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Novel interactions of non-pollinating ants with pollinators and fruit consumers in a tropical forest

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Abstract The tropical ants *Ectatomma ruidum* and *E. tuberculatum* (Formicidae) regularly patrol leaves, flowers, and fruits of the understory shrub, *Psychotria limonensis* (Rubiaceae), on Barro Colorado Island, Panama. Ant and pollinator exclusion experiments elucidated both positive and negative effects of ant attendance on plant reproductive success, including pollination, fruit set, fruit loss, and fruit removal. Ants did not pollinate flowers but did contribute to higher pollination success, probably by increasing the relocation frequency of winged pollinators and thus the rate of flower visitation. Ants also prevented fruit loss to herbivorous insects which were common during the early stages of fruit development. Thus, ant attendance strongly improved both pollination and fruit set whereby plants with ants set more fruit per flower and also lost fewer fruits during fruit maturation. In contrast, ants had a negative effect on the removal of ripe fruits by avian frugivores. Thus, ant attendance has a non-trivial influence on plant reproduction, this interaction being beneficial at some stages of the plant reproductive cycle and carrying costs at another stage. A tight ecological or co-evolved relationship between these *Ectatomma* spp. and *P. limonensis* is unlikely given that ant attendance of plants is detrimental to fruit removal.

Key words Ant attendance · *Ectatomma* · Plant reproductive success · *Psychotria limonensis*

Introduction

Ants which live in and around plants have the potential to increase or decrease plant fitness depending upon their interactions with herbivores, pollinators, and seed dispersers (Levey and Byrne 1993; Willmer and Stone 1997). Site fidelity is common in ants, providing temporal consistency and the opportunity for ants to influence plant reproductive success during multiple stages of the reproductive cycle (Horvitz and Schemske 1984). Furthermore, ants can be beneficial at some stages and detrimental at others, leading to a complex network of interactions among ants, herbivores, and pollinators during flowering and fruit development (Schemske and Horvitz 1988). Less understood is the effect of pugnacious ants on vertebrate frugivores (Davidson 1988). A complete analysis of ant effects on plant reproductive success requires an examination of the cost/benefit balance during all stages of the plant reproductive cycle including flowering, fruit development, and fruit removal.

Many plants provide extrafloral nectaries to attract pugnacious ants that protect leaves, flowers, and developing fruits from insect herbivory and parasitism (Janzen 1966; Bentley 1977). For example, the aspen sunflower, *Helianthella quinquenervis*, uses extrafloral nectar to attract ants that protect seeds from larval infestation by preventing oviposition by several species of insects (Inouye and Taylor 1979). Another interesting case of the benefit of ant attendants comes from the tropical herb *Calathea ovandensis* which appears to increase flower production when its extrafloral nectaries are stimulated by ant activity (Horvitz and Schemske 1988). However, ants will also forage on plants for floral nectar in the absence of extrafloral nectaries (Ramsey 1995).

Effective flower pollination by ants foraging for flower nectar has been documented in a limited number of cases. These ant-pollinated plants exhibit a suite of characters such that (1) plants occur particularly in dry/open Mediterranean habitats, (2) ants outnumber win-

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ged pollinators during the flowering season, and (3) floral nectaries are accessible to flightless ants (Hickman 1974; Peakall and Beattie 1991; Gómez and Zamora 1992; Ramsey 1995; Gómez et al. 1996). In most cases, however, ants do not transfer viable pollen due to either lack of movement among self-incompatible plants (Hölldobler and Wilson 1990) or pollen destruction from metapleural gland secretions (Beattie et al. 1985).

Many ants are pugnacious and predatory towards other insects and even much larger vertebrates. These aggressive behaviors are likely to deter other insects and vertebrates from plant reproductive structures. Deterrence by ants would be beneficial when preventing herbivores from consuming developing fruits, but may be detrimental when potential pollinators and seed dispersers avoid flowers and fruits due to ant attendance. Schemske and Horvitz (1988) used path analysis to test for direct and indirect effects of ants and herbivores on flowers, initiated fruits, and ripe-fruit number. The influence of ants on mature-fruit number was slight, but positive. Studies of ant-epiphyte gardens suggest that ants may also deter vertebrate frugivores as demonstrated by many rotting fruits on trees (Davidson 1988).

