SPATIAL STRUCTURING AND FLORAL AVOIDANCE BEHAVIOR PREVENT ANT–POLLINATOR CONFLICT IN A MEXICAN ANT-ACACIA

Nigel E. Raine,[1,4] Pat Willmer,[2] and Graham N. Stone[3]

[1]*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[2]*School of Biology, University of St. Andrews, Bute Medical Building, St. Andrews KY19 9PS, UK*
[3]*Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK*

Abstract. Ant-acacias represent a classic insect–plant mutualism: the ants defend the plant from attack by herbivores, and in return are provided with trophic rewards and living space within swollen thorns. A potential drawback of this and other ant–plant mutualisms is that ant-guards may drive away useful insects, particularly pollinators. We assess the potential for ant–pollinator conflict in a Mexican ant-acacia, *Acacia hindsii*. This *Acacia* is guarded by a highly aggressive ant (*Pseudomyrmex veneficus*), which resides entirely within the host plant canopy and is provided with extrafloral nectar and protein-rich Beltian bodies. *Acacia hindsii* flowers released their pollen from 0700 to 0830 hours, and were visited by pollinators from 0730 to 1300 hours. Over the same time period ants maintained high activity levels at extrafloral nectaries on young leaves. Although daily activity of ants and pollinators overlapped substantially in time, it was largely separated in space: resources exploited by the ants are predominantly concentrated within new growth, while inflorescences are present only on shoots from the previous year. Ants nevertheless visit extrafloral nectaries on older leaves near inflorescences, and there is thus potential for ant–pollinator conflict. Bioassays of ant behavior showed young *A. hindsii* inflorescences induce an avoidance response in its ant-guards, which reinforces spatial segregation between ants and pollinators. This effect is absent from buds or postreproductive flowers. Young flowers of two non-myrmecophilous *Acacia* species also induced significant (though less potent) repellent effects, suggesting a general role for ant-repellents in *Acacia*, with selective enhancement in myrmecophilous species.

Key words: Acacia hindsii; ant-plant; ant–pollinator conflict; ant-repellent; México; mutualism; pollination; Pseudomyrmex veneficus.

INTRODUCTION

Ant-acacias harbor ant-guards in expanded stipular thorns (pseudogalls) and provide them with varying levels of nutritive reward (Janzen 1966, Hocking 1970); all ant-acacias provide ant-guards with nectar from extrafloral nectaries on the leaf rachis and petiole, and neotropical species also provide protein-rich Beltian bodies on the pinnule-tips of young leaves. In return, the ants provide a highly effective defense against vertebrate and invertebrate herbivores, and in some cases also against smothering plants and fire (Janzen 1966, 1967*a*, Hocking 1970, Madden and Young 1992, Agrawal and Rutter 1998, Stapley 1998). Not all of the impacts of ant-guards on their hosts are benign, however. In *Acacia drepanolobium*, ant-guards manipulate the growth form of their host tree, pruning axillary and terminal shoots and reducing seed set (Young et al. 1997, Stanton et al. 1999). More generally, ant secretions reduce pollen viability (e.g., Beattie et al. 1985,

Wagner 2000), and ants are potential raiders of the floral rewards (pollen and nectar) used to recruit pollinators (Janzen 1977, Davidson and McKey 1993, Ghazoul 2001). Recent work has revealed another potential conflict applicable to many ant-defense mutualisms: aggressive ants could impose a cost to the host tree by driving away useful pollinators (Willmer and Stone 1997). Acacias are largely self-incompatible (Hocking 1970, Janzen 1974, Kenrick and Knox 1989), so admission of pollinators to the flowers is essential for successful seed set. *Acacia* species studied to date in Africa (Tybirk 1993, Stone et al. 1996, 1998) and Australia (Bernhardt and Walker 1984, Bernhardt et al. 1984, Bernhardt 1987) are almost exclusively insect pollinated, and such small flower visitors may be particularly vulnerable to attack by ant-guards.

There are at least three ways in which acacias could minimize the interference caused by ants to insect pollinators. Ant and pollinator activity could be segregated (1) in time and/or (2) in space through patterning of resource provision by the *Acacia*, or (3) ants could be excluded from flowers by chemical and/or mechanical barriers for the period during which pollinators are active. Temporal segregation of ants and pollinators could operate over seasonal and/or daily timescales, the former requiring exclusion of the ants from the trees dur-

ing flowering. There is, however, no evidence in any ant-acacia system for loss of ants coincident with flowering (Young et al. 1997, Stone et al. 1998, Stanton et al. 1999, Palmer et al. 2000). Segregation on a daily timescale would require restriction of ant and bee activity on flowers to different times of day, while spatial partitioning could be achieved by separating flowers from alternative resources (extrafloral nectaries, Beltian bodies, and swollen thorns) exploited by the ants. Finally, a repellent active during pollen release could allow flowers and other resources attractive to ants to be intermixed spatially and temporally, with exclusion of ants from the flowers during the crucial period of pollinator activity. Such a repellent could simultaneously fulfill the additional needs of preserving pollen viability and prevention of harvesting of floral resources by ants.

An alternative hypothesis is that ants avoid flowers for some reason unrelated to the repellent qualities of flowers. Ants could potentially avoid visiting the brightly colored inflorescences because while doing so they become more obvious to potential predators, e.g., insectivorous birds. If such flower avoidance behavior represents an antipredator strategy we might expect to observe (1) very few ant visits to flowers, and (2) avoidance of all flowers, regardless of their age.

