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ABIOTIC FACTORS AND THE CONDITIONAL OUTCOME OF AN ANT–PLANT MUTUALISM

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Abstract. Mutualisms are inserted in a network of direct and indirect biotic interactions built within the framework imposed by the abiotic setting. We carried out an experiment to test how the availability of light and nutrients modulates the interaction strength between *Inga vera* (Mimosoideae), a Neotropical extrafloral-nectary-bearing plant, and its associated ants. From July 2001 to July 2003, 48 plants were grown in an old-field site following a three-factor randomized blocked design where treatments were: ant (ants present and absent), nutrient (without and with nutrient addition), and light (sun and shade). During the experiment, in the absence of ants, plants growing in sunlight were shorter, developed fewer leaves and leaflets, were more damaged by herbivores, had half the total dry biomass, and died more frequently than plants visited by ants. In contrast, ants had no effect on the growth, survival, and total biomass of shaded plants. Therefore small-scale heterogeneity in the light environment turned mutualism (+,+) into commensalism (+,0). This study demonstrates experimentally that the outcome of mutualistic interactions can be conditional upon the abiotic setting.

Key words: ant; biomass; commensalism; conditional outcome; extrafloral nectar; herbivory; Inga vera; *light; mutualism; nutrient;* Selenothrips rubrocinctus; *spatiotemporal variation.*

INTRODUCTION

Mutualisms are inserted in a network of direct and indirect biotic interactions built within the framework imposed by the abiotic setting (Schemske and Horvitz 1988). The degree of dependency between mutualistic partners varies considerably in space and time (Bentley 1976, Barton 1986, Thompson 1988, Maschinski and Whitham 1989, Horvitz and Schemske 1990, Alonso 1998, Bronstein 1998, Rico-Gray et al. 1998, Herre et al. 1999, Moya-Raygoza and Larsen 2001, Adler 2003, Billick and Tonkel 2003, Bronstein et al. 2003). However, our understanding about the causes of such a variation is still limited.

Spatiotemporal variation in ant–plant protective mutualisms has been mostly attributed to shifts in the interplay among biotic agents. For instance, the growth of Amazonian ant-trees of the genus *Tachigali* determines a pattern of ontogenetic succession, involving eight different ant partners, that generates a number of indirect biotic interactions (Fonseca and Benson 2003). Herbivores induce plants to change their defense strategy, altering their levels of secondary compounds and the quantity and quality of extrafloral nectar and food bodies, which will, in turn, modify both ant and herbivore abundance (Agrawal and Rutter 1998, Linsenmair et al. 2001, Wäckers et al. 2001). Experiments manipulating the presence of ants have demonstrated a profound impact on plant fitness and on the fate of their herbivores (Janzen 1966, Bentley 1976, Huxley 1978, Koptur 1984, Vasconcelos 1991, Fonseca 1994, de la Fuente and Marquis 1999). The experimental exclusion of ant predators in *Piper cenocladum* (Piperaceae), a Central American myrmecophyte, produces a top-down effect on the food web, increasing the abundance of patrolling ants and the levels of defense to the plants (Letourneau and Dyer 1998, Dyer and Letourneau 1999).

Nevertheless the support for the hypothesis that abiotic factors can also be a relevant force determining the outcome of mutualistic interactions has been mostly anecdotal (Bentley 1977, Davidson and McKey 1993, Bronstein 1994, 1998). Several studies that recorded spatiotemporal variation of ant–plant mutualisms attributed the observed patterns to hypothetical variation in climate conditions (Alonso 1998, Rico-Gray et al. 1998). Other studies have attributed interhabitat differences in ant–plant mutualisms to abiotic factors, however, with no field experimental support. For instance, light availability in forest gaps has been suggested as an important factor defining ant affiliation in the *Cecropia–Azteca* system (Davidson and Fisher 1991, but see Folgarait and Davidson 1994, 1995). In the extrafloral-nectary-bearing plant *Stryphnodendron microstachyum* (Fabaceae), individuals growing under the canopy of a secondary forest, which faced a higher herbivore density, produced more nectar and attracted more ants than plants at an open pasture (de la Fuente and Marquis 1999). Recently however, in an experiment with *Macaranga triloba* (Euphorbiaceae), it has

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been demonstrated that soil fertilization increased the production of food bodies and nectar to the ants, producing a significant decrease in herbivory levels (Heil et al. 2001).

This study provides an experimental test of the hypothesis that abiotic factors can modulate the interaction strength between ant–plant mutualistic partners. In particular, we test how the availability of light and nutrients modulates the interaction strength between *Inga vera* (Mimosoideae), an extrafloral-nectary-bearing plant, and its associated ants.

