

The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree

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Abstract. Three recent meta-analyses of protective plant–ant mutualisms report a surprisingly weak relationship between herbivore protection and measured demographic benefits to ant-plants, suggesting high tolerance for herbivory, substantial costs of ant-mediated defense, and/or benefits that are realized episodically rather than continuously. Experimental manipulations of protective ant–plant associations typically last for less than a year, yet virtually all specialized myrmecophytes are long-lived perennials for which the costs and benefits of maintaining ant symbionts could accrue at different rates over the host’s lifetime. To complement long-term monitoring studies, we experimentally excluded each of four ant symbionts from their long-lived myrmecophyte host trees (*Acacia drepanolobium*) for 4.5 years. Ant species varied in their effectiveness against herbivores and in their effects on intermediate-term growth and reproduction, but the level of herbivore protection provided was a poor predictor of the net impact they had on host trees. Removal of the three *Crematogaster* species resulted in cumulative gains in host tree growth and/or reproduction over the course of the experiment, despite the fact that two of those species significantly reduce chronic herbivore damage. In contrast, although *T. penzigi* is a relatively poor defender, the low cost of maintaining this ant symbiont apparently eliminated negative impacts on overall tree growth and reproduction, resulting in enhanced allocation to new branch growth by the final census. *Acacia drepanolobium* is evidently highly tolerant of herbivory by insects and small browsers, and the costs of maintaining *Crematogaster* colonies exceeded the benefits received during the study. No experimental trees were killed by elephants, but elephant damage was uniquely associated with reduced tree growth, and at least one ant species (*C. mimosae*) strongly deterred elephant browsing. We hypothesize that rare but catastrophic damage by elephants may be more important than chronic herbivory in maintaining the costly myrmecophyte habit in this system.

Key words: *Acacia drepanolobium*; ant-plant; *Crematogaster*; herbivory; mutualism costs and benefits; myrmecophyte; plant defense; Tetraoponera.

INTRODUCTION

Our understanding of mutualisms, once framed by the concept that these interactions are specialized and unconditionally beneficial associations between pairs of species, has been enriched over the past several decades by several important realizations. First, theoretical analyses have shown that the interests of mutualist partners often do not coincide, especially in systems where associations are transient or are reestablished each generation (Trivers 1971, Axelrod and Hamilton 1981, Doebeli and Knowlton 1998, reviewed in Sachs et al. 2004). Second, it now appears that most mutualistic interactions involve guilds of alternative partner species on one or both sides of the interaction (reviewed in

Stanton 2003, Bascompte and Jordano 2007). These two findings lead to the third emerging generalization: the fitness consequences of mutualism are likely to change in magnitude, and even in sign, depending both on the ecological context and the identity of partners (Bronstein 1994, Thompson 2005, Palmer et al. 2010). Nonetheless, three recent meta-analyses report that protective plant–ants very rarely have negative effects on their plant partners (Chamberlain and Holland 2009, Rosumek et al. 2009; Trager et al. 2010), countering an expectation that the costs of supporting ant defenders will sometimes exceed the benefits that ant-plants receive (Bronstein et al. 2006).

As a general rule, greater ant-related investment by ant-plants increases local ant densities and confers greater herbivore protection. Ant-based plant protection systems range widely in specificity and intimacy, from myrmecophilic plants that entice ants to patrol their vulnerable aboveground tissues, to obligate symbioses in which myrmecophytic host plants provide

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presumably costly domatia and food resources to their resident ant colonies (reviewed in Koptur 1992, Davidson and McKey 1993, Heil and McKey 2003). Synthetic analyses conducted across these varied systems reveal that ants generally reduce herbivory on their plant partners, but the protection they provide tends to be greater in obligate ant–myrmecophyte associations than in more facultative interactions between ants and myrmecophiles (reviewed in Heil et al. 2001, Chamberlain and Holland 2009, Rosumek et al. 2009; Trager et al. 2010).

Despite the consistency of published work demonstrating that symbiotic ants can be effective plant defenders, our understanding of the potential costs and benefits to myrmecophytes of maintaining plant–ants, and of the selective pressures that maintain these relationships, remains incomplete. First, many studies of ant effects on myrmecophytes have simply compared naturally occupied and unoccupied plants. Because low-quality hosts may be less attractive to, or more likely to be abandoned by, symbiotic plant–ants (e.g., Palmer et al. 2010), ant-occupancy manipulations are required to make unbiased estimates of the direct effects of ants on host partners (Agrawal and Rutter 1998, Chamberlain and Holland 2009). Second, although myrmecophytes are generally long-lived perennial shrubs or trees, very few studies have either maintained ant-exclusion treatments for more than a year (but see Letourneau 1998) or conducted long-term monitoring of individual myrmecophytes and their ant associates (but see Palmer et al. 2010). These empirical limitations have almost certainly impaired our understanding of the demographic costs and benefits of the myrmecophyte habit.

The small minority of studies that have measured effects of experimental ant exclusion on whole-plant growth and/or reproduction, as well as on herbivore damage, indicate that ant impacts on short-term plant performance are generally weaker (and less ubiquitously positive) than their suppression of herbivory (Chamberlain and Holland 2009, Rosumek et al. 2009; Trager et al. 2010). Factors that may contribute to this apparent mismatch between ant protection and host performance include high costs associated with ant occupancy and high tolerance for herbivore damage. Moreover, whereas ant effects on herbivore damage are measured over relatively short-term experiments, the costs and benefits of ant protection accrue over the host's lifetime (Bronstein 2001), and may have their greatest impacts during rare or catastrophic events (Cochard and Agosti 2008, Palmer et al. 2008b). Longer-term manipulation experiments, ideally coordinated with long-term monitoring, are needed to estimate the demographic costs and benefits of ant symbionts that may manifest at different time scales over a myrmecophyte's lifetime.