To date, potential ant–pollinator conflict has been studied in one African ant-acacia (Willmer and Stone 1997). On *Acacia zanzibarica*, daily activity of pollinators and the ant-guard *Crematogaster sjostedti* overlapped in time and space, and ants were excluded from flowers by a floral ant-repellent. Although insects elsewhere on guarded *A. zanzibarica* trees were rapidly attacked, pollinators foraged unhindered through the period of pollen release. Despite the extensive work on *Acacia cornigera* and other neotropical ant-acacia mutualisms, very little is known about ant–pollinator interactions in these systems. Neotropical and African ant-acacia mutualisms are thought to have evolved independently (Davidson and McKey 1993, McKey and Davidson 1993, Miller and Bayer 2001), and it is of interest to ask whether similar solutions to the potential problem of ant–pollinator conflict are found in both systems. Recent work has revealed ant-repellent flowers in a neotropical ant-acacia, *A. collinsii* (Ghazoul 2001), but the temporal and spatial dynamics of ant and pollinator activity in this system remain unknown. Here we assess the potential for ant–pollinator conflict in a Mexican ant-acacia, *Acacia hindsii*, and address the following specific questions:

1) To what extent do ant and pollinator activity on *A. hindsii* overlap in time and space? Is there potential for ant–pollinator conflict?
2) Does *A. hindsii* possess a floral ant-repellent? If so, how does repellence change with flower age and how persistent is the effect?
3) Do sympatric non-myrmecophilous acacias have

ant-repellent flowers? If so, how does the magnitude of the effect compare to that induced by *A. hindsii* on *P. veneficus* ants?

METHODS

The study site and study species

A. hindsii Benth. (*Acacia* subgenus *Acacia*, Fabaceae: Mimosoideae) is a tree 6–10 m tall native to the Pacific coast of México (Janzen 1974, McVaugh 1989). Data were gathered between 5 May and 6 June 2000 in coastal tropical dry deciduous forest near the Chamela Biological Station of the Universidad Nacional Autónoma de México, in Jalisco State, México (19°29.91′ N, 105°02.67′ W). *A. hindsii* flowers at the end of the dry season (between April and July), producing large numbers of yellow spicate inflorescences. At Chamela, *A. hindsii* accommodates colonies of a single ant-guard species, *Pseudomyrmex veneficus* Wheeler (Formicidae: Pseudomyrmecinae) within swollen thorns, and provides them with extrafloral nectar from nectaries on the rachis and petiole of the leaves, and protein-rich Beltian bodies. The latter are presented on the tips of the pinnules of new leaves, and once harvested are not replaced. Datasets for pollen release, pollinator visitation, ant activity, extrafloral nectar secretion, and the spatial distribution of resources used by ants were generated for the same two trees on multiple dates, with additional trees scored for pollen release and pollinator activity. Numbers of trees and sampling days for specific variables are given in the figure legends.

Inflorescences from two sympatric non-myrmecophilous *Acacia* species, *A. macracantha* (Humb. & Bonpl. Ex. Willd.) and *A. angustissima* (Mill.), were tested for ant-repellent properties and compared to those shown by *A. hindsii* (see Experiment 3 below): these *Acacia* species belong to the subgenera *Acacia* and *Aculeiferum*, respectively.

Floral rewards and pollinator activity

In contrast to the two non-myrmecophilous *Acacia* species, which produce relatively small volumes of floral nectar, *A. hindsii* flowers do not secrete nectar (Raine 2001). Therefore pollen is the only reward available to pollinators visiting *A. hindsii* flowers, and any potential for ant theft of floral nectar is removed. *Acacia* pollen is presented in the form of compound polyads (Knox and Kenrick 1982), and *A. hindsii* produces eight polyads per anther. Pollen release in *A. hindsii* was quantified by tracking changes in the relative abundance of pollen on the inflorescence surface through time, using methods described in detail by Stone et al. (1996, 1998). Sampled inflorescences were rolled lightly over the sticky side of a piece of clear adhesive tape, and the ratio of polyads to anthers scored (for six fields of view per inflorescence) under a light microscope at 70× magnification. The ratio rises as polyads are re-

leased during dehiscence, and falls as pollen is removed by flower visitors (Stone et al. 1996, 1998). Four inflorescences from each tree were scored at hourly intervals from 0600 to 1800 hours, different inflorescences were sampled at each time interval, and the mean for each tree and time interval was calculated.

Pollinator activity was quantified hourly from 0700 to 1700 hours by watching a consistent group of 12–15 inflorescences per tree for 30-min periods, repeated for three individual trees for two days of observation per tree, totaling six observation days. Voucher specimens of flower visitor taxa were identified with reference to extensive collections held at the Chamela Biological Station, and have subsequently been deposited in the Hope Entomological Collection, University of Oxford, UK.

Extrafloral nectar secretion by Acacia hindsii

Each leaf bears 2–22 extrafloral nectaries along the rachis and petiole. Nectar secretion rates were quantified by analyzing changes in nectar standing crop over time on leaves from which ants were excluded using banding grease. All nectaries on a leaf were emptied every 15 min with a 1-μL micropipette (Camlab, Cambridge, UK), and the nectar volume per leaf calculated from the length of the nectar column. A consistent set of 15–17 leaves was sampled for each of two trees for two days. The sucrose concentration in the sampled nectar varied between 40% and 70%. Here we present summary data on nectar secretion rates; variation among leaf age classes in the quantity and quality of nectar will be discussed in detail elsewhere. Very few flying insects were observed to harvest extrafloral nectar, and on days in which nectar secretion was quantified, care was taken to monitor the study leaves to prevent nectar theft by wasps and flies.

