Research Article

Plant Killing by Mutualistic Ants Increases the Density of Host Species Seedlings in the Dry Forest of Costa Rica

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Some species of plant-mutualistic ants kill the vegetation growing in the vicinities of their host plant, creating an area of bare ground (clearing). The reduced competition in the clearing may facilitate the establishment of host species sprouts (clones and seedlings), which in turn benefits the ants with additional food and shelter (“sprout-establishment hypothesis”). To test this hypothesis, the density and origin of Acacia collinsii sprouts growing inside clearings and in the vicinities of acacia plants without clearings were compared. Also, to assess the pruning selectivity of acacia ants (Pseudomyrmex spinicola), seedlings were transplanted into clearings. The reaction of ants towards unrewarding acacia seedlings (without food and shelter) was also tested. The density of acacia sprouts growing inside clearings was almost twice that in the vicinities of host plants without clearings, and sprouts were inhabited by nestmates of the colony that made the clearing. Clones and seedlings were found in similar proportions in the clearings, and ants did not kill unrewarding acacia seedlings or seedlings unrelated to their host. The benefit reported here for the ants could be in conflict with the host plant, especially when the plant has rhizomalous reproduction.

1. Introduction

In obligatory ant-plant mutualisms, the ants obtain food and shelter from their host, and, in exchange, they defend the plant against herbivores [1, 2]. In addition to attacking insect or vertebrate herbivores, some ants (henceforth, resident ants) also kill plants in the vicinity of their host tree by biting or poisoning [1, 3–10], functioning as allelopathic agents [4]. By killing neighboring plants, the ants leave an area of bare ground around the host plant (henceforth, “clearing”; Figure 1).

Two main functions have been attributed to the clearings: (1) isolating the colony and (2) reducing competition for the host plant. The isolation that the clearings provide to the host tree may also prevent invasions from other ants by reducing or eliminating access venues to the colony that other invasive ants might use to enter the tree. Davidson et al. [11] noted that most ant species that kill nearby vegetation defend themselves by stinging and are unable to defend themselves against ants that are chemically defended (i.e., ants that are stingless and use chemical sprays). Empirical data showed contradictory results: Triplaris trees inhabited by Pseudomyrmex ants that were artificially connected to neighboring vegetation had more invading ants than isolated ones, but connected trees of Cordia nodosa associated with Allomerus ants did not have more invasions [11]. Also, Crematogaster ants living in nonwaxy species of Macaranga trees pruned more intensively than those living on trees with waxy barriers [9], presumably because ants in waxy trees are already isolated from potential intruder ants, which have difficulty walking on waxy surfaces.

Ants can also enhance growth of their host plant and obtain more resources for their colony by pruning nearby vegetation. Any plant growing close to the host acacia is a competitor for light, water, nutrients, or space. Therefore, killing neighboring plants or vines growing on the acacia reduces host plant competition [4]. Janzen suggested this hypothesis based on observations of acacias weakened or dying under the shadow of vines or trees. Besides the observations on Acacia by Janzen, it is known that Tococa trees inhabited by Myrmelachista ants die when the ants do
pruning behavior was favoring clones of the host acacia near acacias without clearings. To understand whether the sprouts were occupied by ants more often than sprouts growing that made the clearing; (3) whether sprouts in a clearing whether the sprouts were inhabited by the same colony of sprouts near host plants with or without clearings; (2) establishment of acacia sprouts, by comparing the density of acacia sprouts, (dispersed seeds versus vegetative growth from the host tree, it may facilitate the establishment of seedlings growing inside the clearings is very limited. For several ant species, we know that they kill any plant inside the clearings except for seedlings of the mutualistic species, but little is known about the seedling’s origin (dispersed seeds versus vegetative growth from the host plant), or how specific are ants in identifying or favoring host seedlings over seedlings of other plants. The ants kill any plant that is in contact with their host tree, apparently either to prevent the growth of encroaching vegetation, or to minimize invader entry points. However, we do not know how they react when the contacting plant is an unrewarding seedling (i.e., a seedling that does not provide food or shelter), and whether they evaluate the long-term potential benefits of keeping a seedling that is not producing any reward against the short-term potential threat of having a seedling with leaves or branches in contact with the plant.

