant-colony growth saturate as levels of herbivory approach zero (Fonseca 1993). Such models predict that the plant should make intermediate investments in ant mutualists to maximize the plant's own net benefits, and these predictions can be generalized to other mutualistic interactions (Bronstein 1998). In addition, mutualists that make high investments in rewards are predicted to be more commonly exploited by non-beneficial partners (Bronstein 2001b).

Contrary to these predictions, two recent studies have found evidence that net benefits for plants from mutualisms with phytoecious ants increase by means of positive feedback between plant investments and ant

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services. Frederickson and Gordon (2009) reported that two myrmecophytic plant species in the Amazon grew faster when they were inhabited by mutualistic ants, but grew more slowly when inhabited by non-mutualistic ants. Heil et al. (2009) reported that myrmecophytic *Acacia* plant species that offered higher space and food rewards were more frequently associated with mutualistic ant species, and that plant species that offered higher rewards benefited from better ant defense against folivory. These two studies provide examples of positive feedback, when higher investments by plants benefit ant-colony growth, which may increase ant fitness, and more effective ant services benefit plant growth, which may increase plant fitness. Such positive feedback, or “partner fidelity” (Bronstein 2001b), could stabilize mutualistic interactions over evolutionary time.

In the vast majority of symbiotic ant–plant mutualisms, ants tend hemipteran insects of the superfamily Coccoidea, collectively known as “coccoids” (Davidson and McKey 1993). Coccoids feed on plant phloem and produce sugar-rich waste, known as “honeydew,” that ants consume. Ant–plant–hemipteran mutualisms generate net benefits for plants when ants protect them from herbivores more harmful than the hemipterans themselves. In non-symbiotic ant–plant interactions, in which ants visit plants but maintain colonies elsewhere, coccoids and other hemipterans can provide indirect benefits to plants by attracting defensive ants (see review in Styrsky and Eubanks [2007]). However, it has been argued that in symbiotic ant–plant associations, coccoids impose greater direct costs than indirect benefits to the host plant (e.g., Becerra and Venable 1989), partly because host plants could better control their level of investment in ants by producing food for ants directly (McKey et al. 2005).

Here we asked whether there are benefits to plants, mediated by ants, from increased investment in symbiotic coccoids. We investigated interactions among *Cordia alliodora* (Ruiz and Pavón) Oken (Boraginaceae), *Azteca pittieri* Forel (Dolichoderinae), symbiotic coccoids, and foliar herbivores in two Neotropical dry forests. *C. alliodora* is a Neotropical myrmecophytic tree with domatia at stem nodes that are commonly inhabited by *Azteca* ants. Leaves grow in whorls near growing shoots where new domatia are produced. *C. alliodora* offers no plant-produced food rewards for ants, and *Azteca* ants always host coccoids inside the domatia where the ants nest (Wheeler 1942, Tillberg 2004; E. G. Pringle, *personal observation*). As early as 1902, the naturalist C. H. Tyler Townsend wrote to the entomologist W. M. Wheeler that the *C. alliodora* system constituted “a most striking instance of the interrelations and mutual dependence of plant, ant, and coccid” (Wheeler 1942:15–16).

Coccoids would benefit plants indirectly if they were associated with higher quantity or quality of ant defense against foliar herbivores, or with beneficial tissue-specific patterns of defense to the plant. For example,

the optimal-defense hypothesis (McKey 1979, Rhoades 1979) proposes that plants should make larger investments in anti-herbivore defense in more valuable and vulnerable tissues, which would result in a spatially heterogeneous pattern of defense among plant tissues. Young leaves are typically more valuable than old leaves to a plant because they provide more photosynthate (Harper 1989), and they are more vulnerable and attractive than old leaves to herbivores because they are less tough and more nutritious (Coley and Kursar 1996).

We asked the following questions about ant defense of tree leaves and its relation to interactions with coccoids: (1) Do *A. pittieri* ants defend *C. alliodora* leaves against herbivores? (2) Is the effectiveness of ant defense related to the quantity or density of ant defenders? (3) Is the number of coccoids associated with the quantity of ant defenders? (4) Are there indirect effects of coccoids on foliar herbivory, mediated by ant-colony size? (5) Does the quantity of carbohydrate rewards produced by coccoids positively affect ant defense per capita? Finally, (6) Do ants preferentially defend young leaves, and, if so, could this behavior be explained by an association of coccoids with young leaves?