An additional stage at which ants can influence plant fitness is through the dispersal of seeds. The most specialized seeds for ant dispersal contain lipid-rich accessory structures known as elaiosomes (Beattie 1985). To ants, these nutritious structures are functionally analogous to the fleshy fruits that attract vertebrate dispersers. Furthermore, ants can disperse seeds by either or both of two modes: (1) primary dispersal in which seeds are removed directly from plants and (2) secondary dispersal in which seeds are removed from dung piles of other primary dispersers such as birds and mammals (Beattie 1985; Davidson 1988; Levey and Byrne 1993).

The primary focus of this study was to determine the effects of the foraging behavior of predatory ants on the reproductive success of a tropical shrub over its entire reproductive cycle, namely pollination, fruit development, and fruit removal. Data were collected for immature fruit set from flower number, fruit damage, and fruit removal and compared to ant distribution and behavior.

Materials and methods

This study was carried out at the Smithsonian Tropical Research Institute's field station on Barro Colorado Island (BCI), Panama

Table 1 Ant presence and behaviors on *Psychotria limonensis*. Values are means (%), with standard deviations in parentheses) of the incidence of *Ectatomma* and their foraging behaviors on all plants to which ants had access. The presence of *Ectatomma* on

	<i>Ectatomma</i> present (1995)	<i>Ectatomma</i> present (1996)	Predatory posturing	Aphid tending	Nectar consumption	Fruit juice consumption
Flowers	64.3 (49.7)	68.20 (30.90)	30.31 (25.20)	0.69 (3.71)	27.68 (29.03)	–
Immature fruits	25.6 (44.2)	12.33 (19.05)	12.33 (19.05)	2.85 (7.50)	–	2.75 (5.89)
Mature fruits	25.0 (44.7)	16.26 (21.65)	18.05 (17.17)	6.64 (12.39)	–	0.65 (4.81)

(9°09'N, 79°51'W) which receives an average of 2600 mm of rain per year. *Psychotria limonensis* is a common shrub in the understory of BCI and displays flowers and fruits throughout most of the year, but with a peak in the early wet season (Croat 1978; S.J. Wright, unpublished data). The inflorescences are terminal, and there can be several per individual and up to 98 fresh florets per inflorescence at one time. *P. limonensis* flowers are visited by a diverse assemblage of potential pollinators including ants, winged hymenopterans, coleopterans, lepidopterans, and hummingbirds. It is not known if this species is self-compatible, but there are both self-compatible and self-incompatible plants in the genus (Bawa and Beach 1983). Plants have from one to three fruit clumps per individual, and a clump can have 1 to more than 200 berries. Common avian frugivores in the understory of BCI include tanagers, manakins, neotropical migrants, and many omnivorous birds that include fruits in their diets (Poulin and Lefebvre 1996). Emetic data reveal that *P. limonensis* seeds are ingested by manakins and migratory thrushes (B. Poulin, G. Lefebvre, and S.J. Wright, unpublished data).

The tropical ants *Ectatomma ruidum* and *E. tuberculatum* are frequently found foraging on *P. limonensis* during flowering and fruiting. Four foraging behaviors by *Ectatomma* were commonly observed during flowering and fruiting in *P. limonensis*: (1) nectar consumption, (2) water consumption off fruits, (3) aphid tending, and (4) depredating insects. Furthermore, *E. ruidum* typically forages within 1.5–2 m of its nest entrances (Lachaud 1990) which are often located near the base of *P. limonensis* plants on BCI (personal observation). However, this plant species does not provide the extrafloral nectaries or space for brood rearing that typically attract ant attendants (Janzen 1966; Jaffe et al. 1989), and during the pilot survey to this study, *Ectatomma* spp. were observed on 64% and 25% of flowering and fruiting plants, respectively (Table 1). Thus, the association among these species is common but not ubiquitous, and is relatively long in duration.