METHODS

Study site

The experiment was carried out in the experimental field station of the Universidade do Vale do Rio dos Sinos (29°47′31″ S and 51°09′07″ W), Rio Grande do Sul, Brazil. The regional climate is classified as subtropical (Holdridge 1967), the average annual temperature fluctuates between 24°C in January and 14°C in July, and the mean annual precipitation is 1346 mm. The relief is smooth at an altitude of 60 m, dominated by acid soils ($\text{pH} = 4.7$) with low levels of phosphorus (1.9 mg/L) and potassium (39 mg/L). The vegetation is an old field dominated by the grasses *Lolium perenne*, *Andropogon bicornis*, and *Andropogon leucostachyus* and herbs of the genera *Eryngium* (Apicaceae) and *Desmodium* (Fabaceae). The field was cut four months before the beginning of the experiment.

Study species

Inga (Mimosoideae) is a neotropical genus with 258 species (Pennington 1997), which mostly are trees with parapinnate leaves, displaying one prominent extrafloral nectary between each pair of opposite leaflets. *Inga vera* Willd. is a semideciduous, fast-growing tree, up to 25 m in height, which develops well in wet and sunny sites and shows compound leaves with 2–16 leaflets. *I. vera* has a wide distribution in the neotropics, from Mexico to Uruguay, occurring mostly in lowland rain forests and riverbanks. The population in the study site belongs to the subspecies *I. vera* Willd. *affinis* (DC) T. D. Penn (Pennington 1997). Field observation has indicated that different populations of *I. vera* are associated with different ant species.

Experimental design

The experiment followed a three-factor, randomized, complete blocks design with the following treatments: ant (ants present and absent), nutrient (without and with nutrient addition), and light (sun and shade). Six replicate blocks containing the eight treatment combinations were located in an area of 1000 m^2. Blocks were 8 m from each other, and within each block individual plants were 4 m from the nearest neighbor. In March 2001, seeds from six mature *Inga vera* plants were collected and cultivated outdoors under natural conditions. In July 2001, six sets of eight seedlings of similar size were transplanted to the experimental area, the seedlings being assigned randomly to the treatments. At the beginning of the experiment there was no significant difference in height, number of leaves, and leaflets among treatments.

Plants designated as the ‘‘ants present’’ group could be freely visited by ants, but plants from the ‘‘ants absent’’ treatment received, as frequently as necessary, the application of a sticky gel on the base of their stems, which prevented access for the ants to the plant surface (Bryonline, Bryonline Indústria e Comércio de Produtos Químicos Ltda., São Paulo, Brazil). Plants in the ‘‘without nutrient addition’’ group developed under natural soil conditions while the ‘‘with nutrient addition’’ plants received in July 2001, as suggested by Bartz et al. (1995), the application of potassium (4 g $\text{KCl}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) and phosphorus (12 g $\text{P}_2\text{O}_3\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$), as well as calcium carbonate (100 g $\text{CaCO}_3\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) to control soil acidity. We refrained from adding nitrogen to the plants, since this could potentially reduce the nodulation of nitrogen-fixer (N-fixer) bacteria (e.g., Voisin et al. 2002). Plants in the ‘‘sun’’ treatment were exposed to natural levels of solar radiation, while the ‘‘shaded’’ treatment plants were protected from the sun by a 1-m^2 black mesh that reduced light intensity by 70%.

Data collection and analyses

From September 2001 to June 2003 we performed monthly snapshot censuses to record the abundance and composition of ants and herbivores per plant. Both ant and herbivore censuses were performed during the mornings of sunny to midclouded days within a time frame of three hours. For each plant, the number of individuals of each species was recorded by direct counting, taking care to exclude insects that arrived in the plant after the beginning of the counting. A small number of individuals of each ant and herbivore species were collected to allow identification, and the sampled specimens were deposited in the entomological collection of the Universidade do Vale do Rio dos Sinos.

In order to evaluate whether *Inga vera* could rely on ants throughout the whole year, the mean number of ants per month was calculated and plotted during the 20 months of the experiment. Furthermore, to test whether ant abundance was synchronized with the availability of resources offered by the plants, a correlation was applied between the mean number of ants and the monthly mean production of new leaflets, which is proportional to the number of active extrafloral nectaries. The same temporal analysis was performed for the herbivores.

Plant growth and phenology were followed during the 20 months of the experiment. Plant height was recorded monthly to the nearest centimeter. The number of leaves and leaflets were determined by direct counting. The production of new leaves and leaflets was recorded once a month by following the emergence of reddish or light green leaves and leaflets. Leaf and leaflet loss were recorded monthly by comparing the number of leaves and leaflets between the time t (excluding the new leaves) and $t-1$.

The level of herbivory of each plant was estimated monthly by dividing the sum of the herbivory level score for all leaflets by the total number of leaflets. The herbivory level score of each leaflet was visually estimated according to the following classes of damage: 0, 0%; 1, 1–5%; 2, 6–25%; 3, 26–50%; 4, 51–75%; 5, 76–99%; 6, 100% (Fonseca 1994). Damage included removal of the leaf surface by chewing herbivores and tissue necrosis due to piercing and sucking species, as well as mines and galls compromising photosynthesis. Furthermore, when a whole leaflet falls, which can easily be recognized by a scar in the leaf rachis, it is recorded as a six. Therefore the herbivory level varies from zero, when all leaves are intact, to six, when the plant becomes completely defoliated.