STUDY SYSTEM

The study was conducted in the Chamela-Cuixmala Biosphere Reserve (19°30' N, 105°02' W) in Jalisco, Mexico, and in the Area de Conservación Guanacaste, Sector Santa Rosa (10°50' N, 85°36' W), in Guanacaste, Costa Rica. Both sites, hereafter “Chamela” and “Santa Rosa,” consist of Pacific coastal seasonally dry tropical forest, in which rainy seasons alternate with severe dry seasons. The rainy season lasts approximately from July to October in Chamela and from May to November in Santa Rosa. The most common ant species to occupy *Cordia alliodora* domatia at both sites is *Azteca pittieri*, although trees may also be inhabited by *Cephalotes setulifer* (Santa Rosa), *Crematogaster* spp. (both sites), *Pseudomyrmex viduus* (Santa Rosa), and *Camponotus* sp. (Santa Rosa), listed in decreasing relative abundance. Founding queens of *A. pittieri* chew into unoccupied domatia. As the ant colony and tree grow, worker ants chew openings in other domatia and the colony spreads throughout the tree. Colonies of *A. pittieri* usually have one queen and are limited to a single tree (E. G. Pringle, *personal observation*). *A. pittieri* ants patrol the leaves and stems of *C. alliodora* and attack herbivores and other intruders, biting and chasing them off the tree (Tillberg 2004; E. G. Pringle, R. Dirzo, and D. M. Gordon, *personal observations*). Reports differ on whether *Azteca* ants significantly reduce herbivory on *C. alliodora* leaves (Wheeler 1942, Tillberg 2004).

A. pittieri ants host both Coccidae (soft scale insects) and Pseudococcidae (mealybugs) inside domatia (Tillberg 2004) and eat their honeydew (E. G. Pringle, *personal observation*). Individual trees are usually inhabited by several species of coccids, including

Cryptostigma reticulolaminae (both sites), *Cryptostigma inquilina* and *Aztecalecanium* sp. (Chamela), and Coccidae species in the *Toumeyella* group (Santa Rosa). Pseudococcid species include *Paraputo cuatensis* (Chamela) and *Paraputo larai* (Santa Rosa) (all identifications were made by T. Kondo, CORPOICA, Palmira, Colombia). There is no evidence that the ants are harvesting coccoids as a protein source, although this behavior has been reported in other systems (Carroll and Janzen 1973, Buckley 1987). *A. pittieri* ants do not catch and eat live herbivores, and they appear to obtain protein primarily from dead insects and guano that occasionally fall onto the plant (E. G. Pringle, *personal observation*). Other species of *Azteca* have been shown to be nitrogen limited (Davidson 2005).

METHODS

Ant defense of plants in ant-exclusion experiments

To test whether ants defend the plant from herbivory, we excluded ants from some leaves and compared herbivory on these leaves to herbivory on control leaves that ants were allowed to access. Experiments were conducted over three weeks in the late rainy season in 2007 (August in Santa Rosa, September–October in Chamela) and in the early rainy season in 2008 (May–June in Santa Rosa, July–August in Chamela). *Azteca pittieri*-occupied *Cordia alliodora* trees between 2 m and 8 m were selected at both sites, and individual branches within trees were haphazardly assigned to control or exclusion treatments (at each site, $n = 20$ trees, six control–exclusion branch pairs per tree in 2007, and $n = 40$ trees, two control–exclusion branch pairs per tree in 2008). On branches receiving the exclusion treatment, a sticky barrier of Tanglefoot (Tanglefoot Company, Grand Rapids, Michigan, USA) was applied in 1.5-cm bands around the circumference of the branch between each terminal ant-occupied domatium and the leaf whorls just below it.

Percentage leaf area eaten (%LAE) during the experiment was calculated as: [(area eaten after the experiment – area eaten before the experiment)/total leaf area] $\times 100$. To test whether herbivory was greater on exclusion or control leaves, averages of %LAE of exclusion and control leaves for each tree were compared as matched-pair replicates. Matched pairs were compared using one-sample Wilcoxon sign-rank tests. For additional methods, see Appendix A.

Relationships among standing herbivory, ant-colony size, and plant investments

To test whether benefits and costs to the plant of its mutualism with ants are related to the quantity of ant defenders, we conducted measurements of standing herbivory, ant-colony size, nesting-space availability, and number of coccoids. To estimate the overall effectiveness of ant colonies at reducing herbivory, we measured standing herbivory levels at each site on the same 20 trees per site for which we conducted colony-

size measurements (see next paragraph), approximately one month after the 2008 rainy season had begun. Standing levels of herbivory were estimated by assigning leaves to a value corresponding to percentage leaf area eaten: 0 = 0%, 1 = >1–6%, 2 = >6–12%, 3 = >12–25%, 4 = >25–50%, 5 = >50–100%, for about 100 individual leaves, from at least three branches per tree. An index of herbivory, the average standing level of herbivory per leaf for each tree, 0–5, was then estimated as the sum, for each category, of the product of the number of leaves in that category and its value, divided by the total number of leaves (Dirzo and Domínguez 1995). Herbivory levels were Box-Cox power-transformed prior to analysis.