E. ruidum frequently forages for nectar and other liquid forms of food (e.g., fruit juice and rainwater), which it brings back to the nest (Pratt 1989). It also has an excellent ability to learn the location of nectar resources (Schatz et al. 1994, 1995), and demonstrates spatial fidelity to food resources (Passera et al. 1994). As with many ponerine ants, *E. ruidum* and *E. tuberculatum* are predators of other insects (Wheeler 1986) and thus may deter pollination and/or fruit damage by insects (this study).

Initial data on the abundance of *Ectatomma* ants on the flowers and fruits of *P. limonensis* were gathered in July 1995. Eight transects (0.5 km long) were censused along trails, and all plants within 2 m of either side of the trail were included. Flower number, fruit number, and ant abundance were noted over 3–5 min. Observations and experiments ran in the following year from April to August 1996.

Pollination and fruit set

Exclusion experiments were used to assess the effects of ants and winged flower visitors on *P. limonensis* pollination. Sixty-one marked plants were randomly assigned to one of the following treatments: (1) “ants only” excluded winged visitors but allowed ants access to flowers; (2) “winged only” excluded ants but allowed flying animals to access the inflorescence; (3) “no visitors” excluded both ants and winged flower visitors; (4) “all visitors”, in which both

plants is expressed as the percentage of plants containing the ants on a trail survey in 1995 ($n = 57$) and on the experimental plants in 1996 for which ant access was not restricted ($n = 54$)

ants and winged flower visitors had access. Initially, the two treatments that included winged pollinators ("winged only" and "all visitors") each had 17 plants and the two treatments that excluded winged visitors ("ants only" and "no visitors") each contained 15 plants; however, some plants were trampled by coatis (*Nasua narica*) or damaged from branch falls during the experiment.

To exclude ants from inflorescences, I taped a 5- to 7-cm piece of paper lengthwise around the stem below the inflorescence and then copiously covered the paper with Tanglefoot. I removed the leaves, vines, and stems of neighboring plants that were in contact with the experimental individual to prevent arboreal passage of ants. Covering inflorescences with a cloth mesh (bridal veil material, 1-mm mesh size) excluded winged visitors from flower stigmas. To allow ant access to the "ants only" treatment, I tied the mesh bag around a cardboard tube (diameter 4 cm), located >6 cm below the inflorescence, which provided ample space around the stem for traveling ants. Occasionally, winged insects managed to get to an inflorescence either by cutting a hole in the exterior mesh or by flying up through the ant passage. Similarly, some ants were able to cross to the ant exclusion treatments ("winged only" and "no visitors") when neighboring plants shifted during windstorms. When ants were found on the ant exclusion treatments, their presence was noted, the ants were removed, and the plant was modified by removing leaves or branches to prevent further invasion.

Every 6 days for 108 days (30 April–15 August 1996), I observed each of the 61 plants for 2–4 min and recorded the number of flowers, ants, winged flower visitors (classified to order), and the number of developing fruits on each plant. Following the definition of Gómez and Zamora (1992), I defined flower visitors as "any insect that could make contact with the anthers and/or stigma." Ant behaviors were also recorded.

I continued to monitor and maintain flowering plant treatments after the last flowers dropped for up to 75 days of subsequent fruit development. As the inflorescence period ended, I recorded the number of fruits that developed and noted any continuing presence of *Ectatomma* species on *P. limonensis*. Fruit production was assessed as fruit set, defined as the ratio of the number of fruits per plant relative to the number of flowers per plant. Fruit set was analyzed in two ways: (1) maximum fruit set, defined as the ratio of the greatest number of immature fruits recorded on any one census relative to the maximum number of flowers recorded on a particular census; (2) final fruit set, defined as the number of immature fruits remaining at the end of the study relative to the maximum number of flowers recorded on a particular census. Comparison of these measures accounted for loss of fruits.