The rate of herbivory (percentage area eaten per day) was estimated bimonthly based on six randomly chosen mature leaflets and six randomly chosen immature leaflets per plant, dependent on the availability. Herbivory rate (H) was estimated for each leaflet according to the Eq. $H = [(Ef/Af - Ei/Ai) \times 100]/T$, where A is the leaflet area, E is the area eaten, and T is the number of days between the beginning (i) and end (f) of the observation period (~30 days). Rates of herbivory per plant were transformed ($\ln [1 + 1000H]$) to homogenize the variance. Leaflet area was estimated from the length (l) and the width (w) of each leaflet, according to the regression $A = 0.6741\ lw + 0.1581$ ($N = 98$ leaflets, $r^2 = 0.996$; areas recorded by the software Scion Image 2000, for Windows 4.0.2, Scion Corporation, Frederick, Maryland USA). Since the production of new leaves in *Inga* was strongly seasonal, and some plants had the number of leaves substantially reduced during the experiment, the design for the analysis of the rate of herbivory became incomplete and unbalanced. Therefore the data were analyzed by leaflet maturity level and season (spring, September–November; summer, December–February; autumn, March–May; and winter, June–August) with the use of factorial ANOVA with blocks.

In July 2003, the experiment was ended, since some plants, with a total lifetime of 28 months, were reaching the mesh ceiling. Plants were then taken to the laboratory for the determination of biomass allocation. Roots and shoots were dried at 60°C for two weeks and weighed with a precision up to 10^{-4} g. Total plant mass was calculated as the sum of root and shoot mass. The shoot: root ratio was calculated as the dry mass of the shoot divided by the dry mass of the root. Differences in biomass allocation were tested with a factorial ANOVA, with blocks.

Repeated-measures factorial ANOVAs of three treatments in blocks were used to investigate the effect of the independent factors ant, nutrient, and light on the dependent variables plant height; number of leaves and leaflets; production and loss of leaves and leaflets; herbivory level; and number of herbivores and ants. Dependent variables were $\log_{10}(x + 1)$ transformed to meet parametric assumptions. Repeated-measures analysis requires the subjects to be present at all times. Since some plants died during the experiment, the presence of missing data in our data set would eliminate those plants from the analyses, creating an undesirable bias. Thus we replaced the missing data with zeros, meaning that after dying, the plants stabilized with no leaves, leaflets, or accumulated biomass. For herbivory level, the missing values were replaced by six, the highest score of herbivory level. All statistics were done in Systat (2000).

RESULTS

Ants

Camponotus fastigatus (Roger 1863) and *Crematogaster quadriformis* (Roger 1863) were the main ant species associated with *Inga vera* in the study area. Of the 12 ant species recorded during the censuses, these two species together corresponded to 89% of the total number of ants collected ($N = 440$ and 604, respectively, from a total of 1177 recorded individuals), the remaining species being rare (<40 recorded individuals per species). Despite its abundance, *Crematogaster quadriformis* was recorded in only three plants while *Camponotus fastigatus* was present in 88% of the 24 plants to which it had access. The total number of ants recorded per plant during the experiment did not vary significantly between treatments.

There was a strong temporal synchrony between the dynamic of leaflet production and the abundance of *Camponotus fastigatus* ($r = 0.749$, $P < 0.001$) and *Crematogaster quadriformis* ($r = 0.713$, $P < 0.001$, considering only the phenology of the three plants on which this species occurred) during the 20 months of experiment (Fig. 1). Leaflet production reached its peak during the summer (December–February) and fell abruptly during the winter (June–August). The number of ants per plant was low at the beginning of the experiment but increased in the second year. For instance, only 5.0 ± 1.2 ants/plant (mean ± SE) were recorded during the three months of the first summer, while during the second summer the plants had an average of 28.7 ± 17.4 ants/plant (paired t test, $t = 2.87$, df = 21, $P = 0.009$).

Herbivores

The exotic red-banded thrips *Selenothrips rubrocinctus* (Giard) (Thysanoptera) was the main herbivore of *Inga vera* at the study area. This species alone, with 2970 individuals recorded, represented 96% of the total number of herbivores collected ($N = 3090$). The other herbivores associated with *I. vera* were less common:

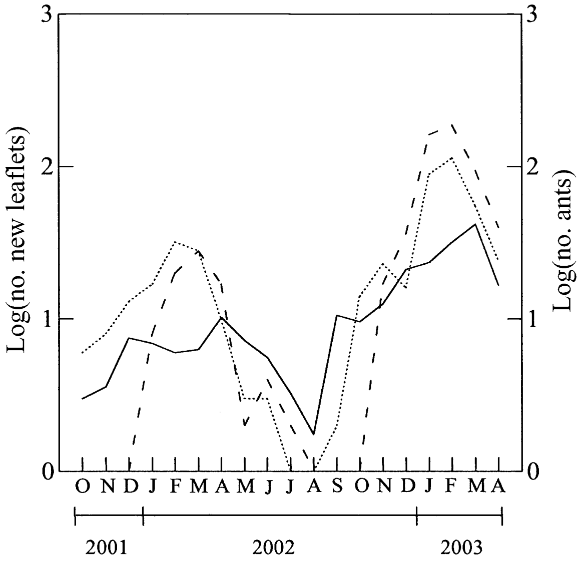

FIG. 1. The mean number of leaflets produced per plant (solid line) and the mean abundance of *Camponotus fastigatus* (dotted line) and *Crematogaster quadriformis* (dashed line) recorded in *Inga vera* from October 2001 to April 2003. Note that only plants with free access for the ants are included.

Orthoptera ($N = 38$), Hemiptera ($N = 36$), Coleoptera ($N = 20$), Isopoda ($N = 15$), Lepidoptera ($N = 10$), and Gastropoda ($N = 1$). Red-banded thrips are sap feeders that obtain their resources by continuously piercing the leaf epidermis with their mouthparts, causing cell damage, dehydration, tissue necrosis, and leaf loss. The temporal dynamic of thrips during the 20 months of the experiment was not correlated with the production of new leaflets ($r = -0.09$, df = 17, $P > 0.05$). The population increased rapidly at the beginning of the first autumn and decreased steadily at the end of the winter, to reappear in even higher densities just before the autumn of the second year. Indeed the mean (± 1 SE) number of thrips per plant increased by a factor of 15 from the first autumn (0.9 ± 0.6 thrips/plant) to the second autumn (15.3 ± 5.3 thrips/plant) of the experiment (paired t test, $t = 6.38$, df = 47, $P < 0.001$).

A factorial ANOVA showed that the mean number of thrips per month during the experiment was significantly lower on plants with ants ($\bar{X}_{ap} = 4.8 \pm 2.0$, $\bar{X}_{aa} = 1.4 \pm 0.5$ thrips/mo; $F_{1,35} = 4.245$, $P < 0.05$; ap, ants present; aa, ants absent) and exposed to the sun ($\bar{X}_{sun} = 0.6 \pm 0.3$, $\bar{X}_{shade} = 5.6 \pm 2.0$; $F_{1,35} = 21.694$, $P < 0.001$; Fig. 2). However, the number of herbivores, excluding thrips, was not affected by the treatments.

Plant height

Inga vera plants growing in the sun in the presence of ants ended the experiment with a mean height of 42.2 ± 5.9 cm, almost twice as high as plants without ants in the same light environment ($\bar{X} = 23.9 \pm 10.1$ cm). In contrast, shaded plants reached ~66.5 ± 8.0 cm, independent of the presence of ants or the addition of nutrients (Fig. 3A). A repeated-measures factorial ANOVA showed that the interaction of ant × light × time was highly significant, but nutrient addition did not affect plant height in any way (Table 1).

Vegetative phenology

In the sun, at the end of the experiment, the number of leaves of *Inga vera* plants with ants ($\bar{X} = 19.8 \pm 6.4$ leaves) was 2.5 times higher than the number of leaves exhibited by plants without ants ($\bar{X} = 8.0 \pm 4.0$ leaves). In the shade, however, the presence of ants did not affect the final number of leaves (Fig. 3B). A similar pattern was found for the number of leaflets (Table 1, Appendix). Repeated-measures factorial ANOVA showed that the interaction ant × light × time was highly significant for both the number of leaves and leaflets (Table 1). Fertilized plants had more leaves than unfertilized ones at the end of the experiment; however, the analysis failed to detect any interaction between the nutrient treatment and either the light or the ant treatment (Fig. 3B, Table 1).

The production of leaves and leaflets presented similar patterns (Fig. 3C, Appendix). In the sun, plants with ants produced on average 1.8 ± 0.4 leaves/mo and 17.3 ± 5.3 leaflets/mo, while plants without ants produced only 1.2 ± 0.3 leaves/mo and 9.0 ± 6.4 leaflets/mo. In contrast, in the shade, the presence of ants did not cause any significant effect (Fig. 3C). Accordingly, the interaction ant × light × time was significant for both leaves and leaflets (Table 1). Fertilized plants produced more leaves than unfertilized plants, but this factor acted in an additive manner in relation to the other treatments (Table 1).