Colony-size measurements were conducted in Santa Rosa (Costa Rica) in June 2008 and in Chamela (Mexico) in July 2008. All ants and coccoids were counted, and all domatia were measured, in six trees per site, and these values were estimated for an additional 14 trees per site. At each site, six trees spanning approximately equal sizes, from 1.6 to 4.5 m in height, were cut down and collected. To ensure that ants were in the domatia and not patrolling, trees were cut down in the rain, when *A. pittieri* ants are not active, or sprayed heavily with water from spray bottles for 10 minutes until ants had retreated into domatia. All domatia except for three subterminal “estimator” domatia (see Appendix B), including openings in the main trunk, were collected in large, press-seal plastic bags with alcohol-soaked paper towels and left for 12 h until ants had suffocated. All domatia were counted, and the widest internal length and width were measured from each using calipers. From each domatium, all ant workers and coccoids were counted. Worker ants that escaped from domatia and were loose in the bags were also counted. Domatia of all sizes appeared similarly shaped, so domatia volumes were estimated as if they were cylinders. We estimated space, number of ants, and number of coccoids from partial collections of an additional 14 trees per site (Appendix B). In all analyses, we used the observed values of nesting space, ants, and coccoids from six collected trees per site and the corrected estimates from the 14 partially collected trees per site. To standardize by tree size in calculating ant density, coccoid density, and nesting space, we divided the total values for each of these by the sum of the basal diameter and diameter at breast height (dbh; measured at 1.4 m) for each tree. All measurements were Box-Cox power-transformed before regression analyses.

To compare the importance of total number and density of ants in the tree, we conducted regression analyses of these measurements separately. All regression analyses were conducted as general linear models (GLMs). Three factors were employed for each model: the independent variable, site (Chamela or Santa Rosa), and the interaction effect of the independent variable and site. Interaction effects were all nonsignificant, so data from both sites are presented together in pooled

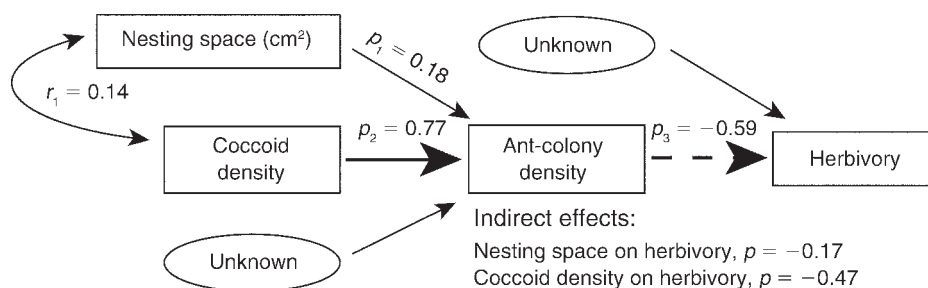


FIG. 1. Path diagram of the direct and indirect effects of colony-size variables of the symbiotic ant *Azteca pittieri* on standing herbivory of *Cordia alliodora* leaves. Solid arrows indicate positive relationships; the dashed arrow indicates a negative relationship. Larger arrows indicate significant pathways ($P < 0.0001$; Appendix E: Table E1); path coefficients, p , are presented. "Unknown" variables include all other possible sources of variation in the response variables (Appendix E: Table E1). Hemipteran coccoids produce honeydew on which ants feed.

analyses. Additional experiments examined the mechanisms of changes in defense with ant-colony size and are reported in Appendix C.

Indirect effects of plant investments

Using path analysis of colony-size measurements, we asked whether investments by the plant, in nesting space and phloem used by coccoids, were proportional to the benefits of more effective ant defense by larger colonies and reduced foliar herbivory (Fig. 1). Path analysis on colony-size measurements standardized by tree size was conducted by calculating path coefficients, standardized standard-least-squares partial regression coefficients, and R^2 values using multiple- or single-factor linear regressions. Compound paths were calculated subsequently by multiplying component path coefficients and summing across distinct, possible paths (Sokal and Rohlf 1995). For example, the indirect effect of nesting space on herbivory was calculated as: $p_1 \times p_3 + r_1 \times p_2 \times p_3$ (Fig. 1). Nesting space and number of coccoids were treated as fixed factors. Their mutual non-independence was accounted for by their correlation coefficient, r , and by a multiple regression of the number of worker ants by both factors to calculate path coefficients, p , and explained variation, R^2 . Neither direction nor significance of the paths changed when sites were analyzed separately, so analyses with data pooled from both sites are presented here.

Experimental effects of carbohydrates on ant behavior

To test whether per capita ant behavior changed with differential access to coccoid honeydew, we kept ants in the laboratory and controlled the carbohydrate concentration of their diets for three weeks. For detailed methods, see Appendix D. Briefly, we paired groups of ants from two domatia collected in June and July 2008 from six trees in Santa Rosa, and nine trees in Chamela, and assigned each group to one of two experimental diet treatments for three weeks. One treatment was a high-carbohydrate diet, 70% weight mass by volume sugar solution, and the other treatment was a low-carbohydrate

diet, 2–3% weight mass by volume. Typical sugar concentration in coccoid honeydew may exceed 70% (Ewart and Metcalf 1956), so the 70% sugar diet simulated natural conditions for a well-fed colony. The 2–3% sugar diet simulated starvation conditions, when small amounts of concentrated honeydew intake are severely diluted by water intake. Water and protein sources were provided ad libitum.