In this paper, I address several aspects of the sprout establishment hypothesis in the obligatory mutualism of acacia plants (Acacia collinsii) with Pseudomyrmex spinicola ants. I tested (1) whether the clearings favored the establishment of acacia sprouts, by comparing the density of sprouts near host plants with or without clearings; (2) whether the sprouts were inhabited by the same colony that made the clearing; (3) whether sprouts in a clearing were occupied by ants more often than sprouts growing near acacias without clearings. To understand whether the pruning behavior was favoring clones of the host acacia over seedlings coming from other plants, I evaluated the proportion of the sprouts established in the clearings that were growing from seeds or from rhizomes of the host plant. Additionally, I experimentally tested whether ants allowed saplings that were not clones or seedlings from their host tree to grow in the clearings. And finally, I evaluated whether ants kill or protect acacia seedlings that were not offering any immediate reward (nectar, protein bodies, or swollen thorns) but were functioning as bridges for potential intruders.

2. Methods and Materials

2.1. Study Site. The investigation was conducted in the dry forest of Palo Verde National Park (10° 21’ N, 85 21’ W) in Guanacaste, Costa Rica. Palo Verde has a mean annual rainfall of 1500 mm and elevation ranging from 0 to 100 m. This area has a well-defined dry season from November to May and a rainy season from June to October. A. collinsii plants inhabited by P. spinicola occur in secondary growth forest where the lianas and vines are very common. These ants defend the colony against predators or intruders by stinging, and they kill vegetation around their host by biting and cutting the leaves or stems, producing a circular clearing around the host plant (radii between ~30 cm up to 2 m; [3], this paper). In Palo Verde, it is possible to find acacias with clearings and acacias without clearings (normally inhabited by other Pseudomyrmex species, by the chemically defended Crematogaster brevispinosa, and few trees without ants). A. collinsii trees are able to reproduce sexually by seeds, or vegetatively by rhizomes that produce clones of the adult plant [3]. In the study site of the present investigation, the invasion threat for resident ants comes from both arboreal ants, which could displace them from the tree, and army ants that can predate on the brood. The sampling was carried out during the wet season of 2007 and 2008.

2.2. Density of Acacia Sprouts. In June 2007, I searched for solitary Acacia collinsii trees with clearings and inhabited by P. spinicola ants (Figure 1), and for acacia trees without clearings, to compare the density of acacia sprouts growing in the vicinity of the plant. A plant was considered to be solitary when it was separated by more than 4 m from another conspecific adult. Almost all of these clearings were approximately circular, so the radius was used as an estimate of the clearing’s size. For each acacia, radii were measured (±0.5 cm) from the trunk of the acacia (hereafter, central acacia) to the edge of the cleared area in the four cardinal directions, and the mean was used to calculate the area of the corresponding circle. I estimated the size of the acacia (n = 60) by measuring the diameter of the acacia (±0.05 cm) at the ground level and counted all the acacia sprouts with swollen spines growing inside the clearing. For acacias without clearings (n = 48), I counted all sprouts growing within 1 m of the acacia. I used the estimated area of the clearing to calculate the density of acacia sprouts per square centimeter. Means ± standard deviations are presented. The density of acacia sprouts was compared with nonparametric ANCOVA test using ranks, following the procedure of McSweeney and Porter [15] and Conover and Iman [16].
because the response variable did not meet normality and homoscedasticity. This statistical method takes into account the within-group and total-group regression when adjusting the dependent variable to the covariate [17]. The clearing (presence or absence) was considered a fixed factor, and plant diameter was used as a covariate. The covariate was included because the size of the plant could be correlated with the number of saplings growing into de clearing, for example, older plants could have more sprouts.

2.3. Inhabitants of the Sprouts Growing in the Clearing or Near Acacias without Clearings. To test whether ants on the sprouts were from the same colony that made the clearing, the sprouts from the density measurements were observed for ants. If the sprout had ants, I identified the species and verified whether they belonged to the same colony of ants on the central acacia. Colony identity was deduced by observing whether the ants on the sprout walked in a line back and forth between the central acacia and the sprout. If I did not find ants walking on the ground at that moment, they were encouraged to walk onto a stick from the sprout, allowing them to walk onto the central acacia. I checked whether the resident ants let the intruder walk on the tree or attacked it by biting and stinging (these ants recognize nestmates by chemical cues [18]). As control, I induced ants from the central acacia to walk on a stick and then reintroduced them to the central acacia, in different place from where I placed the ant coming from the nearby sprout. The frequency of sprouts with nestmates, ants from other species, or unoccupied were compared by chi-square tests separately for plants with clearings or without clearings.