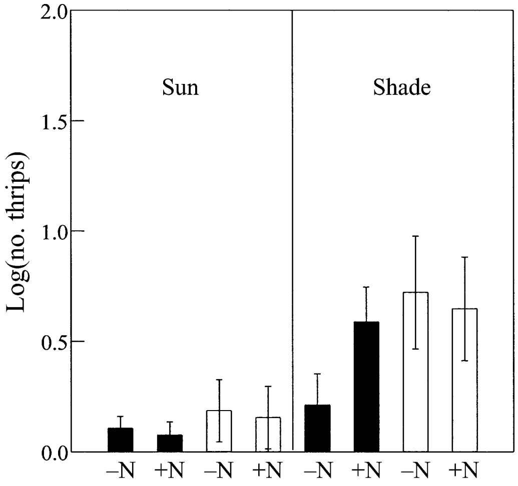

FIG. 2. Abundance (mean ± SE) of thrips recorded in *Inga vera* plants during the plant life span. Treatments are natural light conditions (sun) and light intensity reduced (shade); ants present (solid bars) and ants absent (open bars); natural soil nutrient levels (−N) and nutrients potassium and phosphorus added (+N). (See *Methods* for details.) Error bars represent ±SE.

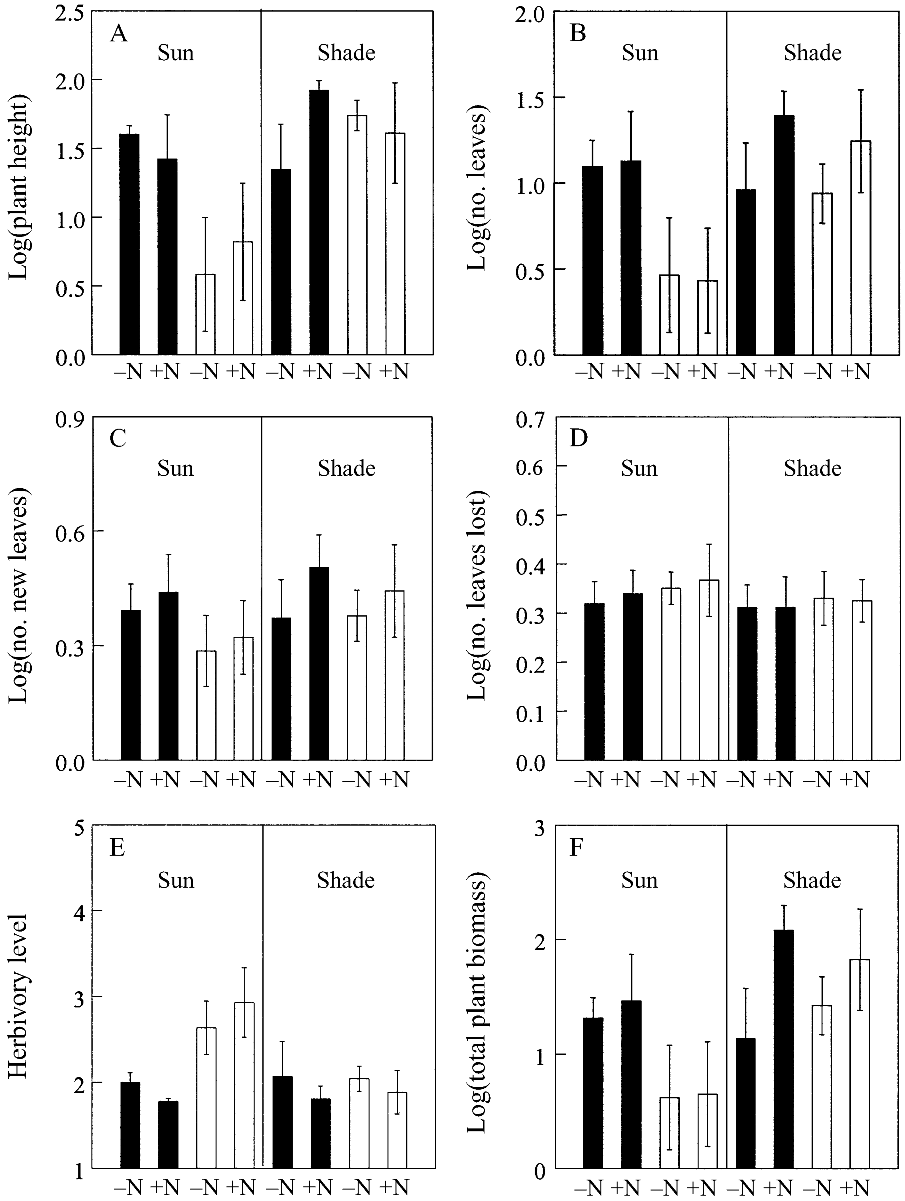

FIG. 3. Performance of *Inga vera* plants. Values (log-transformed except in panel E) are means ± SE. (A) Height (originally measured in centimeters) at the end of the experimental period. (B) Number of leaves at the end of the experimental period. (C) Number of immature leaves produced per month during the plant's life span. (D) Number of leaves lost during the plant's life span. (E) Herbivory level during the plant's life span. Herbivory level measures the degree of defoliation of a plant, varying from 0 (all leaves intact) to 6 (completely defoliated). (F) Total plant biomass (measured in grams) at the end of experimental period. Treatments are natural light conditions (sun) and light intensity reduced (shade); ants present (solid bars) and ants absent (open bars); natural soil nutrient levels (−N) and nutrients potassium and phosphorus added (+N). (See *Methods* for details.)