To test for differences between treatments in ant behavior, after three weeks of experimental diets, we conducted two behavioral assays in the laboratory on each experimental group of ants. We measured aggression toward an herbivore intruder and overall activity levels. Aggression was measured by placing a third-instar caterpillar, *Cropia templada* Schaus (Noctuidae), a *C. alliodora* herbivore that is common at both sites, in each container that contained a group of ants with a given diet treatment. Aggression assays were conducted 24 h or 48 h after the activity assay in Santa Rosa and Chamela, respectively. We recorded whether the caterpillar was contacted by ants, and the total number of ant attacks following detection over a 10-min period. An encounter was characterized as an attack if an ant touched the caterpillar with its mandibles and appeared to bite. If the ant backed up, out of contact with the caterpillar, and then bit it again, this was counted as another attack.

Activity was measured using methods adapted from Grover et al. (2007). To elicit patrolling behavior, we placed a three-dimensional structure analogous to a tree branch, three 7-cm-long bamboo skewers arranged as a tripod with a paper platform on top, in each container, across from the vial and feeding stations at 09:00 h on the day of the assay. Counts of the number of ants anywhere on the structure were made at 5, 10, and 15 min, and at 1, 2, 4, 6, and 8 h from the time at which the structure was introduced, and these counts were averaged over all time points.

Counts of the number of live ants in each container were made once after the first week of this experiment in Santa Rosa, to compare mortality between 70%-sugar

and 2%-sugar treatments. Subsequently, to generate more accurate counts and to avoid disturbing the ants excessively, we waited until the end of all behavioral assays to count the number of ants in the containers. Following behavioral assays, all ants were counted by removing live ants from each container one by one. Total ant counts also included ants that died during caterpillar attacks. The number of ants was used to determine per capita activity and aggression. Data were pooled from both sites and analyzed using matched-pair, two-sample Wilcoxon sign-rank tests. For these analyses, we did not specify “site” as a factor because of the paired design of the experiment.

*Spatiotemporal variability in defense
and symbiont densities*

To investigate whether ant defense is spatiotemporally matched to valuable, vulnerable plant parts, we asked whether ants preferentially defend young leaves associated with young domatia. To test this, we compared ant aggression on young and old leaves. We placed Arctiid caterpillars (similar to, but not identified as, *Hypercompe*) on young leaves within one branch node of a green, terminal domatium and on old leaves within one branch node of an older, woody domatium in Chamela in July 2006. We measured the time ants took to contact caterpillars, total number of ant attacks on caterpillars, and whether the caterpillars were chased completely off the tree within 5 min. To examine whether coccoid and ant densities varied with domatium age, we collected ~18 branches from 10 *C. alliodora* trees in Chamela in October 2007 and coded terminal, subterminal, and tertiary domatia along each branch as youngest, intermediate, and oldest, respectively ($n = 50$ domatia). Densities of coccoids, which live along domatium walls, were calculated per square centimeter of domatium surface area, whereas densities of ants, which can fill entire domatia, were calculated per cubic centimeter of domatium volume. Measurements and counts were conducted as described above for measurements of colony size. Densities were log-transformed and compared among domatia ages by ANOVA and post hoc Tukey hsd.

Statistical analyses

All statistical analyses were performed in JMP 7.0.2 (SAS Institute 2009) or R 2.8.1 (CRAN-r-project; R Development Core Team 2008). Distributions of residuals were tested for normality using Shapiro-Wilk tests.

RESULTS

*Relationships among herbivory, ant-colony size,
and plant investments*

Azteca pittieri ants significantly reduced herbivory in exclusion experiments in both the early and late rainy season in Chamela (matched-pair Wilcoxon, early $P < 0.01$, late $P < 0.04$; Fig. 2). Ants did not reduce herbivory in exclusion experiments in Santa Rosa at

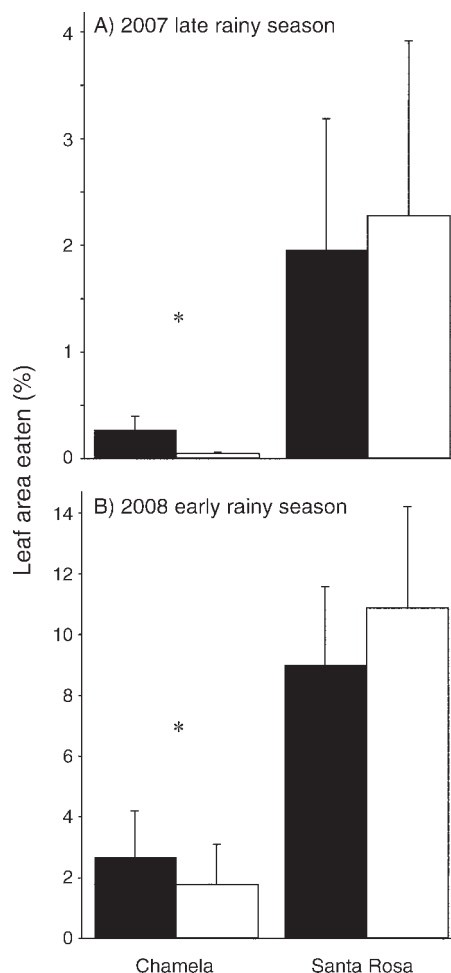


FIG. 2. Percentage of leaf area eaten (mean + SE) after three weeks of ant-exclusion experiments in (A) late rainy season 2007 and (B) early rainy season 2008, at two Pacific coast seasonally dry tropical forest sites: Chamela (Mexico) and Santa Rosa (Costa Rica). Black bars represent ant-exclusion treatments in which ants were excluded from leaves with Tanglefoot; open bars represent control treatments in which ants continued to have access to leaves. For matched-pair Wilcoxon tests, an asterisk (*) indicates $P < 0.05$; high SEs indicate large intercolonial differences. Note the difference in scale between panels (A) and (B).