In this study, we experimentally removed each of four species within an East African guild of acacia–ants from their long-lived myrmecophyte host trees. We main-

tained treatments for almost five years so that we could compare the effects of different ant species on host tree growth, reproduction and herbivore damage over multiple growing seasons. The experiment was initiated on trees of a single, common size class, allowing us to interpret our findings within the context of demographic models constructed for this population based on long-term monitoring of all *A. drepanolobium* size and age classes (Palmer et al. 2010).

MATERIALS AND METHODS

Study system

This work was conducted in semiarid, savanna–bushlands at the Mpala Research Centre, in Kenya's Laikipia District (0°17' N, 37°52' E). The study site (1800 m elevation) is underlain by heavy clay “black cotton” soils (Ahn and Geiger 1987), and the canopy is dominated by a single myrmecophyte acacia species (Taiti 1992, Young et al. 1997, 1998). *Acacia* (*Senegalia*) *drepanolobium* is a relatively small tree, rarely exceeding 6 m in height in our study area. The habitat supports numerous large herbivores and browsers, including elephants. *Acacia drepanolobium* is well-defended, producing sharp, 2–7 cm stipular thorn pairs along its branches, a subset of which share a hollow, inflated base in which ants nest. The bipinnate leaves bear one to three extrafloral nectaries along their central axis, but do not produce food bodies. Investment by *A. drepanolobium* in structural and ant-related defenses decreases under reduced browsing pressure (Young and Okello 1998, Huntzinger et al. 2004, Palmer et al. 2008a).

Four species of acacia–ants are the principal symbionts of *A. drepanolobium* in East Africa (Hocking 1970), and all co-occur at fine spatial scales in our study area (Young et al. 1997, Palmer et al. 2000, Palmer 2003). *Tetraponera penzigi*, *Crematogaster nigriceps*, and *C. mimosae* nest exclusively within swollen thorns, whereas *C. sjostedti* nests within trunk and stem cavities created by wood-boring beetles (Palmer et al. 2008a). *Tetraponera penzigi* colonies are most common on small trees < 1.5 m in height, and typically control only a single canopy. In contrast, *Crematogaster* colonies often span multiple, mature trees. Intense competition for hosts among ant colonies results in ant species turnover as trees grow over time, with the largest colonies (formed by *C. sjostedti*) tending to displace colonies of intermediate and small size formed by *C. mimosae*, *C. nigriceps*, and *T. penzigi* (Palmer et al. 2000, 2010, Palmer 2004). Early experimental studies of *A. drepanolobium* showed that its symbiotic ants and/or sharp thorns can deter some small to medium-sized browsers (Milewski et al. 1991, Madden and Young 1992, Stapley 1998). More recent work indicates that megaherbivores (particularly elephants) play a critical role in the maintenance and ecological success of this ant–tree association (Palmer et al. 2008a, Goheen and Palmer 2010).

Experimental design

In March 2005, we walked transects through the study area to select healthy experimental trees 1.5–2 m tall. All four acacia-ant species are common on trees of this height, which we estimate to be 30–40 years of age (Young et al. 1997, Palmer et al. 2010). Once we found two or more closely size-matched trees, we searched exhaustively to complete one replicate of the experiment, consisting of eight trees of similar size (two trees for each of the four symbiotic ant species). In all, we established 12 replicates spread across 15–20 ha of the study area. Within a replicate, one of the two trees occupied by each ant species was assigned at random to the ant removal treatment, with the other tree designated as a control. Prior to removing ants, we measured maximum height of living canopy tissue and stem diameter (at 20–30 cm) on each tree. The aggressiveness of the resident ant colony was estimated by two observers, each of whom sharply tapped a swollen thorn and counted the ants recruiting to this disturbance within one minute (also see Palmer and Brody 2007, Palmer et al. 2008a).

After measurements were complete, trees assigned to the ant removal treatment were disturbed vigorously to draw ants out of swollen thorns, and then heavily fogged using a pyrethrin-based insecticide (0.6% alpha-cypermethrine), which has a outdoor half-life of 1–4 weeks. Insecticide was also injected into the interiors of swollen thorn domatia and into stem cavities. Recolonization of ant-removal trees by crawling ants was prevented by encircling their stems with a duct tape strip liberally smeared with Tangletrap sticky barrier (The Tanglefoot Company, Grand Rapids, Michigan, USA) and by pruning and trimming any vegetation touching the tree. Control trees were vigorously disturbed in the same manner, insecticide was applied to four 2.5-cm² areas on each tree stem near the ground where ant activity was low, and then a duct tape strip (without the sticky barrier) was installed. After 48 hours, ant workers readily moved back and forth over the duct tape and the sprayed test areas, suggesting that the residual impacts of these treatments were relatively minor. For the next month, some limited spraying was repeated on ant removal trees to kill persistent ants. Over the next 4.5 years, treatments were maintained by trimming vegetation around all trees, restoring sticky barriers and injecting small amounts (< 1 mL) of insecticide into domatia that had been colonized through the air by foundress ant queens.