Seed dispersal

Fruit maturation can last for several months in *P. limonensis*, and there is a high degree of flowering and fruiting asynchrony. Ant effects on fruit removal were studied through experiments conducted concurrently with pollination experiments and involving a different set of individual plants. The effect of ants on fruit removal was examined through manipulation of ant presence with two treatments: (1) ant exclusion ($n = 24$) and (2) ant inclusion ($n = 25$). Ants were excluded from access to fruits by applying a patch of Tanglefoot on the stem as described above.

Mature fruits of *P. limonensis* are red with high reflectance spectra (>10%) above 600 nm and immature fruits are green and inconspicuous (<10% reflectance) across all wavelengths visible to birds (D.L. Altshuler, unpublished data). All crops were randomly thinned to 40 fruits of which at least half were mature at the onset of monitoring. All other mature crops, on either the same plant or its neighbors, located within 2 m of the focal fruit clump, were removed. As other *P. limonensis* fruits in the 2-m vicinity matured, these were also removed. Green, immature fruits of *P. limonensis* were not removed because these are not likely to function as a cue to fruit availability.

Every 3 days I censused plants for 2–4 min and recorded the number of fruits removed, partially eaten, desiccated, or fallen, as well as the number of ants present on each fruit stalk and nearby

flower or immature fruit stalks. I cleared the large leaves underneath fruit clumps to observe fallen fruits.

Statistical analysis

Experimental analyses were conducted with SYSTAT (Wilkinson 1990) using the general linear models. If needed, response variables were transformed (as the square root of the variable + 1) to meet the assumptions of normality for parametric tests. The responses of fruit set and pollinator abundances were tested using a two-way analysis of variance for the effects of ant exclusion and winged-pollinator exclusion. The effect of ants on fruit loss was compared using a one-way analysis of variance between the two treatments that included winged pollinators. Fruit removal, damage, and desiccation were compared by a two-way analysis of variance for the effects of ant exclusion and treatment duration. All post hoc analyses were Fisher's least-significance-difference tests for effects of ant/pollinator exclusion treatments. Differences in observed ant behaviors between the ant-inclusion pollinator treatments were compared during flowering and fruit development using Kruskal-Wallis non-parametric tests.

Results

Pollination and fruit set

Ants engaged in one of three behaviors on flowering plants: (1) predatory behavior, which was characterized by open mandibles and a completely still posture, (2) tending aphids, and (3) consuming nectar from flowers (Table 1). However, the two ant inclusion treatments, "ants only" and "all visitors," did not differ in the frequencies of the three ant behaviors (all $P > 0.30$). Ant behaviors during fruit development were similar except that ants foraged for water droplets on fruits instead of nectar from flowers. These behaviors also did not differ between treatments (all $P > 0.30$).

Flowers of *P. limonensis* were visited predominantly by insects in the orders Coleoptera, Hymenoptera, and Lepidoptera, although I also observed a small number of Homoptera ($n = 2$) and Orthoptera ($n = 1$). Ant effects on pollinator distribution were tested by pooling all flower visitors for the effects of ant presence and pollinator presence (treatments) on the number of pollinators observed on each plant during the flowering period (Table 2). Pairwise comparisons revealed that the "winged only" treatment had significantly more flower visitors than each of the other treatments (all $P < 0.01$) and that none of the other treatments differed statisti-

Table 2 Effects of ants and pollinators on the number of winged flower visitors to *P. limonensis*. ANOVA performed on the square root of fruit set + 1

Source	df	MS	F	P
Main effects				
Ant exclusion	1	2.840	5.839	0.019
Pollinator exclusion	1	6.146	12.635	0.001
Interaction effect				
Ant exclusion × pollinator exclusion	1	1.336	2.746	0.103
Error	57	0.486		

cally (Table 3). Thus, the “all visitors” treatment had a statistically similar number of observed winged flower visitors as the two treatments that cut off access to those volant insects.