either time (early $P = 0.2$, late $P = 0.4$; Fig. 2). Herbivore pressure, the herbivory inflicted on leaves when ants were excluded, was considerably higher in Santa Rosa than in Chamela, and higher in the early rainy season than in the late at both sites.

Herbivory was negatively related to ant density. Standing herbivory levels were negatively related to the density of worker ants, i.e., the total number of ants divided by tree size ($R^2 = 0.56$, $F_{3,36} = 15.11$, $P < 0.0001$; for ant density, $P < 0.001$; for site, $P < 0.0003$; for ant density \times site, $P = 0.5$; see Fig. 3A). Standing herbivory levels were not related to the total number of worker ants ($R^2 = 0.38$, $F_{3,36} = 7.37$, $P < 0.0007$; for number of

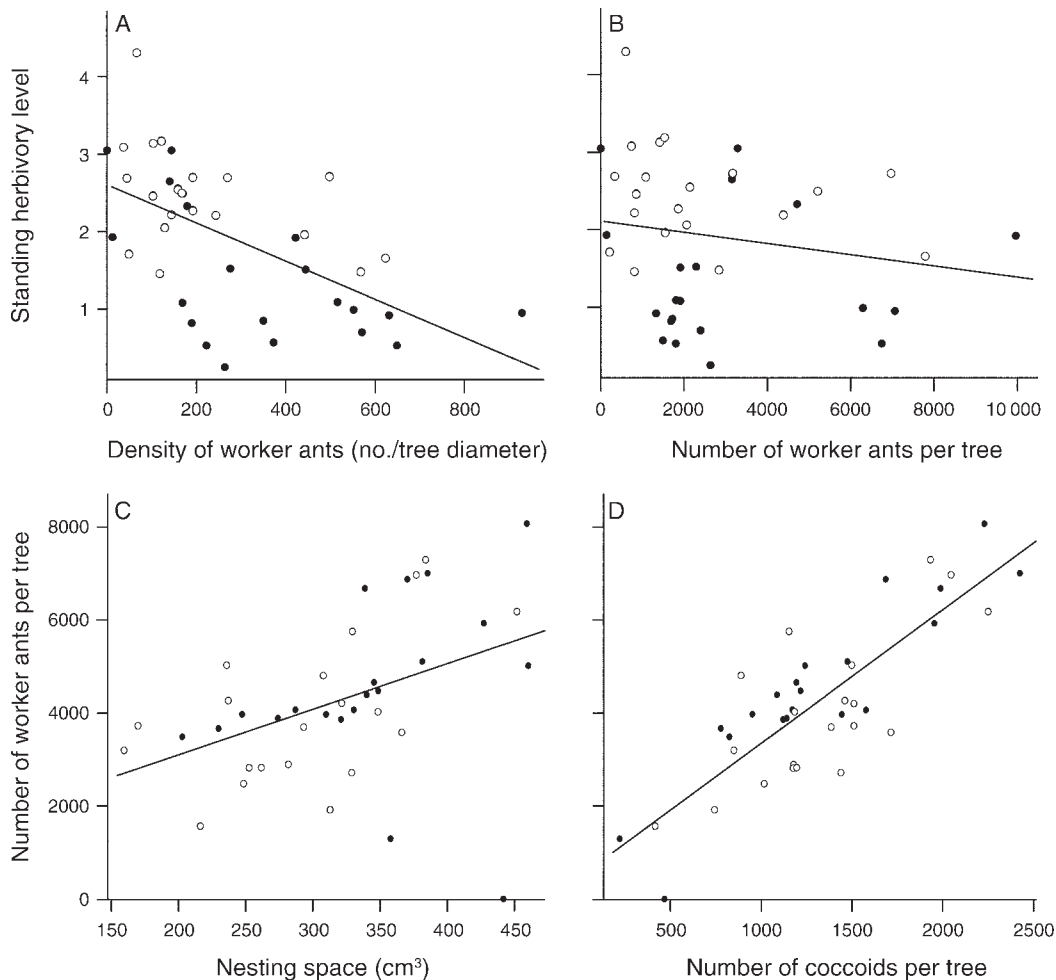


FIG. 3. (A, B) Relationship between average standing herbivory levels per leaf per tree, estimated as the average among five categories of percentage of leaf area eaten, and (A) the density of worker ants, calculated as the total number of worker ants divided by tree diameters ($R^2 = 0.34$, $P < 0.0001$), and (B) the total number of worker ants in each tree ($R^2 = 0.06$, $P = 0.13$). For panels (A) and (B), graphs are shown with raw numbers; analyses were conducted on power-transformed data. (C, D) Relationship between the total number of worker ants in each tree and (C) total nesting space inside tree domatia ($R^2 = 0.18$, $P < 0.006$) and (D) the total number of coccoids ($R^2 = 0.68$, $P < 0.0001$). For panels (C) and (D), all values are power-transformed. For all graphs, solid circles represent points from Chamela, and open circles represent points from Santa Rosa.