Tree canopy height and stem diameter were remeasured in September 2005, August 2006, January 2008, and August 2009. In September 2005, we tested the effectiveness of the removal treatment by remeasuring ant recruitment to disturbance. Because some fruits persist in the canopy for a year or more, we were able to determine whether a tree had reproduced since the previous survey by noting the presence of flowering nodes and/or persistent fruits at each census. At posttreatment surveys, we counted all branch apices

that had been browsed and sites of attack by other herbivores for whole trees. Sites of attack by wood-boring beetles were identified by oviposition scars left by female cerambycids and by sap-exuding wounds in the stem, indicating feeding by stem-boring beetle larvae. We also counted stem galls and discrete clusters of leaf galls in a subset of surveys, but we have not yet identified the insects responsible for those damage categories. At each census we recorded the presence of browsing damage by elephants, and on the final survey, the diameters of all elephant-killed stems were recorded. Damage to leaves could not be measured consistently among surveys, since leaflets abscise during dry seasons. By August 2009, the growth of many trees made treatment maintenance and further surveys impractical.

Statistical analysis

During the experiment, eight control trees were taken over by other ant species; all were dropped from statistical analyses for this paper. One control tree could not be relocated after the second survey, and another control tree occupied by *C. sjostedti* died, leaving a final sample size of 48 ant-removal trees and 38 control trees. Statistical analyses and tests for the normality of residuals were conducted using SAS v.9.2 (SAS Institute 2002). The Shapiro-Wilk *W* statistic exceeded 0.90 for all parametric analyses reported here, an indication that the data quite closely conformed to the assumptions for valid hypothesis-testing.

1. *Ant response to disturbance before and after removal treatments.*—Separate, fixed-factor ANOVA models (the GLM procedure) were used to test for effects of initial ant species, ant removal treatment and their interaction on ant response to disturbance (the mean of two counts per tree), either before or after the removal treatment was initiated. For both the pre- and post-treatment analyses, we performed preplanned tests for significant differences between the control and ant-removal treatments within each ant species (implemented using the SLICE option in GLM).

2. *Tree growth over time.*—Multivariate analysis of variance was used to test for the effects of initial ant species, removal treatment and their interaction on two final tree size measures—stem diameter and canopy height (GLM procedure; MANOVA option). Despite our efforts to match trees by initial size, some systematic variation was apparent, and so we included initial stem diameter and canopy height as covariates in the MANOVA. Repeated measures analyses of variance were used to model each measure of tree size over successive surveys, based on the between-subject effects of initial ant species, removal treatment, and their interaction. We performed planned comparisons between the control and removal trees for each ant species at each census. To assess allocation to photosynthetic tissues (and thus to future growth potential), we used ANOVA to ask how recent branch growth for whole trees at the final census in 2009 was predicted by final

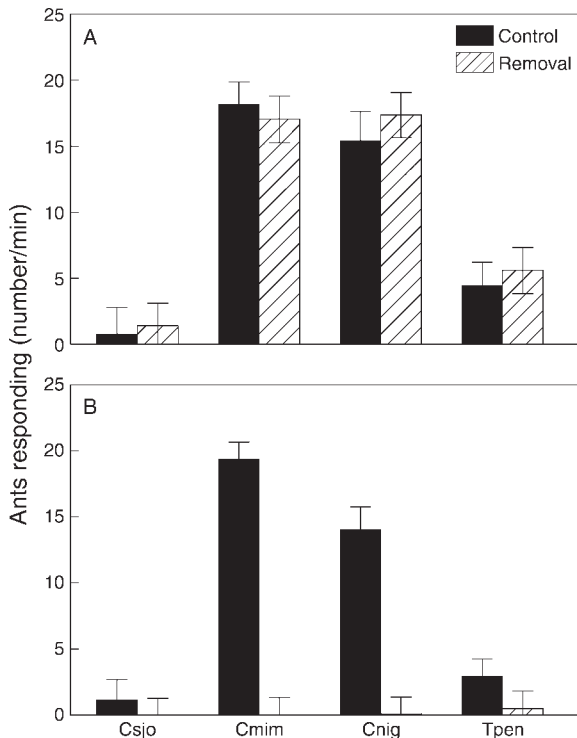


FIG. 1. Numbers of acacia-ants responding to a standardized disturbance of *A. drepanolobium* trees assigned to ant-removal and control treatments. (A) Ant activity measured prior to treatment initiation. (B) Ant activity measured six months after treatments were initiated. Trees were occupied by one of four species of ant symbionts, abbreviated as follows: Csjo, *Crematogaster sjostedti*; Cmim, *C. mimosae*; Cnig, *C. nigriceps*; Tpen, *Tetraponera penzigi*. Values are least-square means \pm SE. Sample size is 9–12 trees per ant species and treatment category.

stem diameter (a covariate), as well as by ant species, ant removal treatment and the ant \times treatment interaction.

3. *Tree reproduction*.—Cochran-Mantel-Haenzel (CMS) statistics were used to compare control and ant-removal trees with respect to whether or not they reproduced over the course of the experiment (FREQ procedure; CMS option). Two-way contingency tables were stratified over initial ant species to test for the overall effect of ant removal on tree reproduction, and the Breslow-Day test was used to test for odds-ratio variation among ant species. Two-tailed Fisher's Exact Tests were then used to test for effects of removing each ant species on tree reproduction.