The maximum number of flowers observed among the four treatment groups varied significantly (ANOVA: $F_{3,57} = 3.438$, $P < 0.05$) but only among the following treatments: the “no visitors” treatment had a greater number of flowers on average than did the “all visitors” and “winged only” treatments (post hoc test, $P < 0.05$). However, the “no visitors” treatment barely set fruit because access to these flowers was prohibited by Tanglefoot and bridal veil. Therefore, differences in fruit set did not result from differences in the number of flowers on plants in different treatments.

All plants lacking a Tanglefoot barrier were visited by both *E. ruidum* and/or *E. tuberculatum* individuals during the study, and all plants lacking mesh barriers were visited by winged pollinators and set fruit. Plants that excluded access to winged flower visitors (“ants only” and “no visitors”) produced few fruits (Fig. 1). The maximum production of fruits relative to the maximum number of flowers recorded (maximum fruit set) was affected by both ant and pollinator treatments (Table 4). Post hoc analysis revealed significant mean differences between all the pairwise treatment comparisons except for the two treatments that excluded winged flower visitors (Fig. 1). The differences among treatments were also significant for final fruit set (Table 4), with identical significance relationships but different probabilities (Fig. 1). In no cases were there significant differences between the “ants only” and “no visitors” treatments. However, the “all visitors” treatment had significantly higher fruit set in both analyses when compared to the “winged only” treatment. Fruit set ratios were typically higher than 100% for plants with a high degree of pollination because the maximum number of flowers recorded at any one census was less than the total number of flowers available for pollination.

Fruit loss

The number of unripe fruits present at the end of the study (final fruit set) was lower than the largest number of fruits

(maximum fruit set) recorded on plants (Fig. 1). The decrease in immature fruit number over the course of the study resulted from herbivory of unripe fruits. However, only plants in the “winged only” and “all visitors” treatments had winged pollinators and set appreciable numbers of fruits. These treatments differed in either excluding or including, respectively, ant attendants. The “all visitors” treatment that included ants lost a lower percentage of fruits [mean = $13.5 \pm 6.0\%$ (SE)] than the “winged only” treatment that excluded ant attendants [mean = $30.9 \pm 5.8\%$ (SE)]. Thus, significantly more developing fruits were lost to herbivores when ants were absent (ANOVA: $F_{1,29} = 4.239$, $P < 0.05$).

Fruit removal

Three mutually exclusive outcomes were possible for a given fruit: (1) fruit damage resulting from partial consumption or penetration of the fruit coat by ants to gain access to fruit juices; (2) fruit desiccation and/or dropping to the ground resulting from lack of removal and

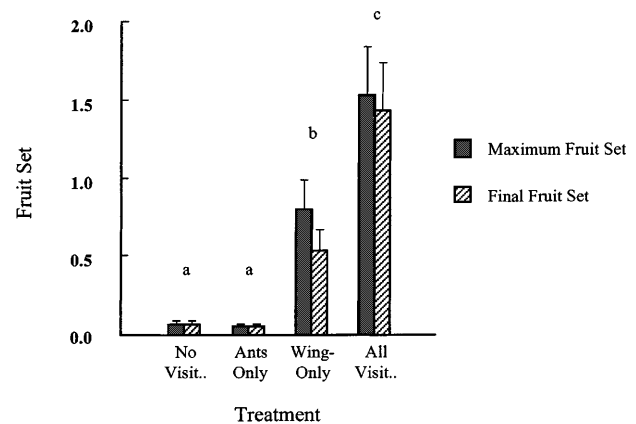


Fig. 1 The effects of ant and pollinator inclusion/exclusion on the maximum and final immature fruit sets. Columns represent means and bars are standard errors. Immature fruit set is the ratio of the number of immature fruits to the maximum number of flowers recorded at any given census. Lowercase letters indicate statistically significant differences ($P < 0.05$) among treatments according to Fisher’s least-significant-difference test with the following relationship: $a < b < c$. Treatments with the same letter are statistically indistinguishable