ants, $P = 0.3$; for site, $P < 0.0002$; for number of ants \times site, $P = 0.9$; Fig. 3B). Results of experiments showing that larger ant colonies defend the tree more effectively are reported in Appendix C.

The number of ants was more strongly related to the number of coccoids than to available nesting space in domatia. The total number of worker ants in a tree was positively related to the total amount of nesting space ($R^2 = 0.20$, $F_{3,36} = 2.97$, $P < 0.05$; Fig. 3C) and to the total numbers of coccoids ($R^2 = 0.72$, $F_{3,36} = 30.37$, $P < 0.0001$; Fig. 3D). However, when domatia space and number of coccoids were considered simultaneously in multiple regression, only the number of coccoids significantly predicted ant-colony size ($R^2 = 0.69$, $F_{2,37} = 41.09$, $P < 0.0001$; for nesting space, $P = 0.5$; for number of coccoids, $P < 0.0001$). Similarly, ant densities were positively related to coccoid densities ($R^2 = 0.69$,

$F_{3,36} = 26.92$, $P < 0.0001$), but not to trees' standardized nesting space, i.e., the total nesting space divided by tree size ($R^2 = 0.10$, $F_{3,36} = 1.41$, $P = 0.3$).

Path analysis indicated that coccoid density had stronger indirect effects on reduced herbivory of *Cordia alliodora* leaves than did nesting space (Fig. 1; Appendix E; Table E1).

Experimental effects of carbohydrates on ant behavior

When ants were fed carbohydrate-rich diets for three weeks, they were more aggressive toward caterpillars. Ants in high-carbohydrate treatments attacked caterpillars more often per capita than did ants in low-carbohydrate treatments (Fig. 4A; paired Wilcoxon sign-rank, $P < 0.04$). Per capita patrolling activity, the average number of ants exploring an experimental structure divided by the number of ants, did not differ

between high- and low-carbohydrate treatments (Fig. 4B; paired Wilcoxon sign-rank, $P = 0.8$). Significantly more ants from the low-carbohydrate than high-carbohydrate treatment died in the first week of the experiment (matched pair t test; $t = -3.49$, $df = 5$, $P < 0.02$).

*Spatiotemporal variability in defense
and symbiont densities*

Ant defense was significantly more effective on younger leaves. In a field experiment, the time to detection of caterpillars was significantly shorter on younger leaves, where more ants were observed patrolling (Fig. 5A; $Z = 3.42$, $df = 19$, $P < 0.0006$). Once ants contacted a caterpillar, it was attacked significantly more often on young leaves than on old leaves (Fig. 5B; $Z = -2.81$, $df = 19$, $P < 0.005$). Within 5 minutes, ants chased caterpillars off 33% of young leaves, but off only 5% of old leaves ($Z = -2.33$, $df = 19$, $P < 0.02$).

Mean densities of coccoids were significantly higher in the youngest, terminal domatia than in either type of older domatium (Fig. 5C). Mean densities of worker ants were higher, but not significantly so, in the youngest domatia (Fig. 5D).

DISCUSSION

Ant-exclusion experiments showed that *Azteca pittieri* could significantly reduce herbivory on *Cordia alliodora* leaves in two Neotropical dry forests. The effectiveness of ant defense and resulting average rates of herbivory were context-dependent. Rates of herbivory and herbivory pressure were higher in the early rainy season than in the late rainy season, and higher in Santa Rosa (Costa Rica) than in Chamela (Mexico). Ants significantly reduced herbivory in Chamela, and not in Santa Rosa. The context dependence of the value of ant defense in this system may help to explain conflicting previous reports about the effectiveness of defense by *Azteca* ants of *C. alliodora* leaves (Wheeler 1942, Tillberg 2004).

Higher ant densities, and not total number of ants, were associated with reduced herbivory. At both sites, ants appeared to reduce herbivory to 10% of leaf area or less when colonies reached densities greater than 500 workers/1-cm-diameter (basal diameter + dbh) tree (Fig. 3A). However, colonies of *A. pittieri* were generally much smaller in Santa Rosa than in Chamela, which may explain why ants did not reduce herbivory overall in the exclusion experiments in Santa Rosa.