4. *Damage to trees*.—MANOVA was used to test for variation in multiple types of herbivore damage that was associated with ant species, removal treatment and their interaction. Each damage type was not measured in all surveys, so we analyzed the *mean* score for each of five damage types recorded over the entire experiment for each tree. Using mean damage scores dramatically improved the normality of residuals. Dependent variables in the MANOVA model were means for: number of beetle damage sites (four surveys), number of

browsed apices (four surveys), number of stem galls (three surveys), number of leaf gall clusters (two surveys), and total diameter of large branches removed by elephants (final survey only). Because larger trees are also larger targets for herbivores, we included each tree's stem diameter in 2009 as a covariate, so statistical effects of ant species and ant removal treatment on damage represent impacts on herbivory that are independent of overall tree size. To follow up on earlier results demonstrating that *C. sjostedti* facilitates host tree attack by cerambycid beetles (Palmer et al. 2008a), we used repeated measures ANOVA to model how the number of beetle attack sites (per tree initially occupied by *C. sjostedti*) was influenced by removal treatment, final tree diameter and the time at which each survey was conducted. Stratified categorical analysis (FREQ procedure; CMS option) was used to test for effects of initial ant species and ant removal treatment on the probability that a tree was damaged by elephants over the course of the experiment. Fisher's exact test was used to test for effects of removing each ant species on elephant browsing. Finally, we used Akaike's information criterion (AIC) to compare all possible multiple linear regression models, in which the means for all five damage types and initial stem diameter were used to predict final tree diameter, pooled across all ant species and treatments.

RESULTS

Effectiveness of ant exclusion.—The ant removal treatment was effective in reducing ant densities, especially for the two species that respond most aggressively to superficial disturbance (Fig. 1). Prior to ant removal, ant recruitment to disturbance varied dramatically among the four ant species ($F_{3,76} = 41.09$, $P < 0.0001$), but was equivalent between trees assigned to the treatment and control groups ($P = 0.6108$ for the effect of treatment; $P = 0.8504$ for the treatment \times ant interaction). Six months into the experiment, ant recruitment to our standardized disturbance was significantly reduced on removal trees ($F_{1,76} = 18.65$, $P < 0.0001$). Effects of the removal treatment varied among ants ($F_{3,76} = 19.68$, $P < 0.0001$); numbers of responding workers were dramatically reduced for *C. mimosae* and *C. nigriceps* ($P < 0.0001$ in each case), but not significantly so for the two less aggressive ant species.

Tree growth and reproduction.—A MANOVA for initial tree height and stem diameter revealed significant initial size variation among trees occupied by different acacia-ant species ($F_{3,72} = 3.72$; $P = 0.0018$). There was no significant variation in initial height among trees occupied by different ants or assigned to the removal or control treatments ($F_{7,82} = 0.95$; $P = 0.4738$). In contrast, trees occupied by *T. penzigi* had significantly smaller stem diameters than those occupied by the three *Crematogaster* species ($F_{3,75} = 7.30$; $P = 0.0002$), reflecting the younger average age of host trees occupied by this species (Palmer et al. 2010). Initial stem diameter did not differ between

control and removal treatment groups ($P = 0.3665$), and for neither tree size measure was there a significant ant species \times treatment interaction.

On average, ant removal stimulated tree growth over the course of the experiment, although this response was strongest in trees formerly occupied by *C. sjostedti* and *C. nigriceps* (Fig. 2). MANOVA demonstrated significant stimulation of host tree growth by ant removal ($F_{2,72} = 10.74$; $P < 0.0001$), but subsequent repeated measures ANOVA models showed that tree height and stem diameter responded at somewhat different time scales to the experimental manipulations. Over the 4.5-year experiment, trees from which symbiotic ants had been removed increased in height faster than control trees (Fig. 2A; time \times removal treatment $F_{4,72} = 56.66$; $P = 0.0037$). There was no significant interaction between ant species and removal treatment in affecting height growth over time in the full model ($P = 0.4910$). In contrast, the stimulatory effect of ant removal on tree diameter growth (time \times removal treatment $P = 0.0004$) varied significantly among ant species (Fig. 2B; time \times ant \times removal treatment $F_{9,175} = 2.48$; $P = 0.0111$) and took longer to manifest. After 4.5 years, trees from which *C. sjostedti* and *C. nigriceps* had been removed had more robust stems than control trees (a priori planned comparisons, $P = 0.0599$ and 0.0102 , respectively), whereas removal of *C. mimosae* led to marginally greater diameter growth by the final census ($P = 0.1657$). Removal of *T. penzigi* had no effect on final stem diameter ($P = 0.9838$), but this ant species did appear to enhance the future growth potential of its host trees. For a given stem diameter at the final census, total new branch growth produced by control and ant-removal trees was indistinguishable for the three *Crematogaster* species, but control trees occupied by *T. penzigi* showed 78% more recent branch growth than trees from which this species had been removed ($F_{1,21} = 9.58$; $P = 0.0028$).