Another prediction is that sprouts in a clearing should be more often occupied by ants, than sprouts growing close to acacias without clearings. The density of sprouts occupied by ants (response variable) and the density of unoccupied sprouts (response variable) were compared by separate ANCOVA tests using ranks, where the fixed factor and covariate were clearing (presence or absence) and the plant diameter, respectively.

2.4. Identity of the Acacia Sprouts Established in a Clearing. To test whether plant killing was favoring host clones over seedlings (i.e., that ants were favoring the vegetative growth of their host plant), I determined the proportion of sprouts corresponding to clones and seedlings, on the clearings of 22 acacias. On each clearing, I dug about 25 cm deep around the main acacia and around the sprouts, looking for rhizomes. When rhizomes were found, they were followed to elucidate connections between the main acacia and the sprouts. Sprouts were classified as “independent” when its main root entered vertically into the ground, and it was not attached to a rhizome. When a horizontal rhizome between the sprout and the central acacia was found, the sprout was classified as a “clone of the main acacia”. I classified some sprouts as “having horizontal roots” when the sprout lacked a main root growing vertical into the ground, and it was attached to a horizontal rhizome making an inverted “T” or an “L” with the stem but was not connected to the central acacia. In each clearing, the main acacia’s diameter and the distance (±0.5 cm) to the sprouts around it were measured. After corroborating that the diameter of the plant was not correlated with the proportion of clones (Spearman correlation, \( R = -0.20, P = 0.37 \)), a Wilcoxon matched pairs test was used to compare the quantity of sprouts coming from seeds and those that were clones of the acacia. Means and standard deviations are presented.

Because the relation between established seedlings and the main acacia was impossible to determine with the previous observations, I performed field experiments where I placed acacia seedlings of known origin inside clearings and tested the reaction of the ants. I planted seeds of A. collinsii in bags with soil, and grew them on partially shaded tables protected from large herbivores (deer, horses, and cows). The A. collinsii seeds came from trees that were more than five kilometers away from the experimental trees. In similar bags, I also planted stolons of Oplismenus sp. (Poaceae) with 7-8 leaves, a grass native of the study area that is regularly killed by Pseudomyrmex ants. When the acacias had six or seven leaves (3-4 weeks after planting), they were introduced into the clearing of 12 solitary acacias (hereafter, experimental trees) that had less than three young acacias growing naturally in the clearing, to determine whether the ants will kill them or allow them to grow. In the clearing of each experimental tree, I placed two bags with A. collinsii seedlings at half of the clearings, and two bags with Oplismenus stolons as controls. Within each clearing, I placed bags with conspecific plants diametrically opposed from each other. Ants were able to walk on the vertical surface of the plastic bag without impediment. After placing bagged plants, I observed them for 10 days twice a day for 2 minutes, checking for P. spinicola ants pruning or chewing the plant. I checked the plants one more time after 20 days from the setup of the experiment. The experimental placement of plants in the clearings allowed me to evaluate the reaction of the ants to acacia seedlings that were not clones or seedlings from their host tree.

2.5. Unrewarding Acacia Seedlings in Contact with the Host Tree. To understand whether the decision of pruning a sprout changed when the acacia sprout also represented a potential bridge for intruders, I placed two plants in bags in contact with the trunk of solitary acacias inhabited by P. spinicola ants. One of the plants in the pair was an acacia seedling, and the other was either an Oplismenus grass stolon (7-8 developed leaves, \( n = 22 \)) or a Coursetia caribaea shrub seedling (\( n = 12 \)). I used the C. caribaea (Fabaceae) seedlings (approx. 15 cm height) because it is a woody species with pinnate leaves like the acacia. Both plant species are common in the study area. Acacia seedlings used in the experiment did not have nectaries, food bodies or swollen spines, and, therefore, did not offer food or shelter to the ants. The diameter of the experimental acacias ranged from 2 to 2.5 cm at ground level. For all the plants, I placed the bag next to the trunk and arranged the leaves such that the second leaf touched the acacia. All colonies were sampled only once, and all bagged plants were used for a single colony. Similar observations to the previous experiment were done for 8 days.
3. Results