Ant activity patterns

General ant traffic was quantified using instantaneous counts of ants on five 50-cm branch sections in each of four trees (including those for which pollinator visitation was quantified). Counts were made every 90 min for two days, from 0700 to 1900 hours for each branch section, each of which contained occupied pseudogalls, leaves (both new and old), and flowers. Detailed data on spatial and temporal patterning of ant activity were collected for two trees. We recorded instantaneous hourly counts from 0730 to 1830 hours of (a) the numbers of ants visiting each of buds, young flowers, and old flowers on ten 10 cm long flower shoots, and the numbers of ants visiting nectaries on the associated old leaves; (b) the numbers of ants visiting nectaries on a constant set of young leaves (12 leaf groups, each containing four leaves, randomly distributed within the portion of the canopy accessible from the ground). We also made hourly counts from 0800 to 2000 hours of the number of ants transporting Beltian bodies across a standard marked area of stem

(5 × 10 cm) in a 15-min observation period. Beltian body transport along the stem is tightly coupled to harvesting on the leaves (N. E. Raine and G. N. Stone, *unpublished manuscript*), and provides a good estimate of harvesting activity.

Spatial distribution of ant-utilized resources within the canopy

We scored the distribution of three resources (flowers, swollen thorns, and young leaves bearing both extrafloral nectaries and Beltian bodies) throughout two large branches of a single *A. hindsii* tree. Data collection was extremely labor intensive, and only one tree was sampled; patterns appeared similar for the other trees studied. We reduced each branch to a series of unbranched sections (treated as replicates in subsequent analyses), and recorded the numbers of each resource type per unit length. The relationship between the densities of flowers and new leaves was assessed using a general linear model in which flower densities were $\log_e$ transformed to meet the assumptions of the analysis. All statistical procedures were conducted using the SAS system (SAS 1999).

Experimental analyses of ant repellence

The bioassay used.—Flower visits by *Pseudomyrmex veneficus* ants were rare under natural conditions, and the impact of treatment stimuli was thus assessed on major stems with reliably high levels of ant activity. Treatments (stimuli transferred from inflorescences or other plant material by lightly wiping them onto the bark) were applied within 3-cm squares marked onto the bark with water-based pens several days prior to experiments. Experimental squares were arranged in sets of five (blocks in all subsequent analyses), with squares at least 10 cm apart. Two blocks were marked on each of two main stems on the same *A. hindsii*. Analyses of data generated for each stem on a given day were nested to take account of potential stem effects. For each treatment replicate, we recorded the behavior of all *P. veneficus* workers contacting an edge of the square over a 10-min period. Ant responses could be clearly divided between those that (1) progressed uninterrupted into the square (''pass through''), or (2) halted at the treatment boundary and avoided the square either by retreating or bypassing the square (''rejections''). The proportion of ants rejecting a square was then calculated, arcsine square-root transformed (Sokal and Rohlf 1981) prior to analysis, and weighted by the number of ants arriving at the square.

Application of treatments.—Treatments tested for repellent qualities were floral buds, new inflorescences (opening on the day of the experiment), old inflorescences (opening the previous day), leaves, and control (no treatment). Plant material was harvested with forceps and stored in airtight containers at 0830 hours, coincident with maximum pollen dehiscence and assumed to be when any repellent effect of new inflo-

rescences might be strongest. Treatments were applied (according to the schedule required in each experiment) by wiping the stored plant material gently over all bark within the square, taking special care to avoid mechanical damage and any ant responses triggered by associated stimuli (Agrawal 1998). Hence we measured ant responses to those substances that can be transferred to the square by gentle physical contact with each type of plant material. Ant responses were scored 15 min after treatment application. In all experiments, treatments were applied to squares randomly with respect to sequence and position within a block, and each square was used only once per day. Experiments were conducted between 1100 and 1500 hours to ensure high levels of ant activity while reducing any degradation of plant material in storage to a minimum compatible with collection of data.

Experiment 1: Do A. hindsii *flowers repel ants?*— Two replicates of each of five treatments (floral bud, new inflorescence, old inflorescence, leaf, control) were conducted on each of two days. Treatment effects were analyzed using a nested analysis of variance (ANOVA) in a GLM of the form: proportion of ants rejecting a square = day + branch + block (nested within branch) + treatment, with treatment as a categorical variable ($n = 2 \times 2 \times 2 \times 5$). Treatment means were compared using Tukey's test for post hoc comparisons.

Experiment 2: How long do any ant responses to new inflorescences last after a single application?— Proportions of ants rejecting treatment squares were recorded for five treatments. Four treatments were time intervals (15, 30, 60, and 120 min) following application of new inflorescences, while the fifth treatment was a control (no stimulus applied). Two replicates of the five treatments were conducted on each of two days ($n = 2 \times 2 \times 2 \times 5$). Treatment effects were analyzed as in Experiment 1.

Experiment 3: (a) Do non-myrmecophilous Acacia *flowers repel ants, and (b) does repellence persist at constant stimulus strength?*—In this experiment the strength and persistence of *P. veneficus* ant responses to new inflorescences of *A. hindsii* were compared with responses to similar material from two sympatric non-myrmecophilous *Acacia* species, *A. macracantha* and *A. angustissima*. If ant-repellent flowers are restricted to ant-acacias, we expect non-myrmecophilous species to elicit ant responses similar to controls. If ant repellence is a general property of *Acacia* flowers, irrespective of ant association, we expect all acacias to induce ant responses of similar magnitude and persistence. If repellence is a general feature of *Acacia* flowers, but is elevated in ant-acacias, we expect the latter to induce higher rejection rates. The effectiveness of any floral ant-repellent chemical could also be related to the ant species tested. However, as neither *A. macracantha* nor *A. angustissima* have any known mutualistic relationships with ant species, it was impos-