Symbiotic *Crematogaster* colonies substantially reduced the fertility of *A. drepanolobium* trees within this size range. Categorical analysis showed a very significant stimulatory effect of ant removal overall on the probability that a tree reproduced during the experiment (CMS statistic for general association = 8.902, 1 df; $P = 0.0028$). The effects of ant removal were marginally heterogeneous among ant species (Brenslow-Day test $\chi^2 = 6.81$, 3 df; $P = 0.0781$). Only 5% of control trees reproduced (0% for all *Crematogaster*; 22% for *T. penzigi*), in contrast to almost 32% of the trees from which ants were removed. Whereas 17% of *T. penzigi* removal trees reproduced during the experiment, removing *C. mimosae*, *C. nigriceps* and *C. sjostedti* boosted host tree reproduction to 27%, 42%, and 42%, respectively.

Herbivory and browsing damage

Acacia drepanolobium trees that were stripped of their ant symbionts achieved more growth and reproduction

despite their increased vulnerability to herbivore attack (Fig. 3). MANOVA conducted on all five types of herbivore damage revealed that removing ants resulted in much higher overall incidence of attack on trees ($F_{5,70} = 3.95$; $P < 0.0001$), with the effect of the manipulation varying significantly among ant species ($F_{15,194} = 2.46$; $P = 0.0025$). Removing either *C. mimosae* or *C. nigriceps* dramatically increased attack by wood-boring beetles, stem gallers, leaf-gallers, and branch-tip browsers. Uniquely, removal of *C. mimosae* resulted in significantly more destruction of large branches by elephants. In contrast, removal of *T. penzigi* and *C. sjostedti* had little effect on the average levels of attack measured across all surveys.

The weak effect of *C. sjostedti* removal on the average number of beetle attack sites per survey reported here (an 11% decrease; Fig. 3) contrasts with an earlier analysis in which we showed that beetle damage decreased significantly within 18 months of *C. sjostedti* removal, demonstrating facilitation of beetle attack by this ant species (Palmer et al. 2008a). We explored these contrasting results further by conducting a repeated measures analysis just on trees initially occupied by *C. sjostedti*. The analysis confirmed that removing this species significantly reduced beetle attack (for the between-subject effect of ant removal, $F_{1,17} = 7.47$; $P = 0.0142$), but also demonstrated that the effect of *C. sjostedti* removal changed marginally over time (census \times treatment interaction $F_{3,15} = 2.99$; $P = 0.0646$), and that final tree diameter was strongly and positively associated with greater beetle attack overall ($F_{1,17} = 17.45$; $P = 0.0006$). In sum, the short-term facilitation of beetle attack by *C. sjostedti* disappeared in the final survey, by which time beetle-ravaged, *C. sjostedti*-occupied control trees were shrinking in size (Fig. 2), while removal trees were displaying vigorous growth and attracting intensive beetle attack. Our results also hint at the long-term demographic costs of occupancy by *C. sjostedti* (also see Palmer et al. 2008a, 2010): the one tree that died during this experiment was a control tree from which *C. sjostedti* symbionts were not removed.

Elephant damage to our experimental trees was spotty, but potentially important to long-term fitness. Over the course of the experiment, the probability of experiencing elephant damage ranged from 43% for *C. sjostedti* trees to 13% for *C. nigriceps* trees. There was no overall effect of ant removal on elephant browsing, with damage being recorded on 25% and 28% of control and ant-removal trees, respectively (CMS statistic for general association = 0.0337, 1 df; $P = 0.8543$). The effects of ant removal on elephant browsing were marginally heterogeneous among ants (Brenslow-Day test $\chi^2 = 7.3217$, 3 df; $P = 0.0623$). For three ant species, experimental removal had no effect on the likelihood of elephant damage (Fisher's exact test $P > 0.35$ in all cases). Only in the case of *C. mimosae*, where ant removal caused a more than 10-fold increase in elephant browsing