Ants significantly decreased leaf loss in *Inga vera*. Plants with ants lost only 2.4 ± 0.7 leaves/mo and 13.8 ± 4.4 leaflets/mo, while plants without ants lost on average 3.6 ± 0.6 leaves/mo and 33.5 ± 8.0 leaflets/mo. Repeated-measures factorial ANOVA showed that ant presence was significant for both leaves and leaflets (Fig. 3D, Table 1). Furthermore, light was also important in determining leaf loss; however, its effect was additive in relation to the presence of the ants (Fig. 3D, Table 1).

Herbivory level

In the sun, mean (±SE) herbivory level of plants growing under the presence of ants ($\bar{X}$ = 2.3 ± 0.4) was two times lower than the herbivory level of plants without ants ($\bar{X}$ = 4.9 ± 0.5). The presence of ants,

TABLE 1. Summary of F values generated by repeated-measures ANOVA performed on plant-size, vegetative-phenology, and herbivory-pressure attributes of *Inga vera*; treatments are ant (ants present and absent), nutrient (without and with nutrient addition), and light (sun and shade).

		Plant size		
Source of variation	df	Height	No. of leaves	No. of leaflets
Between subjects				
Block	5	1.447	3.566*	2.777
Ant	1	4.445	7.163*	4.355
Nutrient	1	0.037	0.001	0.034
Light	1	7.841*	3.619	6.156
Ant × nutrient	1	0.102	0.064	0.108
Ant × light	1	3.327	4.051	4.202
Nutrient × light	1	0.002	0.843	0.691
Ant × nutrient × light	1	1.651	0.995	0.999
Error between subjects	35			
Within subjects				
Time	19	10.855***	3.903***	8.890***
Time × block	95	1.324	2.133***	2.164***
Time × ant	19	4.310***	3.491***	2.806***
Time × nutrient	19	0.538	2.016*	1.220
Time × light	19	8.137***	5.547***	6.522***
Time × ant × nutrient	19	0.336	0.221	0.359
Time × ant × light	19	4.684***	4.272***	4.100***
Time × nutrient × light	19	0.675	1.085	1.184
Time × ant × nutrient × light	19	0.949	1.047	1.244
Error within subjects	665			

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

however, had no effect on the herbivory level sustained by shady plants (Fig. 3E). A repeated-measures factorial ANOVA showed a significant ant × light × time interaction (Fig. 3E, Table 1).

Herbivory rate

In the winter, mean (±SE) daily rate of herbivory on mature leaflets of plants growing in the sun in the presence of the ants ($\bar{X} = 3.6 \pm 0.4$%/d) was lower than the rate recorded on plants from which the ants were experimentally excluded ($\bar{X} = 4.9 \pm 1.0$%/d). However, the ants did not affect the herbivory rate of shaded plants, resulting in significant ant × light interaction ($F_{1,27} = 4.146$, $P < 0.05$, Appendix). In the shade, fertilized plants had a lower herbivory rate ($\bar{X} = 2.8 \pm 0.3$%/d) than unfertilized ones ($\bar{X} = 4.1 \pm 0.4$%/d). The addition of nutrients, however, caused no effect in the sun, resulting in significant nutrient × light interaction ($F_{1,27} = 5.501$, $P < 0.05$). Immature leaflets did not respond to the treatments (see Appendix).

In the summer, the ant × light interaction was also significant for mature leaflets ($F_{1,32} = 5.278$, $P < 0.05$). In the sun, the rate of herbivory on plants growing with ants ($\bar{X} = 4.0 \pm 0.3$%/d) was lower than without ants ($\bar{X} = 5.4 \pm 0.7$%/d, Appendix), but in the shade the presence of the ants did not cause any effect on herbivory. In this season, the rate of herbivory on immature leaflets exposed to the sun ($\bar{X} = 4.2 \pm 0.2$%/d) was higher than on leaflets growing in the shade (2.4 ± 0.3%/d), this difference being highly significant ($F_{1,29} = 12.614$, $P < 0.001$, Appendix).

In the autumn, the rates of herbivory of mature leaflets with ants (4.2 ± 0.3%/d) were lower than the ones without ants (5.2 ± 0.4%/d) ($F_{1,27} = 5.144$, $P < 0.05$, Appendix). In the spring, the rate of herbivory of immature and mature leaflets was not affected by the treatments.