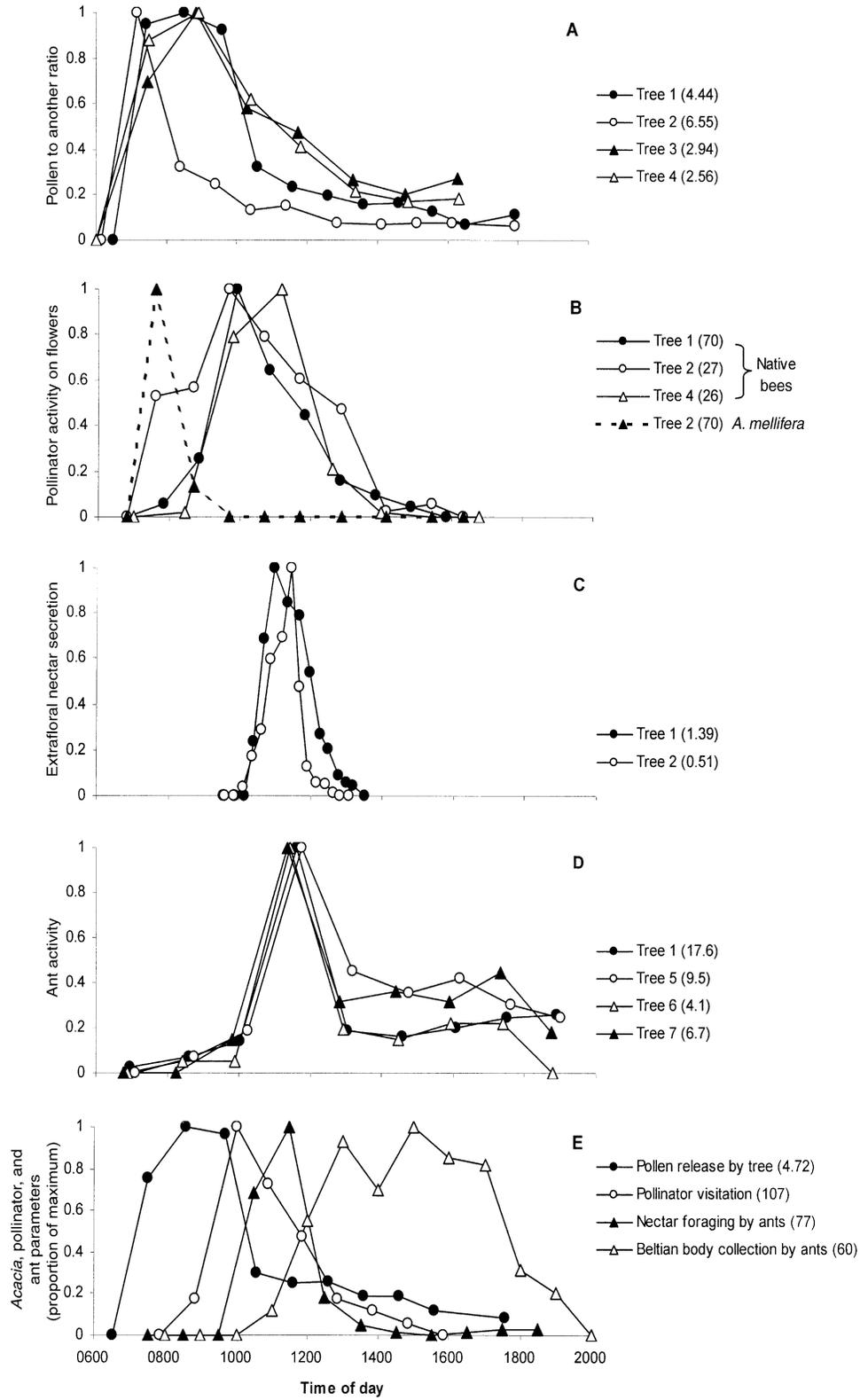sible to conduct the reciprocal comparisons of repellent effects on multiple ant-guard species.

Applying stimuli in an unfamiliar context (on the tree bark rather than on flowers) could potentially elicit a neophobic response (fear of unfamiliar stimuli) (Bernays 1995, Marples et al. 1998) in addition to any genuine repellent effect. Short-term analyses of ant responses may thus confound neophobia and genuine repellence. These two responses can be discriminated by examining the response of individual ants on repeated exposure. At constant stimulus strength a genuine antrepellent should induce a persistent response, while a purely neophobic response is expected to habituate over time.

Experimental treatments were application of new inflorescences from (1) *A. hindsii*, (2) *A. angustissima*, (3) *A. macracantha*, or (4) no inflorescences (control). Stimuli were reapplied to treatment squares on the hour for 5 h (1100–1500 hours), and rejection rates of squares calculated for 5 min of observation 15 min after each round of stimulus application. Frequency of reapplication was guided by the results of Experiment 2. Ant traffic rates were very high throughout the experimental period, and we assume that at least a proportion of individual ants encountered treatment squares repeatedly. The three *Acacia* species differ in the number of anthers per floret and florets per inflorescence (Raine 2001), and because anthers and their contents are a possible source of a repellent stimulus, we adjusted the number of inflorescences applied per square (2 for *A. hindsii*, 10 for *A. angustissima* and *A. macracantha*) to achieve an equivalent number of anthers. The experiment was replicated twice a day for four days, using the same individual tree on all days. Changes in ant responses to treatments over time were analyzed using profile analysis, a variant of MANOVA (Tabachnick and Fidell 1989). This allows us to test whether responses to treatments over time differ from zero slope and from one another. Differences in rejection rates between the first and last time point were also tested using a one-sample *t* test using data for all three acacias combined.