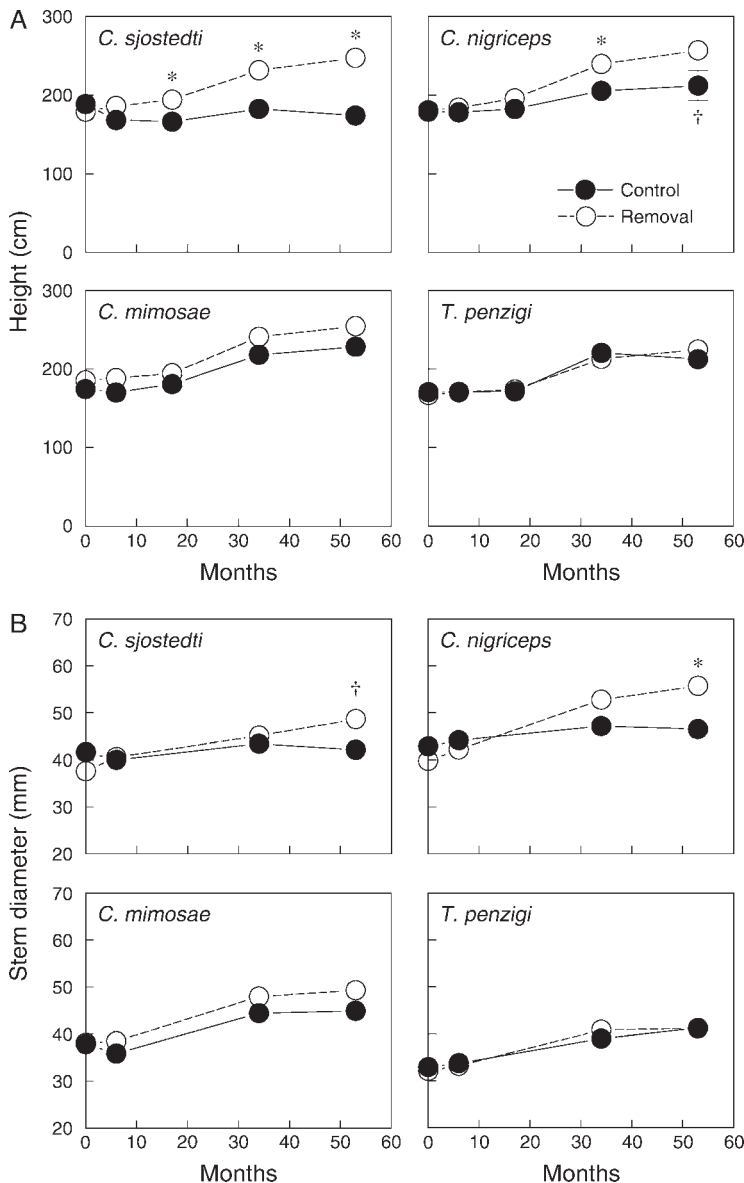


FIG. 2. Growth responses of *A. drepanolobium* trees from which symbiotic ants either were or were not removed: (A) height growth; (B) stem diameter growth. The experiment ran from March 2005 to August 2009. All trees were surveyed at 6, 17, 34, and 53 months after the start of the experiment. Least-square means from repeated-measures ANOVA are shown. Most standard errors are covered by the plotting symbols. Significant or marginally significant differences between removal and control treatments at any given census are shown.

* $P < 0.05$; † $P < 0.10$.

measured in the final 2009 survey (Fig. 3), was the protection of host trees from elephant browsing statistically significant: indeed, no control trees occupied by *C. mimosae* were damaged (Fisher's exact test $P = 0.0373$).

Multiple regression analysis indicated that branch loss to elephants was unique among the five damage measures in predicting reduced tree growth over the course of the experiment. Of all 62 models tested, the "best" model identified by Akaike's information crite-

riorion contained only three predictor variables: initial tree stem diameter, total apices browsed, and total branch diameter lost to elephants. These were also the only three variables that appeared in all eight models with the lowest AIC values, and the total AIC weight for those eight best models was 0.735, indicating their good fit to the data. Elephant browse damage contributed substantially to the fit of the best model, as that model was almost twice as likely to be correct as the regression model containing only initial tree stem diameter and

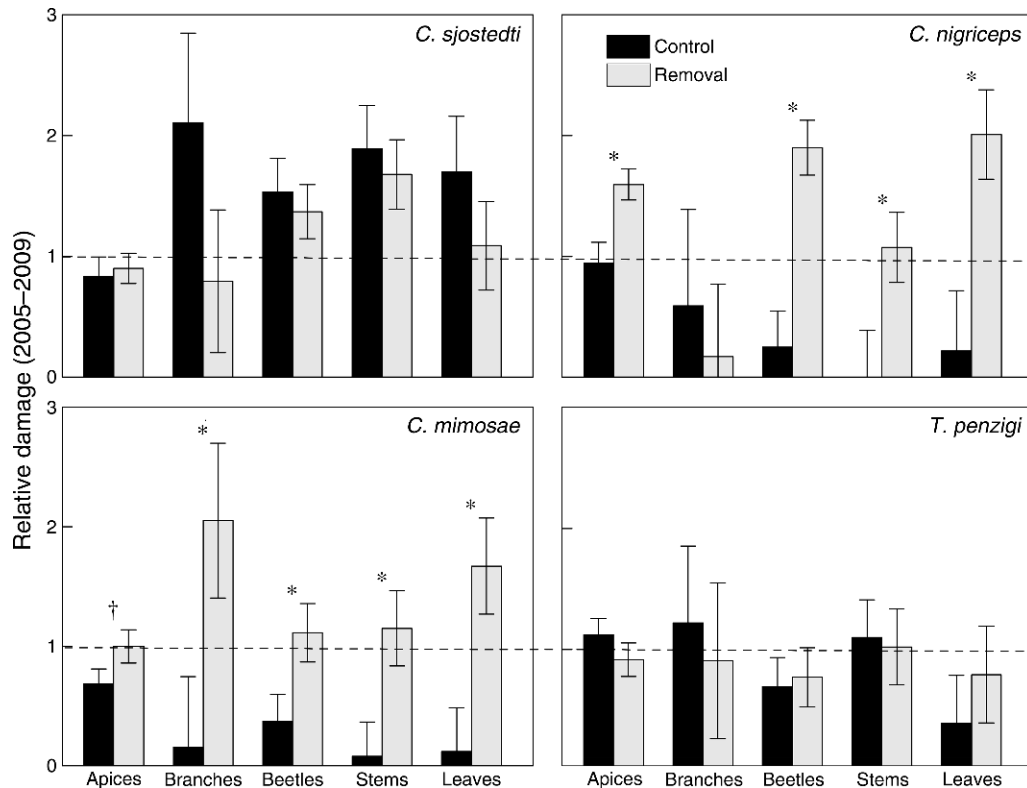


FIG. 3. Standardized damage scores recorded for *A. drepanolobium* trees during a 4.5-year experiment, comparing ant-removal trees to control trees initially occupied by each of four acacia-ant species. MANOVA was used to test for effects of ant species, treatment, and final tree diameter on tree means for five measures of herbivore attack: number of branch apices browsed, total diameter of large branches removed by elephants, number of sites showing damage by wood-boring beetles, number of stem galls, and number of leaf gall clusters. The damage variables were measured on very different scales, and so results of the MANOVA were scaled for visual comparison as follows. For each combination of ant species and ant removal treatment, a damage variable's least-square mean and standard error were normalized by dividing by the experiment-wide means for that variable. Accordingly, treatment groups experiencing greater-than-average or less-than-average damage have normalized values greater or less than 1, respectively. For each damage type, asterisks show significant effects of ant removal in planned comparisons of control and experimental trees initially occupied by each acacia-ant species. Sample size is 9–12 trees per ant-treatment combination.