Table 3 Number of flowers (maximum recorded at any one census), pollinators, and fruits in the four pollination treatments. Maximum flower number differed among treatments (tested by Fisher’s test of least significant difference) according to the significance relationships: $a < c$, but $a = b$ and $b = c$. Winged flower visitor number is the mean number of winged flower visitors observed on plants during the flowering period (this differed among

treatments, tested by Fisher’s test of least significant difference, according to the significance relationship: $1 < 2$). Maximum fruit number is the largest crop of immature fruits observed on any one census. Final fruit number is the crop of immature fruits observed in the final census of the study. Values are means with the SE in parentheses

Treatment	<i>n</i>	Maximum flower number	Winged flower visitor number	Maximum fruit number	Final fruit number
No visitors	15	43.8 (5.24) ^c	0.933 (0.893) ¹	2.333 (5.614)	2.267 (5.485)
Winged only	17	26.7 (4.92) ^a	5.118 (0.839) ²	19.353 (5.273)	13.882 (5.152)
Ants only	14	31.0 (5.42) ^b	0.429 (0.924) ¹	1.571 (5.811)	1.357 (5.678)
All visitors	15	21.1 (5.24) ^a	1.467 (0.893) ¹	29.467 (5.614)	27.933 (5.485)

Table 4 Effects of ants and pollinators on the maximum and final fruit set of *P. limonensis*. ANOVA performed on the square root of fruit set + 1

Source	Maximum fruit set				Final fruit set		
	<i>df</i>	MS	<i>F</i>	<i>P</i>	MS	<i>F</i>	<i>P</i>
Main effects							
Ant exclusion	1	0.200	3.763	0.057	0.316	6.669	0.012
Pollinator exclusion	1	2.542	47.74	0.001	1.796	37.860	0.001
Interaction effect							
Ant exclusion × pollinator exclusion	1	0.214	4.025	0.050	0.342	7.203	0.010
Error	57	0.053			0.047		

aging, and (3) fruit removal by avian frugivores (Fig. 2). *E. ruidum* and *E. tuberculatum* had a significant effect on fruit removal, whereby plants with ant attendants had significantly lower removal than did plants without ants (Table 5). Ants did not affect fruit damage or fruit desiccation (Table 5).

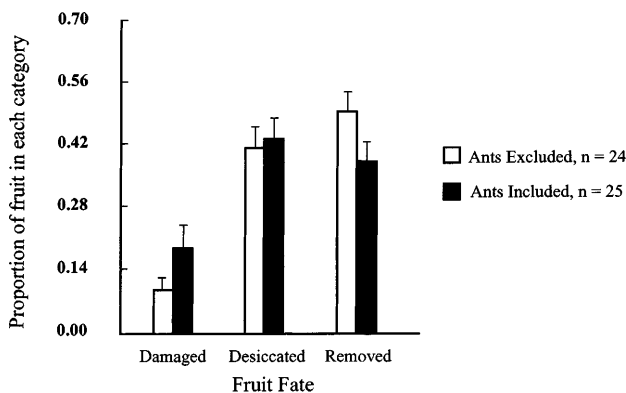
Discussion

The ants *E. ruidum* and *E. tuberculatum* influenced the reproductive success of *P. limonensis* but had opposing effects during different phases of the reproductive cycle. The magnitude of the ant effect also differed among plant phenological stages. This interaction is not a typical mutualism and traditional resources associated with ant-plants such as extrafloral nectaries, seed accessory

structures, and brood housing are absent on *P. limonensis*. Instead, ants used plants as foraging sites for insect prey, aphid-tending and sugar-enriched fluids.