3.1. Density of the Sprouts Growing in the Clearing or Near Acacias without Clearings and Their Inhabitants. There was a higher sprout density in clearings (0.71 ± 0.15 sprouts/cm², n = 69) than in the vicinity of plants without clearings (0.40 ± 0.22 sprouts/cm², n = 35; nonparametric ANCOVA by ranks, $F_{(1, 101)} = 3.73$, $P = 0.05$). When looking at occupied sprouts (i.e., with ants), they were also at higher densities in clearings than close to acacias without clearings (0.46 ± 0.96 sprouts/cm² versus 0.26 ± 0.54 sprouts/cm², respectively; non parametric ANCOVA by ranks, $F_{(1, 101)} = 4.09$, $P = 0.04$). On the other hand, there were similar numbers of unoccupied sprouts (without ants) per square meter within clearings and near acacias without clearings (0.23 ± 1.29 versus 0.13 ± 0.37 sprouts/cm², respectively; ANCOVA by ranks, $F_{(1, 101)} = 0.003$, $P = 0.95$).

The majority (88%) of the A. collinsii sprouts growing inside clearings were occupied by ants from the same colony of the experimental acacia (not treated aggressively by the resident colony); the remaining 12% were unoccupied (Figure 2). The sprouts near acacias without clearings showed different proportions of sprouts on each category ($\chi^2 = 11.8$, d.f. = 2, $P = 0.003$): 65% were occupied by ants that were not attacked by the resident colony, 33% were unoccupied, and 2% were occupied by another species of ant (Crematogaster brevispinosa ants inhabited the main acacia, but the sprouts had P. spinicola ants).

3.2. Identity of the Acacia Sprouts Established in a Clearing. Inside the clearings, there was an equal amount of sprouts arising from seeds (4.3 ± 5.3 sprouts per acacia, median = 3) as clones from the acacia (2.9 ± 4.1, median = 1; Wilcoxon paired test, $T = 81$, $P = 0.37$). The results were consistent when the sprouts classified as “having horizontal roots” were incorporated as “clones” in the analysis (Wilcoxon paired test, $T = 98$, $P = 0.54$). It is very likely that these saplings are clones from the acacia, especially when the rhizome they come from was directed towards the central acacia.

None of the acacia seedlings placed in the clearings were pruned or severed, but all Oplismenus grasses were pruned. The majority of the colonies started pruning the grass on the same day that I first observed them on the plant, but some did not do so until after 9 days from the day that ants were first seen on the plants. Two grasses remained uncut by the ants during the first 10 observation days. But, by day 20, the ants had completely severed the main stem of all 24 grasses, whereas all 24 acacias were intact.

3.3. Unrewarding Acacia Seedlings in Contact with the Host Tree. All Oplismenus and C. caribaeaN plants were pruned, whereas all acacia seedlings were not damaged but were defended by the ants. All experimentally placed acacia seedlings were found by the ants on the same day I set them next to the host acacia, except for one of them that did not have ants until the third day. All these colonies were active pruners, because ants began to prune the grasses and the woody seedlings (C. caribaea) on the same day (8 cases) or the day following introduction of these plants (4 cases).