* $P < 0.05$; † $P < 0.10$.

total apices browsed as predictors ($\Delta\text{AIC} = -1.138$; evidence ratio = 1.768). Greater elephant damage was consistently associated with reduced final tree size ($P < 0.05$ in all models), whereas apices browsed (as for all other damage types) was positively related to tree growth, apparently reflecting the greater attraction of large or fast-growing trees as targets for chronic herbivory.

DISCUSSION

The fitness consequences of mutualism hinge on how the resources and services exchanged between partners actually change the demographic rates of participants (Addicott 1986, Doebeli and Knowlton 1998, Bronstein 2001). The most direct way to assess the fitness impacts of associating with a given partner is to perform manipulations so that a species' performance in isolation can be compared to that achieved within the association, but conducting such experiments at demographically informative time scales can present major challenges

when studying long-lived mutualists such as the myrmecophytes that harbor symbiotic ant colonies. This challenge is especially severe when the benefits and costs of associating with a given partner accrue under different conditions, manifest at different intervals, or even change with the age of the participating partners (e.g., Palmer et al. 2010)

Two recent meta-analyses (Chamberlain and Holland 2009; Trager et al. 2010) revealed a surprisingly weak correspondence between ant-mediated reductions in herbivory (the benefit to ant-plants that is most often measured) and increases in plant performance characteristics such as growth and reproduction. This rather loose relationship supports one or both of the following possibilities: first, that supporting ant defenders can have substantial demographic costs (potentially expressed over longer time scales), and second, that ant-plants may be very tolerant of the kinds of herbivore damage most often monitored in these studies. In particular, if the benefits of ant protectors are intermit-

tent, but the costs of supporting those symbionts accrue consistently over time, shorter-term studies will tend to underestimate the selective advantage of the myrmecophyte habit.

Experiments conducted on obligate ant-myrmecophyte systems have consistently demonstrated that the majority of resident ant colonies strongly repel most herbivores (Heil and McKey 2003, Chamberlain and Holland 2009, Rosumek et al. 2009). However, symbionts exhibit other behaviors that could be costly to long-lived host plants, including destruction of reproductive tissues (Yu and Pierce 1998, Gaume et al. 2005), pruning vegetative growth (Stanton et al. 1999), repelling pollinators (Gaume et al. 2005), inducing greater production of nectar or food bodies (Linsenmair et al. 2001), and enhancing attack by herbivores such as coccids and wood-boring beetles (Gaume et al. 1998, Palmer et al. 2008a). In truth, we know remarkably little about how the short-term effects of herbivory and symbiotic ant behaviors contribute to the lifetime fitness of host plants.

The results reported here support the idea that intermediate-term experiments, in concert with long-term demographic monitoring, may be required to capture important costs and benefits of the myrmecophyte habit (also see Heil et al. 2001). Admittedly, our 4.5-year ant-removal experiment represents just a small fraction of the 100+-year lifespan of *A. drepanolobium* (Palmer et al. 2010), but extending our study over multiple growing seasons revealed direct consequences of ant occupancy that would have been missed by shorter-term studies. For example, the stimulatory effects of ant removal on tree height growth and stem growth were only statistically significant after 17 and 41 months, respectively, and it was the relatively heavy reproductive episode at the final census (14 trees in flower) that provided sufficient data to show that at least three of the four ant species suppress reproduction in trees of this size class.

These intermediate-term experimental results are most informative when compared with patterns detected through longer-term monitoring and demographic modeling of growth, survival and reproduction in over 1500 unmanipulated trees (of all size classes) within this same *A. drepanolobium* population (Palmer et al. 2010). For example, the ant removal manipulations reported here demonstrate that the very low rates of reproduction observed in surveyed, unmanipulated trees 1.5–2.5 m in height (<5% per year) can result directly from ant occupancy. Combining experimental and demographic approaches also provides insights into the fitness costs and benefits of this multi-species mutualism. Hosting costly ant symbionts suppresses reproduction by trees 1.5–2.5 m tall, but the long-term monitoring and modeling indicated that the lifetime fitness of trees within this size class is more sensitive to their survival and growth than to reproduction. Accordingly, the ant-associated decreases in host reproduction measured in

this experiment are likely to over-estimate the lifetime fitness costs of supporting ant defenders. Similarly, experimental ant removal revealed that *T. penzigi* is a less costly symbiont to maintain than its *Crematogaster* competitors, and that occupancy by this ant can increase allocation to new growth. This helps to explain a puzzling pattern revealed by long-term monitoring—that occupancy by this relatively poor host defender is associated with enhanced growth, survival and long-term fitness of the younger host trees it most often occupies.