RESULTS

Daily patterning of pollen release and pollinator activity

A. hindsii flowers opened before dawn and dehisced synchronously between 0600 and 0800 hours (Fig. 1A). The principal flower visitors were introduced honey bees (*Apis mellifera*), and native bees including social stingless bees (*Scaptotrigona*, *Trigona*) and members of several solitary genera (*Hylaeus*, Colletidae; *Halictus*, Halictidae; *Ceratina* and *Exomalopsis*, Anthophoridae; *Megachile*, Megachilidae). Flower visitation by bees closely tracked the temporal patterns of pollen availability, with peak visitation occurring very shortly after peak pollen abundance (Fig. 1B). Although honey
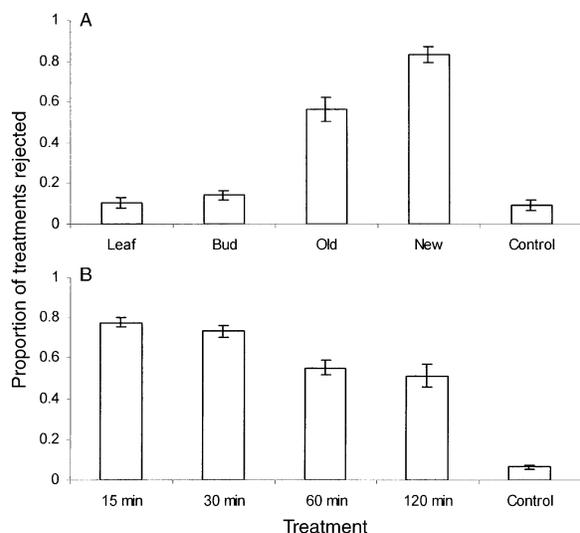
A

- Tree 1 (4.44)
- Tree 2 (6.55)
- Tree 3 (2.94)
- Tree 4 (2.56)

B

- Tree 1 (70)
- Tree 2 (27) } Native bees
- Tree 4 (26)
- Tree 2 (70) *A. mellifera*

C

- Tree 1 (1.39)
- Tree 2 (0.51)

D

- Tree 1 (17.6)
- Tree 5 (9.5)
- Tree 6 (4.1)
- Tree 7 (6.7)

E

- Pollen release by tree (4.72)
- Pollinator visitation (107)
- Nectar foraging by ants (77)
- Beltian body collection by ants (60)

FIG. 2. (A) Experiment 1: mean proportion of experimental treatment squares rejected (±1 SE) by *Pseudomyrmex veneficus* on *A. hindsii* (data for tree 1; 17 and 18 May 2000). Experimental treatments applied to squares were leaves, floral buds, newly opened inflorescences, old inflorescences, and control (no treatment). (B) Experiment 2: mean proportion of experimental treatment squares rejected (±1 SE) by *Pseudomyrmex veneficus* observed at time points (*t*) after a single treatment with a young *A. hindsii* inflorescence (data for tree 1; 21 and 24 May 2000).

bee activity declined to low levels by 0900 hours, native bees continued to visit flowers until 1300 hours. There was close correspondence between pollinator activity and the fall in pollen standing crop (compare Fig. 1A, B).

Temporal patterning of extrafloral nectar secretion and ant activity

Extrafloral nectar was secreted in a brief, discrete burst from 1000–1330 hours, peaking at 1100–1130 hours (Fig. 1C). During the study period, activity by *Pseudomyrmex veneficus* was almost entirely diurnal, and any ants observed outside pseudogalls before 0630 or after 2100 hours were seen on the young leaves. The diurnal pattern of ant activity outside pseudogalls showed a distinct peak around 1130 hours for all trees studied (Fig. 1D). Although honey bees had ceased foraging by the time ants became active outside pseu-

dogalls, daily activity patterns of ants and native pollinators overlapped substantially in the late morning (Fig. 1B, D). The majority of ants active outside pseudogalls during the period of intense pollinator activity were harvesting extrafloral nectar, with peak ant activity coinciding with peak nectar secretion (compare Fig. 1C, D). The coincidence between peak ant activity and nectar secretion was true for both young and old leaves (close to the flowers), although the number of ants active on the latter was considerably lower. Harvesting of Beltian bodies was restricted to the afternoon (Fig. 1E). Although ant activity was observed in regions of the canopy bearing flowers, ant visits to new flowers were extremely rare (only two were observed, both after 1430 hours), and we observed no encounters between ants and pollinators. We also observed no evidence to suggest that insectivorous birds, or other predators, might be deterring ants from visiting flowers.

The spatial distribution of resources within the canopy

Abundance of inflorescences and new leaves is significantly negatively correlated (General Linear Model [GLM]: $\log_e$ flowers $= -0.0135$(new leaves) $- 2.64$; $F_{313,1} = 39.80$, $P < 0.0001$). Almost all stem sections (307 of 315) bore either young leaves or flowers, but not both, such that these resources are effectively partitioned spatially within the *A. hindsii* canopy. The majority of occupied pseudogalls were also spatially associated with young leaves. Despite such spatial separation, young growth and inflorescences are often in close proximity, and ants were observed visiting nectaries on older leaves close to the *Acacia* flowers.

Experimental analyses of ant repellence

Experiment 1: Do A. hindsii *flowers repel ants?*— Ant responses to *A. hindsii* tissues differed significantly among treatments ($F_{4,31} = 76.37$, $P < 0.0001$; Fig. 2A), with a significantly higher proportion of ants rejecting squares treated with new ($P < 0.05$) than old inflorescences, buds, and leaves. Ant rejection rates of both flower treatments were significantly higher (4–10 times) than all other treatments, and bud, leaf, and control treatments did not differ significantly (Table 1A).

Experiment 2: How long do repellent effects last after a single treatment application?—Ant rejection of

FIG. 1. Resource provision, pollinator visitation, and ant activity through time for *A. hindsii* (see *Methods* for quantification techniques used). To enable comparison between different trees, and of temporal patterns in different types of data, all data sets have been constrained to vary between 0 and 1 by dividing by the maximum value in each data set. This scaling value is given in parentheses for each data set in the relevant key to plotting symbols. (A) Mean pollen availability through time for four *A. hindsii* individuals. Means are over three days for tree 1, and two days for trees 2–4. (B) Mean numbers of visits by honeybees (dashed line) and native bee taxa (solid lines) per 30-min observation period for three *A. hindsii* individuals (means are over two days for each tree). (C) Mean nectar secretion rates per leaf (microliters secreted per leaf in 15 min) for two *A. hindsii* individuals (means are over two days for each tree). (D) Mean instantaneous counts of ant activity over the same two days for four *A. hindsii* individuals. (E) Relative timing of pollen release, pollinator visitation, and ant collection of resources (nectar and Beltian bodies) for a single *A. hindsii* (tree 1) on 12 May 2000.