Although larger trees did generally have more ants, at both sites the number of coccoids explained ant-colony sizes and densities better than did nesting space. The slope of the positive relationship between the number of ants and coccoids (Fig. 3D) suggests that, on average, approximately three ants depend on each coccoid. It is not possible to conclude from these data whether the number of ants in a colony is limited by the supply of food from coccoids, or whether ants maintain the number of coccoids at suitable levels to support the

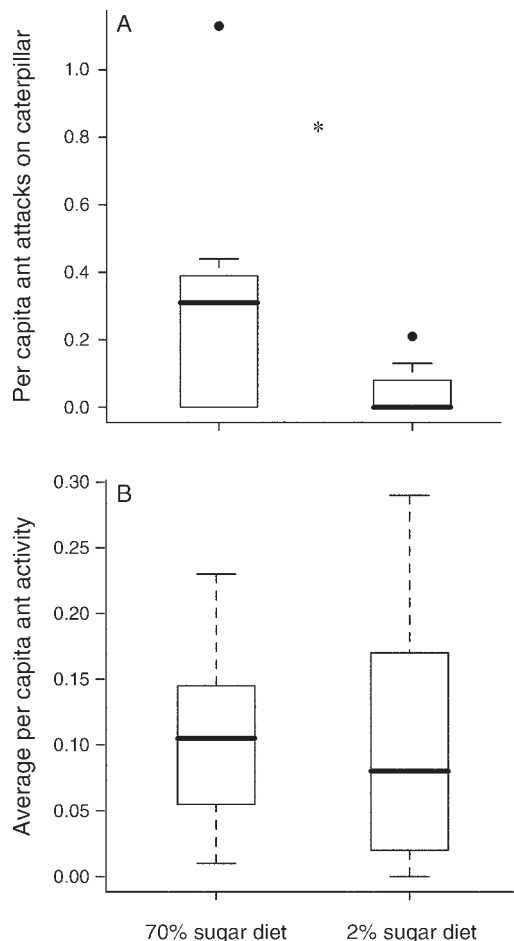


FIG. 4. Effect of two experimentally controlled diets on ant aggression and activity. The heavy line across the box is the median, the top and bottom of the box are the first and third quartile of the data, respectively, and the whiskers extend to the most extreme data point that is no more than $1.5\times$ the length of the box away from the median. Points indicate outliers; the asterisk (*) indicates $P < 0.04$ by Wilcoxon sign-rank. (A) Per capita attacks on a *Cropia templada* caterpillar over a 10-min period. (B) Average per capita activity of ants is the average number of ants exploring an experimental structure at eight time points over 8 h.

colony without excess honeydew production. A surplus of honeydew in humid, tropical environments can be associated with growth of lethal pathogens (Queiroz and Oliveira 2001). Whatever the direction of causality, the numbers of ants and coccoids were so tightly correlated that we conclude that larger ant colonies are associated with higher tree investment in coccoids. Larger ant colonies were associated with better defense against foliar herbivores (Appendix C). Accordingly, of the two investments that trees make in ant symbionts, nesting space and coccoids, only density of coccoids had strong indirect effects on foliar herbivory, mediated by direct effects on ant-colony size. Increasing costs of phloem loss to support larger ant colonies could be offset by increasing benefits of ant defense.