In this study, the costs of maintaining resident ant colonies often exceeded the intermediate-term benefits *A. drepanolobium* host trees gained from their symbionts. Averaged over all four acacia-ant species, trees from which ants were removed suffered greater attack from multiple herbivore types, but nonetheless grew faster and were more likely to reproduce, compared with control trees. We also observed specific responses of both trees and herbivores to removal of each of the four ant species, providing insights into the specific ecological costs and benefits of hosting symbiotic ants for trees of this size class. Most importantly, the degree to which ants deterred most herbivores was a very poor predictor of cumulative host tree growth and reproduction. The near-term demographic boon of ant removal was most obvious (and least surprising) in trees occupied by *C. nigriceps*, which is a good defender but also a pruning parasitic sterilizer (Stanton et al. 1999), and in those occupied by *C. sjostedti*, a species that facilitates beetle attack (Palmer et al. 2008a). More unexpected was our discovery that removal of *C. mimosae* (an aggressive symbiont that effectively limits all types of herbivore damage we measured) also increased host tree reproduction and had a tendency to enhance tree growth.

The contrasting effects of removing *Crematogaster* and *T. penzigi* ants imply that *A. drepanolobium* trees experience significant ecological costs due to some combination of extrafloral nectar production and the tending of phloem-feeding insects by ant symbionts. In contrast to the *Crematogaster* species, which actively feed at extrafloral nectaries, *T. penzigi* workers actively remove all nectaries from developing leaves (Young et al. 1997, Palmer et al. 2002). In addition, whereas *C. mimosae* and *C. sjostedti* tend scale insects inside tree domatia and cavities, *T. penzigi* trees are conspicuously free of these phloem feeders. In contrast to *C. mimosae*, an excellent host defender, *T. penzigi* did not reduce any type of herbivore attack we scored, but also did not reduce host tree reproduction, and even bolstered the production of new canopy growth by the final census. This result, in concert with long-term monitoring of *A. drepanolobium* in the study area, provides mechanistic insights into demographic models indicating that *T. penzigi* strongly benefits lifetime host tree fitness (Palmer et al. 2010).

Some authors have suggested that high tolerance to herbivory in ant-plants may reduce the costs of

exploitation by ants that are poor defenders, and thus help to stabilize these multi-species mutualisms (Edwards and Yu 2008, Edwards 2009; Trager et al. 2010). *Acacia drepanolobium* appears to fit this pattern: it can produce compensatory growth via enhanced photosynthesis after browsing (Gadd et al. 2001, King and Caylor 2010), and healthy trees often coppice vigorously after fire or major stem damage (Okello et al. 2001, 2008). In this experiment, chronic levels of branch-tip browsing, galling, and stem-boring had no measurable effects on tree growth, reducing the demographic benefits trees gained from ant-mediated protection. The one exception to this pattern of nearly complete compensation was browsing damage caused by elephants: trees that lost branch tissue to elephant feeding experienced reduced growth over the course of our experiment.

We suggest that episodic but potentially catastrophic damage from elephants may play an important role in maintaining the myrmecophyte habit in this system. Elephants remove large canopy sections when they browse on *A. drepanolobium*, and often knock down medium to large trees while feeding. Whereas vigorous trees may resprout after such catastrophic damage, senescent or heavily beetle-damaged trees often do not survive. Several independent lines of evidence indicate that symbiotic ants can have profound effects on these elephant-tree interactions. Goheen and Palmer (2010) showed that captive elephants are deterred from feeding on *A. drepanolobium* by *C. mimosae* (the most common ant symbiont in our study area), and that wild elephants damage non-myrmecophyte acacias more often than *A. drepanolobium*. These authors also compared recent changes in tree canopy cover inside vs. outside of megaherbivore exclosures in the Laikipia area, over a time interval during which regional elephant populations have increased dramatically. They found that myrmecophytic *A. drepanolobium* populations outside of megaherbivore exclosures have not declined with increasing elephant densities, in contrast to canopy cover decreases seen in nearby plant communities dominated by non-myrmecophytic acacias. Finally, we have documented a weakening of this ant-protection mutualism in response to experimental megaherbivore exclusion in our study populations (Young et al. 1998). Within ten years, release from megaherbivore pressure resulted in decreased allocation to extrafloral nectaries and ant domatia by *A. drepanolobium* (Young and Okello 1998, Huntzinger et al. 2004), with concomitant increases in the abundance of *C. sjostedti* (a non-defending, stem-nesting exploiter whose trees experience much higher mortality) at the expense of *C. mimosae* (Palmer et al. 2008a). Taken together, these findings strongly suggest that elephants can act as agents of selection for the high investment that the myrmecophyte habit requires.

Although the landscape-scale effects of elephant feeding on this ant-acacia mutualism can manifest relatively quickly (e.g., after a decade of megaherbivore exclusion), none of our 96 trees experienced catastrophic

elephant browsing over the course of this 4.5-year experimental study. Perhaps consequently, heavy investments in supporting aggressive ant species did not “pay off” within this time frame. However, over the 100+ year lifetime of *A. drepanolobium*, we hypothesize that elephant-mediated selection, occurring episodically over decades, plays a role in favoring trees that pay the high cost of maintaining pugnacious ant colonies. As this ant-plant system is beginning to reveal, understanding the consequences of partner association for long-lived mutualists will require long-term demographic monitoring as well as extended experimental manipulations of symbionts and putative agents of selection conducted at different life stages of the participating species.

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