TABLE 1. Mean proportions of ant-repellent treatment squares rejected by ants in (A) Experiment 1 and (B) Experiment 2.

A) Experiment 1: Do *A. hindsii* flowers repel ants?

Treatment	Tukey's grouping	Mean	1 SE
New inflorescences	A	0.796	0.031
Old inflorescences	B	0.574	0.052
Bud	C	0.144	0.019
Leaf	C	0.073	0.027
Control	C	0.087	0.025

B) Experiment 2: How long do any ant responses to new inflorescences last after a single application?

Time point, t (min)	Tukey's grouping	Mean	1 SE	Initial levels of rejection (%)†
15	A	0.765	0.023	100
30	A	0.746	0.028	97.5
60	B	0.560	0.034	73.2
120	B	0.503	0.057	65.8
Control	C	0.048	0.012	6.3

Note: Means shown are untransformed values. Tukey's groupings are as identified for transformed data, and means with the same grouping letter are not significantly different at the 5% level.

† Ants rejecting the treated square at a given time point as a percentage of rejection levels at the first time point (15 min after stimulus application).

squares treated with new *A. hindsii* inflorescences decreased significantly with time following a single application ($F_{4,31} = 130.53$, $P < 0.0001$: Fig. 2B), with the first significant decrease occurring after 1 h (Table 1B). All inflorescence treatments induced significantly higher rejection rates than controls. Two hours after stimulus application, rejection rates had fallen to 66% of initial levels (Table 1B).

Experiment 3: (a) Do non-myrmecophilous Acacia *flowers repel ants, and (b) does repellence persist at constant stimulus strength?*—Over five rounds (5 h) of stimulus application, flowers of all three *Acacia* species tested showed evidence for a persistent repellent effect relative to controls (Fig. 3). The proportion of ants rejecting squares treated with *A. hindsii* was consistently higher than for either of the non-ant acacias (*A. hindsii* > *A. macracantha* > *A. angustissima* > control: $F_{1,11} > 12.56$, $P < 0.005$ for all differences). For all treatments the overall trend across rounds of stimulus application was a nonsignificant decrease in rejection rate ($T_{32} = 1.62$; $P = 0.115$). There were no significant differences in changes over time among treatments ($F_{12,58} = 1.64$, $P = 0.106$).

DISCUSSION

The extreme rarity of ant visits to flowers, even at times of high ant activity, suggests that the potential for conflict between ant-guards and pollinators in *A. hindsii* has been largely resolved. Here we review the significance of temporal and spatial patterns of resource provision and activity by ant-guards and pollinators,

and then consider the role of floral ant-repellents in *A. hindsii* and sympatric non-myrmecophilous acacias.

Temporal patterns in flowering and the activity of ants and pollinators

A. hindsii is typical of neotropical ant-acacias, including *Acacia cornigera*, in flowering toward the end of the dry season when ant colonies are at their annual minimum size (Janzen 1966). Janzen (1967*b*) has suggested that such seasonal flowering phenology could have been selected to minimize ant–pollinator conflict. However, the persistence of ants on healthy trees throughout the dry season means that any seasonal segregation between ants and pollinators would be at best partial. Moreover, lower rates of leaf (and hence Beltian body) production by the tree under dry season conditions may force ants to forage more actively for alternative sources of protein. In the absence of other segregation mechanisms, the rich supplies of animal prey and protein-rich pollen on flowers would remain vulnerable to raiding by ants. *Formica* ants have been observed to forage in such a way for animal prey on the inflorescences of *Acacia constricta*, which similarly
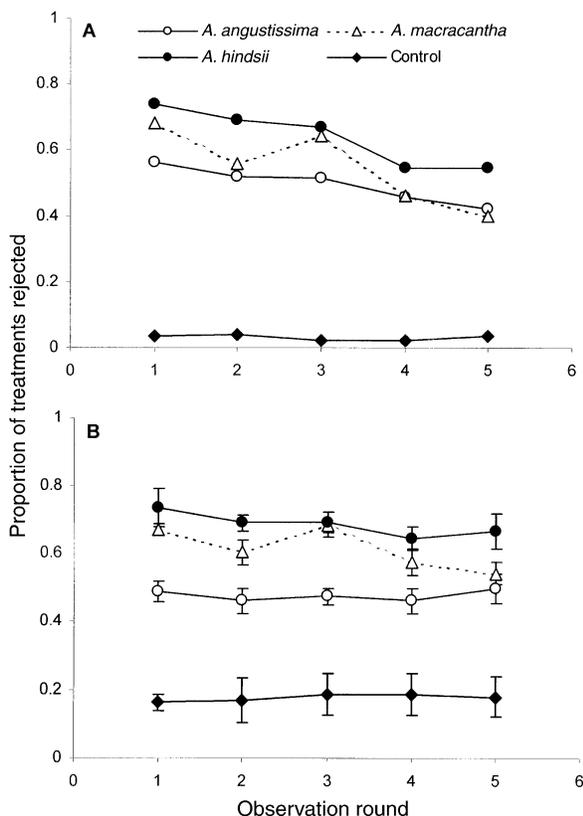

FIG. 3. Experiment 3: mean proportion of squares treated with new inflorescences of three *Acacia* species rejected at constant stimulus strength. No treatment was applied to control squares. (A) Data from day 1 of experiment, and (B) pooled data (means ± 1 SE) for all four days (tree 1; 8, 9, 11, and 13 June 2000).

produce no detectable nectar (Wagner 2000). We have also observed *Camponotus* ants to tend lycaenid larvae on inflorescences of *Acacia brevispica* in Kenya (G. N. Stone and P. Willmer, *unpublished data*).