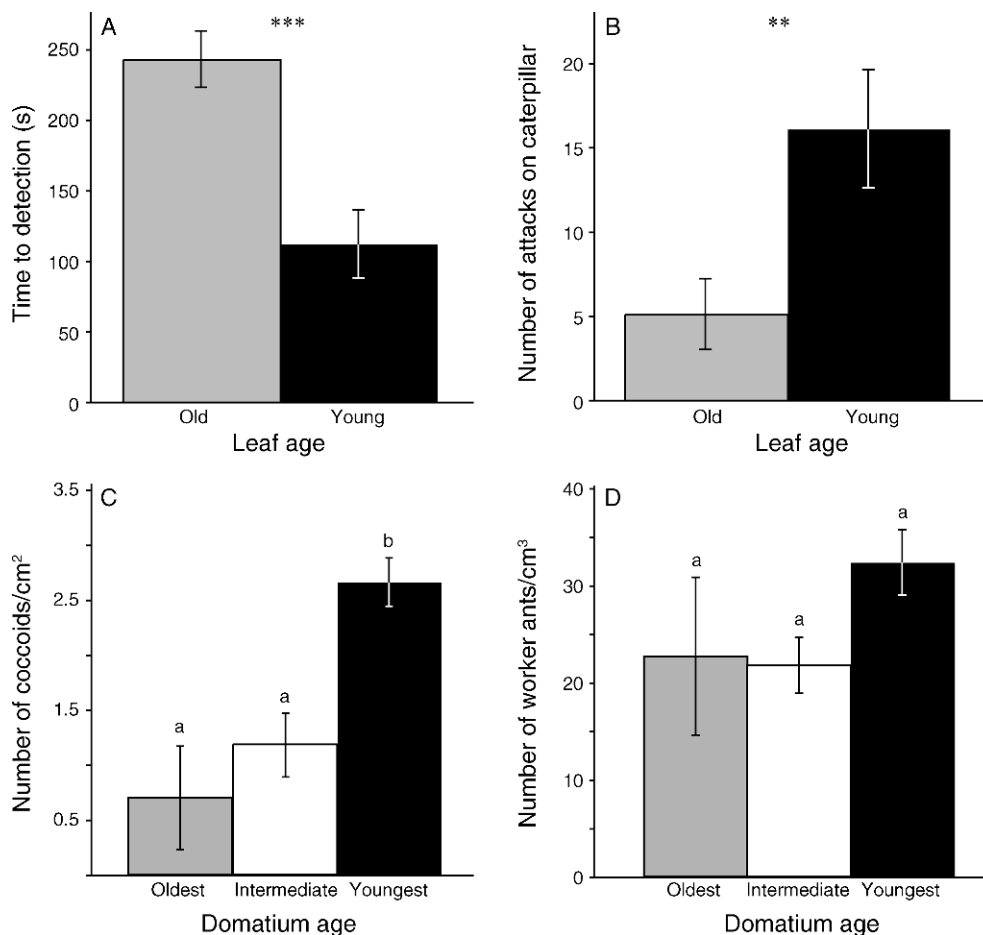


FIG. 5. (A, B) Leaf age and (A) the time necessary for ants to detect a caterpillar and (B) the number of attacks on that caterpillar within 5 min. Gray bars represent old leaves associated with older domatia, and black bars represent young leaves associated with young domatia. Data are means \pm SE. Asterisks indicate results from Wilcoxon rank-sum tests. (C, D) Densities (mean \pm SE) of (C) coccoids and (D) worker ants in domatia, by domatium age. Gray bars represent oldest, white bars represent intermediate, and black bars represent youngest domatia. Bars with different lowercase letters differ significantly (Tukey hsd, $P < 0.05$).

** $P < 0.01$; *** $P < 0.001$.

A. pittieri ants were more aggressive, but not necessarily more active, when supplied with carbohydrate-rich diets over a three-week period. Thus, indirect benefits of coccoids to trees include more effective ant defense when each ant has access to more coccoid honeydew. Grover et al. (2007) showed that carbohydrates fuel aggressive behavior of Argentine ants against other ants. Here we show that this effect extends to aggression by phytoecious ants toward non-ant intruders that antagonize plants. *A. pittieri* workers required abundant carbohydrates to survive, which is further evidence that carbohydrate supply could affect colony size.

We found that *A. pittieri* ants defended young leaves near young domatia more effectively than they defended older leaves near older domatia. This is consistent with the predictions of the optimal-defense hypothesis. Better ant defense of young leaves than of old leaves has also

been observed in other ant-plant symbioses (Heil et al. 2004). We found significantly higher densities of coccoids, and marginally higher densities of worker ants, in young domatia than in older domatia. Hemipteran preference for young plant parts could be explained by easier mechanical access to phloem sieve tubes (Maschwitz and Hänel 1985), lower secondary compounds in the phloem (Gullan 1997), or greater amino acid content of the phloem (Way 1963) in younger tissues. All of these characteristics could arise simply as physiological characteristics of new plant growth. Alternatively, as suggested by the optimal-defense hypothesis, the latter characteristics could arise from specific plant investment in compounds that increase coccoid success, and related effectiveness of ant defense, in young tissues. In either case, greater coccoid success appears to cause preferential dispersal, either independently or carried by ants, to, and/or

higher fecundity on, young tissues. The spatial concentration of ants with greater access to honeydew in young domatia near young leaves could be a general feature of ant–plant mutualisms and help explain why ants tend to defend young leaves more effectively than older leaves.

Conclusions

The results presented here suggest that coccoids indirectly benefit *C. alliodora* trees in three ways. First, more coccoids were associated with larger ant colonies, which provide more defense. Second, the carbohydrates that coccoids supply to ants stimulated ant aggression against herbivores, so coccoids make individual ants more effective defenders. Third, coccoids were more abundant in young domatia near young leaves, and this may account for the more effective ant defense of younger leaves that we observed. Taken together, these indirect benefits of coccoids for plants suggest that there is positive feedback between the plant's investment in a third partner, coccoids, and the effectiveness of ant defense against foliar herbivores. Moreover, we found no evidence for saturating benefits to the plant of ant defense as these investments increased. Nevertheless, the spatial concentration of coccoids in young plant tissues that require the most ant defense indicates the intriguing possibility that plants could partially control their investment in coccoids by investing in compounds that increase coccoid success in young tissues or decrease coccoid success in older tissues. Future studies that measure variation among plant tissues and across plant communities in the availability and content of phloem and honeydew would further elucidate feedback mechanisms among plant, ant, and coccoid mutualists.

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APPENDIX A

Detailed methods for ant-exclusion experiments (*Ecological Archives* E092-004-A1).

APPENDIX B

Estimation of colony-size variables from estimator domatia (*Ecological Archives* E092-004-A2).

APPENDIX C

Quantity of ants and defensive behavior (*Ecological Archives* E092-004-A3).

APPENDIX D

Detailed methods for controlled-diet laboratory experiments (*Ecological Archives* E092-004-A4).

APPENDIX E

A table presenting results from path analysis of indirect effects of plant investments on herbivory (*Ecological Archives* E092-004-A5).