During plant flowering, *E. ruidum* and *E. tuberculatum* did consume flower nectar but did not pollinate the flowers. Bagged flowers to which ants had access set almost no fruits and were statistically indistinguishable from flowers to which ants and winged flower visitors were denied access. Thus, *P. limonensis* is either self-incompatible or does not self-pollinate autonomously.

Pollination of flowers by ants is very rare, despite the high abundance of ants around flowering plants and the antiquity of this relationship (Hölldobler and Wilson 1990). Several reasons can account for the lack of ant pollen transfer by *Ectatomma* among flowers of *P. limonensis*. These ants may have destroyed or damaged plant reproductive parts. This explanation seems unlikely for two reasons: (1) ants were frequently observed consuming flower nectar but no flowers appeared damaged after ant visits, and (2) flowers with ant attendants produced more fruits from winged pollinator visitation (Fig. 1) which would be unlikely if fewer flowers were available. Flower visitation by ants could also be too rare to permit transfer of intraspecific pollen. This is certainly not the case for *Ectatomma* on *Psychotria* plants, the ants being frequent flower visitors at least within the same individual. Ants may also destroy pollen itself by secreting anti-fungal substance through the metapleural glands (Beattie et al. 1985). However, ants can successfully transport pollen if metapleural secretions are not highly toxic to pollen grains or if pollen grains are carried on the legs and thus away from the thorax (Ramsey 1995). This is likely to be the mechanism preventing *Ectatomma* pollination of *P. limonensis* if this plant is self-compatible.

**Fig. 2** Effects of inclusion/exclusion of the ants *Ectatomma ruidum* and *E. tuberculatum* on fruit damage, fruit desiccation, and fruit removal in *Psychotria limonensis*. Columns represent means and bars are standard errors**Table 5** Effects of ant exclusion and duration on fruit removal, desiccation, and damage. Fruit removal and fruit desiccation were transformed as the square root of the response variable + 1. Fruit

Damage was transformed as 1/square root of the response variable + 1

Source	Fruit removal			Fruit desiccation			Fruit damage		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Ant exclusion	1	4.177	0.047	1	0.341	0.562	1	2.587	0.115
Duration	1	1.694	0.200	1	3.364	0.073	1	0.226	0.637
Error	46			46			46		

The ant feature that is most likely to prevent pollination in self-incompatible plants is the tendency of foraging ants to return daily to the same plant, branch, or flower cluster (Hölldobler and Wilson 1990). Thus, ants foraging on large plants such as *P. limonensis* are unlikely to have contacted pollen from other plants. Pollination of self-incompatible plants is most likely to occur in very small plants occurring at high densities.

Despite the lack of pollination by ants, fruit set increased when ants were present on plants along with winged pollinators. The best explanation for the increase in fruit set is that ant attendants affected some aspect of winged-pollinator behavior. Pollinators were rarely observed on plants that contained ants. Furthermore, pollinator abundance in the "all visitors" treatment was statistically indistinguishable from that of plants that denied all access to winged pollinators by bridal veil barriers. In contrast, the "winged only" treatment that denied access to ants but allowed winged flower visitors also had the most winged pollinators. This suggests two hypotheses for the effect of ants on pollinator density: (1) ant predatory activity decreases the pollinator visitation rate among plants with ants, or (2) ant predatory activity decreases pollinator visitation rate to flowers within an individual plant and increases visitation rate among plants. The first hypothesis could be rejected because "all visitors" plants set more fruit than did "winged only" plants, where the opposite would be true if ants deterred pollinators from visiting ant-plants. The observed pattern of fruit set, along with the lack of ant pollination, supports the second hypothesis, namely, predatory ants cause winged pollinators to make shorter foraging trips to fewer flowers on plants with ants and to make more trips among plants.