4. Discussion

The sprout establishment hypothesis proposes that by actively clearing vegetation around the acacia, the ants reduce plant competition, allowing the establishment of host species sprouts, which in turn benefits the ants with additional food and shelter [13]. The study findings support this hypothesis. Fonseca [19] found that nesting space was the main factor limiting colony size for Pseudomyrmex concolor associated with Tachigali trees (Fabaceae), and for seven other ant species that are obligatory mutualists of plants [20]. Food and shelter provided by A. collinsii are crucial for survival and colony growth of P. spinicola [3] and could also limit the colony growth rate or size. Relatively pure stands of acacia trees are common in the dry forest of Central America [3], and they were probably produced because of mutualistic ants killing other vegetation. In these patches, one colony (i.e., offspring of one queen) could occupy all surrounding shoots, and usually two or three colonies occupy larger patches in areas of more than 3 × 6 m [21]. Even beyond pure stands of acacia, colonies established on two neighboring plants (one much larger than the other) are frequently found. Therefore, P. spinicola ants sometimes expand their nest to more than one tree, suggesting that plant resources are limiting the colony size. Concordantly, I observed a higher density of acacia sprouts inside the clearings when compared with the density without clearings. Additionally, the sprouts were occupied by the same ant colony inhabiting the central acacia. However, the sprout-establishment hypothesis alone cannot fully explain this ant behavior, because the number of occupied sprouts in clearings is similar to the number of seedlings available for colonization in plants without clearings. The competition hypothesis proposed by Janzen [4], and the isolation against potential intruders, may also provide other benefits to the colony.

Selective pruning of foreign seedlings but not acacia seedlings placed inside the clearings also concurs with predictions of the sprout-establishment hypothesis. Food
quantity has been shown to be a limiting factor for colony size of *Crematogaster* ants associated with *Macaranga* species [22], and Keeler [23] also found *Pseudomyrmex flavicornis* ants visiting the nectaries of *Ipomoea carnea* plants growing in contact with their host plants and they did not sever it. *Myrmelachista* ants also selectively kill vegetation around their host tree, leaving alive saplings of the two plant species they inhabit [8]. Thus, it is likely that ants may allow other plants to grow close to their host as long as they obtain benefits from the intruder plant. For the acacia ants, Janzen [24] argued that ants would prune acacia sprouts only if they were not offering benefits to the ants, or if they were not clones from their host. However, the results from this investigation show that pruning behavior do not necessarily, or exclusively, benefit the ant’s host plant, and that workers refrained from cutting acacias that were not sprouts from their host. There could be a potential conflict between the host acacia and its resident ants because the clones and seed of the host tree have to compete for the favorable conditions of the clearing with other acacia seedlings. The benefit for the ants could also be detrimental for the plants, because the aggregations of acacia sprouts may also be more vulnerable to predation (Janzen-Connell model [25, 26]), unless the defense of ants counteracts this effect. A study that follows the survival of acacia seedlings, both inside and outside clearings and solitary versus aggregated sprouts, is necessary to evaluate this potential conflict.

The ants still did not kill or prune saplings that were not providing immediate benefits, such as swollen thorns, nectarines, or food bodies, and these may be used as a bridge to get access to the host tree [11]. Potential intruder ants in the study area include workers of other colonies of *P. spinicola*, other acacia ants (*Pseudomyrmex flavicornis, Pseudomyrmex nigrocinctus*, and *Pseudomyrmex nigropilosus*), other arboreal species (*Pseudomyrmex gracilis* and *Crematogaster brevispinosa*) and predatory army ants (*Eciton burchelli parvispinum* and *Neivamyrmex pilosus mexicanus*). Even though other colonies of *Pseudomyrmex* are not predators, they represent potential threats because they use the same shelters (swollen thorns), and are attracted to both glucose-free nectar and Beltian bodies [27, 28]. Invasion of a colony by other *Pseudomyrmex* colonies is likely to occur via branches because of their arboreal habit, and, therefore, the clearings could deter these ants. In the experiments, ants did not kill acacia sprouts in contact with their plant, maybe because other *Pseudomyrmex* ants did not occupy them; hence the risk of invasions from the sprouts was low. The sprout-establishment hypothesis assumes that ants obtain delayed benefits for their pruning behavior, but this delay may be relatively short. The colony could receive the benefits of favoring acacia sprouts in the clearing very rapidly, nectaries and food bodies are produced by the young acacias after 4 or 5 weeks (on their eighth or ninth leaf, Amador-Vargas unpublished data), and more rapidly from clones that develop faster because of the resources they receive from the host plant [3].

More studies that consider the reaction of resident ants to intruder plants of the host’s species are necessary to understand the generality of the results found in this research (but see [14]). We still need to understand in the long-term whether clones are more successful than seedlings inside the clearings (because they are growing faster), and how the ants are able to identify and defend seedlings of the host species when they are not offering rewards.

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