On a daily timescale, both ants and pollinators track the provision of trophic rewards by *A. hindsii* extremely closely, consistent with general observations of bees collecting pollen (e.g., Buchmann and Cane 1989, Stone et al. 1999) and ant recruitment to food sources (Hölldobler 1978, Traniello 1983, Jaffe et al. 1985). Ant-acacias could therefore potentially manipulate ant and pollinator activity patterns to avoid overlap by regulation of daily patterns of reward presentation. Such a mechanism appears to have been important in the evolution of timing of pollen release in communities of acacias that share pollinators (Stone et al. 1996, 1998). However, daily activity patterns of ants and pollinators on *A. hindsii* overlap extensively and there is no evidence in this system of their segregation in daily time. Indeed, simply segregating the daily activity patterns of ants and pollinators on flowers would not preclude ants from potentially disturbing or preventing pollination, for example by robbing floral resources or destroying flowers (Yu and Pierce 1998).

Overlap of ant and pollinator activity patterns may persist because there is no selective pressure to segregate them (possibly because other mechanisms prevent ant–pollinator conflict), and/or because physiological constraints prevent pollen and extrafloral nectar from being presented at different times. Anther dehiscence occurs over a range of conditions and times of day in African acacias (Stone et al. 1996, 1998) and there is no obvious reason why this should be more constrained in *A. hindsii*. Secretion of extrafloral nectar by ant-plants, including acacias, occurs over a wide range of ambient conditions and times of day (Janzen 1967*b*, Bentley 1977*a, b*; N. E. Raine and G. N. Stone, *unpublished manuscript*), and it seems unlikely that nectar release and pollen dehiscence occur at the same time solely because of physiological constraints.

Spatial patterns in activity by ants and pollinators

Simultaneous activity of ant-guards and pollinators is unlikely to result in conflict if they are separated in space. Trophic resources harvested by ants and pollinators are indeed spatially segregated within the *A. hindsii* canopy. Flowers are produced in canopy regions at least one growing (wet) season old, where leaves are morphologically very different from those in new growth. Though these old leaves do possess nectaries, they have fewer of them than young leaves on growing shoots, and do not offer Beltian bodies (N. E. Raine and G. N. Stone, *unpublished manuscript*). Young leaves and newer ant-occupied pseudogalls are concentrated near to the growing shoots of the tree and are the focus for ant activity (Janzen 1967*b*). However, because ants do visit nectaries on older leaves (albeit at lower rates than on younger stem sections; N. E.

Raine and G. N. Stone, *unpublished manuscript*), the scale over which segregation between flowers and other trophic rewards occurs (usually <1 m) probably does not prevent ants from visiting flowers. In fact, spatial overlap between ants and flowers could potentially be important in limiting the damage inflicted by seed predators on developing fruit (Ernst et al. 1989, Traveset 1991, Willmer and Stone 1997). Support for this hypothesis is provided by the observation that in *A. zanzibarica*, where there is no spatial segregation between ants and flowers, levels of ant activity and seed set on a given stem are positively correlated (Willmer and Stone 1997). However, any potential benefits of ant activity in parts of the *A. hindsii* canopy that bear flowers, including effects on seed set, have yet to be investigated.

The distribution of resources within the canopy may not be entirely determined by the host plant. Removal of vegetative and reproductive shoots (castration) of their host plant can be beneficial for the resident ant-guards (Young et al. 1997, Yu and Pierce 1998, Stanton et al. 1999). Thus *P. veneficus* ants could potentially reinforce any degree of segregation which exists between the flower-bearing stems (old growth) and the new growth (producing the majority of trophic rewards) by removing floral buds from new growth, thus promoting the production of trophic rewards. If floral buds were vulnerable to this sort of ant pruning we might expect the plant to protect them, perhaps using a chemical ant-repellent. While our data show no evidence that buds elicited stronger ant avoidance responses than leaves or controls (no treatment), it is possible that buds might be protected from ants by the repellent effect of new inflorescences as a consequence of the racemose presentation of inflorescences in neotropical ant-acacias (see *Are ant-repellents a general feature . . .*).

Floral ant repellence in A. hindsii

Our data show that *A. hindsii* produces a transferable ant-repellent stimulus, concentrated in young flowers (Experiment 1), that has a significant effect on ant behavior for at least 2 h after application (Experiment 2). When stimulus strength is maintained at a constant level there is no sign of significant habituation of the ant response over a period of 5 h (Experiment 3). There are two potential explanations for these responses, which are not mutually exclusive. First, ants may avoid treated squares because *A. hindsii* flowers carry a repellent capable of inducing a persistent aversion response, resistant to habituation at applied stimulus levels. Second, ants may be rejecting squares treated with young flowers because workers are encountering an unfamiliar stimulus to which they have no "hard-wired" aversion, but are showing a neophobic response (Bernays 1995, Marples et al. 1998). While neophobic responses are expected to attenuate through habituation over time at constant stimulus levels, a true repellent should invoke a persistent response. Our data show no

evidence of significant habituation over time intervals of up to 5 h. Although very gradual habituation to long-lasting neophobia cannot be excluded as an explanation, our results are consistent with the presence of a genuine ant-repellent in *A. hindsii* flowers, as demonstrated for *A. zanzibarica* in Tanzania (Willmer and Stone 1997), and for a range of plant species and ant taxa (including the ant-acacia *A. collinsii* and its ant-guards *Pseudomyrmex spinicola* and *P. flavicornis*) by Ghazoul (2001).