Plant biomass

At the end of the experiment, mean (±SE) total dry biomass of plants growing in the sun with the presence of ants ($\bar{X} = 66.8 \pm 35.3$ g) was almost twice the biomass of plants from which the ants were experimentally excluded ($\bar{X} = 35.2 \pm 19.6$ g). In the shade, plants accumulated more biomass, but the effect of the ant presence was smaller ($\bar{X}_{ap} = 121.1 \pm 37.3$ g, $\bar{X}_{aa} = 108.1 \pm 35.8$ g). Indeed, the ant × light interaction was marginally significant for total dry biomass ($F_{1,35} = 3.486$, $P = 0.07$, Fig. 3F), root dry biomass ($F_{1,35} = 3.077$, $P = 0.088$) and shoot: root ratio ($F_{1,35} = 3.952$, $P = 0.055$, Appendix).

Fertilized plants ended the experiment with a significantly higher shoot dry biomass in relation to unfertilized plants ($F_{1,35} = 4.934$, $P = 0.033$, Appendix), and this tendency also occurred for total plant dry biomass ($F_{1,35} = 3.426$, $P = 0.073$) and root dry biomass ($F_{1,35} = 3.587$, $P = 0.067$). Fertilized plants growing in the shade apparently grew better, but this was not significant. Furthermore, we found no evidence for a nutrient × ant interaction.

Mortality

Eleven of the 48 plants that began the experiment in September 2001 died during the 20 months of research.

TABLE 1. Extended.

Vegetative phenology					Herbivory	
df	Leaf production	Leaf lost	Leaflet production	Leaflet lost	df	Herbivory level
5	3.768*	2.573	2.913	1.571	5	1.603
1	5.846	0.122	3.378	1.572	1	8.851**
1	1.304	1.055	1.499	0.137	1	0.065
1	4.377	0.011	6.693	0.836	1	8.080**
1	0.398	1.280	0.300	0.343	1	0.185
1	2.628	0.266	2.925	0.222	1	5.825*
1	1.377	0.655	1.238	0.365	1	0.188
1	0.230	0.158	0.239	0.027	1	0.365
35					35	
18	16.436***	7.932***	21.843***	12.162***	19	26.410***
90	1.594***	1.301	1.661**	1.241	95	1.127
18	1.648	1.757	2.120*	1.784	19	3.912***
18	2.284**	0.710	1.605	0.545	19	0.567
18	2.440***	1.834	2.555**	1.407	19	3.275***
18	0.594	0.766	0.643	0.891	19	0.695
18	2.104**	1.334	2.328**	0.628	19	2.818***
18	0.836	0.771	1.036	1.156	19	0.943
18	1.006	0.759	0.942	0.595	19	1.055
630					665	

The ant treatment had a significant impact on mortality rate, since 9 of the 11 plants that died were not visited by ants (Yates' corrected $\chi^2 = 4.246$, df = 1, $P = 0.039$). Also, 9 of the 11 plants that died were in the sun (Yates' corrected $\chi^2 = 4.246$, df = 1, $P = 0.039$). Indeed, eight *Inga vera* plants that died were growing in the sun without the presence of ants, showing a significant ant × light interaction (Yates' corrected $\chi^2 = 4.626$, df = 1, $P = 0.031$). Six plants that died had suffered nutrient addition and five did not, indicating that mortality rate was not affected by the nutrient treatment (Yates' corrected $\chi^2 = 0.000$, df = 1, $P = 1.000$).

DISCUSSION

Our results on *Inga vera* provide a clear experimental demonstration that the outcome of mutualistic interactions can be conditioned by the abiotic setting. Even over a scale of a few meters, light heterogeneity created strong fitness differences between individuals of *I. vera* by modifying the outcome of their interaction with the visiting ants. While sunny plants had a strong mutualistic relationship (+,+) with their ant partners, shade completely eliminated the benefits of ant protection, with the ants becoming true commensals (+,0).

There are several hypothetical reasons why plants were not receiving benefits from their ant partners in the shade (Rashbrook et al. 1992). First, lower ant density in the shade could have impeded effective defense levels; however, ants were equally abundant in the sun and in the shade. Second, lower herbivore abundance in the shade could have turned ant defense unnecessary; however, the number of herbivores was higher in the shade. Here we favor a third explanation of why the level of herbivory was higher and the plant performance was lower for unprotected *Inga* exposed to the sun, even though we failed to record any herbivore whose density could explain these patterns. The key to this apparent puzzle lies in the behavior of the exotic red-banded thrips, *Selenothrips rubrocinctus*.