Ectatomma is an ant genus belonging to the subfamily Ponerinae whose member species are characteristically aggressive and predacious. Furthermore, *E. tuberculatum* is known to deter herbivory by curculionid beetles (Rico-Gray and Thien 1986 as cited by Davidson and Epstein 1989). Ant presence was associated with both decreased fruit loss and decreased fruit removal suggesting that herbivores and seed dispersers were also repelled by predatory ant behavior. A study of ant-plant interactions in southern Spain found that one plant, *Lepidium subulatum* L. (Brassicaceae), showed a pattern of ant facilitation similar to that of *P. limonensis*: treatments that permitted both ants and winged pollinators set significantly more fruit than did those plants that excluded only ants (Gómez et al. 1996). Similar fitness benefits of ant pugnacious behavior have been reported for plants that provide ant attractants (Janzen 1966; Inouye and Taylor 1979; Beattie 1985), but this is the first report of such activity in the absence of these attractants.

Increased pollen transfer has been found in other plant configurations where nectar consumption is challenging. In particular, it has been shown that winged flower visitors disperse pollen greater distances as flower densities decrease. For example, Melampy (1987) studied pollen dispersal in *Befaria resinosa* (Ericaceae) using

fluorescent dye as a pollen analog. Birds increased individual plant visitation rate at low flower densities because less time was spent at individual plants and more plants were visited.

As *Psychotria* fruits matured, the ants' aggressive behavior made them effectively fruit guards, protecting the unripe embryos from herbivorous and parasitic insects. Typically, twice as many fruits were lost on plants without ant attendants. Therefore ants had a twofold positive effect on fruit set whereby plants set more fruit when ants were present (maximum fruit set) and also lost fewer fruits as these developed (final fruit set).

The primary cost to ant attendance on *P. limonensis* occurs during the period when fruits are ripe and normally removed by vertebrate dispersers. Ant pugnacious behavior deters potential dispersal agents. This pattern has also been suggested for tropical ant-garden epiphytes in which ants tend aphids on fruiting pedicels and deter frugivores (Davidson and Epstein 1989). Strong evidence for this hypothesis comes from observations of large numbers of host tree fruits rotting on branches, despite these being very sweet and palatable (Davidson 1988). Such costs can be mitigated if ants themselves act as seed dispersers although many ant attendants are too small to carry epiphyte seeds (Davidson and Epstein 1989).

The influence of ants on plant fitness is a complex array of costs and benefits of varying magnitudes operating at multiple stages of plant reproduction. For selection to favor *Ectatomma-Psychotria* mutualism, the overall fitness effects of the interaction would need to be positive. The best approach to answering this question is to follow cohorts of plants from flowering through to seed germination. An alternate approach is to use path analysis to assess the effect of each participant during multiple stages of interaction. This approach can also distinguish indirect and direct effects. For example, path analysis was used to detect direct and indirect effects of ant attendants, winged pollinators, and herbivores on the reproductive success of a neotropical herb (Schemske and Horvitz 1988). In this case, ants also increased mature fruit set, though indirectly. The direct effect of ants was to increase flower production by stimulating extrafloral nectaries (Horvitz and Schemske 1988). Direct effects by herbivores on flower number and fruit number and by pollinators on initiated fruit numbers also influenced plant fitness (Schemske and Horvitz 1988). The effects of *Ectatomma* on fruit initiation, mature fruit number, and number of fruits removed are likely to be indirect effects whereby ant aggressive behavior influences the behaviors of pollinators, herbivores, and seed dispersers.

In summary, the interaction among a fruiting shrub, *P. limonensis*, and two ants, *E. ruidum* and *E. tuberculatum*, is mutually beneficial during pollination and fruit set but carries costs to plants during fruit removal. It is not known how these benefits and costs compare to affect total plant fitness. If costs outweigh benefits, it is likely that cooperation in the form of symbiosis beyond

current interaction levels will not evolve. If the seeds of plants in association with ants are not consumed and dispersed by birds, then those interactive plants will not proliferate no matter how much benefit is gained in fruit set. Thus, the overall interaction between *Ectatomma* and *P. limonensis* is positive if the beneficial effects of attendance on pollination and fruit loss surpass the detrimental effect on fruit removal.

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