Although the source of the repellent effect remains unknown, we have demonstrated that young (pollen-rich) flowers are more repellent than old flowers, and hence consider it likely that repellence is associated with floral structures presented at dehiscence. We suggest that incorporation of a repellent in the pollen itself might represent an evolutionary strategy to ensure maximum floral repellence when pollinators visit new flowers. As pollinators remove pollen the flower would become less repellent, allowing ants to resume their protection against flower-eating insects and seed parasites once fertilization has taken place. Because pollinators are also commonly attracted by stimuli associated with dehiscence, such as release of floral scents (Dobson 1987), such a mechanism would synchronize ant and pollinator responses where the timing of dehiscence varies with microclimate (Stone et al. 1998). While such functional explanations are clearly attractive, further work is needed to reveal the basis of ant avoidance of young flowers.

Alternative hypotheses for ant floral avoidance behavior

Despite high levels of diurnal ant activity throughout the *A. hindsii* canopy, flower visits by ants are rare. This suggests that ants avoid flowers, an observation which is compatible with explanations that need not invoke the action of floral ant-repellents. Such floral avoidance behavior could potentially represent either (1) a lack of motivation to visit flowers, or (2) an increased risk of predation associated with flower visits. Ants could potentially be uninterested in visiting flowers, because they do not represent a useful resource. Therefore ant responses where they encounter the chemicals associated with flowers on the stem could represent a reaction to avoid a nonrewarding resource. *A. hindsii* produces trophic rewards (sugar-rich extrafloral nectar and Beltian bodies) specifically for its ant-guards, which we would expect to be both more easily obtained and more attractive to ants than flowers as sources of nutrients. However, during the flowering period, at the end of the dry season, the levels of trophic reward produced by the host tree are likely to be at an annual minimum, and the abundant protein in pollen-rich flowers is a potential alternative source of nutrients. The hypothesis that floral avoidance by ants represents a dismissal of a nonrewarding resource could be falsified by demonstrating that old (or immature)

flowers are visited more often (and/or for longer periods) than young ones as predicted by the repellence hypothesis. Although these data have not yet been collected for *A. hindsii*, this is exactly the pattern of ant behavior seen in the African species *A. zanzibarica*; *Crematogaster* ants make both more and longer visits to old flowers and unopened buds than they do to newly opened inflorescences (Willmer and Stone 1997).

Alternatively, ant floral avoidance behavior could potentially be driven by selection to avoid predation. Dark-colored ants visiting contrasting yellow flowers could be particularly conspicuous to potential predators, such as insectivorous birds, and might be more vulnerable to attack than flying flower visitors. However it seems unlikely (given the abundance of foraging *P. veneficus* on *A. hindsii*) that a flying predator capable of locating ants on flowers could not do so elsewhere in the canopy, and we saw no evidence of significant ant predation in any part of the canopy (including flowers).

Are ant-repellents a general feature of Acacia *flowers?*

Our results closely parallel those for an African ant-acacia, *A. zanzibarica* (Willmer and Stone 1997). African and neotropical members of the *Acacia* subgenus *Acacia* represent independent evolutionary lineages (Miller and Bayer 2001), and the distribution of ant-acacias within these lineages suggests that ant associations have evolved independently in the two regions. All ant-acacias face the same potential conflict between ant-guards and the preservation of both pollen and pollinators, and it is not surprising to find evidence of floral ant repellence in both systems. The ants involved in the two mutualisms are members of distantly related genera (*Pseudomyrmex* for *A. hindsii*, *Crematogaster* for *A. zanzibarica*), and it remains to be seen whether ant behaviors in both systems are in response to similar stimuli. An interesting difference between the two systems lies in the spatial arrangement of inflorescences. *A. hindsii* inflorescences develop in groups arising from a single leafless stem (a raceme), and any ant-repellent stimulus associated with individual inflorescences could thus be potentially shared by other members of the raceme. The first inflorescences to flower are at the base of the raceme, which would allow ant-guards to resume their protection of developing seeds without needing to pass other (potentially repellent) inflorescences still in flower. In addition this potentially offers some protection against ant damage to floral buds, distal on the raceme from the open inflorescences. In contrast to racemose neotropical species, the inflorescences of African ant-acacias arise singly from cushions of buds at the base of stipular spines, and have less apparent potential for shared manipulation of ant behavior.

An alternative to independent evolution of ant-repellents in American and African ant-acacias is that

such floral behavior represents an ancestral state for *Acacia* flowers. We found flowers of two non-ant acacias, *A. macracantha* and *A. angustissima*, to have significant ant-repellent properties, though both were less effective at deterring *Pseudomyrmex* ants than flowers of *A. hindsii*. We believe the most parsimonious explanation for these results is that some degree of floral ant repellency is basal within the genus *Acacia*, and has perhaps been selectively augmented in the two lineages that have evolved mutualistic associations with protective ant-guards. This would parallel the independent expansion of stipular thorns to house ants in both lineages (Hocking 1970).

Ants can reduce plant reproductive output through pollen theft and secretion of substances that reduce pollen viability (e.g., Beattie et al. 1985, Wagner 2000), and are also potential robbers of floral nectar (Janzen 1977, Haber et al. 1981, Ghazoul 2001). Many plants exclude ants from flowers prior to pollination, often through morphological traits (Harley 1991, Federle et al. 1997) and also through the secretion of ant-repellent nectar (Feinsinger and Swarm 1978). *Acacia* inflorescences present pollen over a surface of exposed anthers with no complex floral morphology to exclude ants, suggesting that other protective mechanisms (such as floral repellents) may be required to protect pollen. Although the full taxonomic distribution of floral repellents is currently unknown even within a single genus (*Acacia*), we speculate that other plant lineages with similarly exposed inflorescences may also use floral repellents to protect their pollen from plunder by ants.

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