S. rubrocinctus has been considered one of the major insect pests, attacking worldwide a variety of crops such as cocoa, mango, guava, avocado, and cashew (Peng and Christian 2004). In *Mangifera indica* L., for instance, the attack by red-banded thrips caused severe damage to newly mature leaves that resulted in partially defoliated or denuded trees producing low-quality fruits. Interestingly a high abundance of weaver ants *Oecophylla smaragdina* (Fabricius) was able to decrease shoot damage from 30% to 3% (Peng and Christian 2004). In *Inga vera*, red-banded thrips feed on the leaf undersurface by piercing the epidermis with their mouthparts, causing water loss. We hypothesize that in sunny conditions the thrips suffer higher levels of dehydration, forcing them to increase the water uptake on a per capita basis. For unprotected plants, red-banded thrips would be acting as a major water drain that increases the plant's water stress, jeopardizes photosynthesis, and causes tissue loss. In this situation, even the presence of a small sword of ants would be enough to disturb the feeding behavior of the thrips, reducing their negative impact on the plant's fitness. In contrast, in shade conditions the water stress for both plants and insects apparently was lower, and the impact of *S. rubrocinctus* became negligible. Unfortunately we failed to collect hard physiological and behavioral data to test this mechanism. However, independent of the accuracy of this particular proposed mechanism, the fact remains that the level of herbivory was much higher on unprotected plants exposed to the sun, and that the absence of the ants reduced by half the dry biomass of their mutualistic plant partners, causing plant death.

The addition of nutrients to *I. vera* plants significantly increased the production of leaves, leading to a higher number of leaves per plant. It also affected the herbivory rate of mature leaves during the winter and had a marginal effect on total plant biomass, and on root and shoot allocation. However, the absence of significant interaction between nutrients and ants demonstrated that, at least in this ecological context, nutrient availability did not influence the mutualism between *I. vera* and its ants.

Abiotic conditionality

The efficiency of ants as an antiherbivory defense varies from strong to weak (Janzen 1966, Bentley 1977, Koptur 1984, Vasconcelos 1991, Fonseca 1994, de la Fuente and Marquis 1999, Heil and McKey 2003), and a number of studies have failed to demonstrate that plants benefit from ant-attractive traits, such as extrafloral nectaries (Janzen 1975, O'Dowd and Catchpole 1983, Heads and Lawton 1985, Rashbrook et al. 1992). Although frequently biotic factors, such as ant or herbivore density, are presented as the main reason for the observed differences among ant–plant systems or for the spatiotemporal variation within systems, variation of abiotic factors can be behind such patterns. Abiotic factors can modulate the outcome of mutualism by altering the tritrophic interaction among plants, herbivores, and ants. The availability of light, water, and nutrients are behind major differences in the abundance, richness, and composition of extrafloral-nectary-bearing plants among habitats (Oliveira and Leitão-Filho 1987, Alonso 1998, Rico-Gray et al. 1998, de la Fuente and Marquis 1999). Within a given habitat, small-scale variation in resource distribution can cause major differences in host quality (Heil et al. 2001). According to the resource availability hypothesis, resource levels can also modify the level and type of plant defense (McKey 1984, Coley et al. 1985). In *Cecropia* (Cecropiaceae), light and nutrients affect plant growth, modify the production of food bodies, and possibly determine the identity of associated ants among congeneric hosts (Davidson and Fisher 1991, Folgarait and Davidson 1994, 1995). In *Macaranga triloba* (Euphorbiaceae), soil fertilization increased the production of food bodies and nectar to the ants, producing a significant decrease in herbivory level (Heil et al. 2001).

Abiotic factors can affect the outcome of mutualisms by acting directly on the ants. For instance, ant–plant protective mutualisms are more common in the tropics, and this has been partially attributed to the lower diversity of ants on colder zones (Davidson and McKey 1993). The abundance of ants associated with *Inga vera* did not respond to small-scale differences in nutrient levels and light intensity. However, the seasonal dynamic of the abundance of *Crematogaster quadriformis* and *Camponotus fastigatus* in our subtropical site led the plants to be almost unprotected during the winter months. Although ants still decreased the herbivory rate of mature leaflets in this season, it is conceivable that in sites farther from the equator, the winter will become longer and deeper and the mutualism will break down.

The herbivore fauna can also be directly affected by abiotic factors. Herbivorous insects are sensitive to dehydration and have behavioral mechanisms to avoid water loss. Many have nocturnal activity or, at least, decrease their activity during the warmer hours of the day. Others restrain their foraging activity to shady places and hide themselves from the sun on the bottom surface of the leaves. *Selenothrips rubrocinctus* had a clear preference for *Inga vera* plants growing in the shade. However, whether this is a direct effect of the light factor or an indirect response to the host quality is uncertain. The herbivores fluctuated substantially among seasons, suggesting a relationship with abiotic factors.

Plant dependency on the ants

Plants with extrafloral nectaries are opportunistically associated with guilds of omnivorous ant species that change in composition and abundance in space and time (Bentley 1976). On the other hand, ants associated with extrafloral nectaries rarely rely on a single plant species as their unique food source, feeding on different food resources depending on their availability (Moya-Ragoza and Larsen 2001). Therefore the degree of dependency of a given extrafloral-nectary-bearing plant species on a particular ant species and vice versa is believed to be low (Bentley 1976, 1977). Nevertheless, ecological interactions that are weak in a broad geographic scale can turn out to be strong in local arenas (Thompson 1988, 1994).

In the ecological context of our study site, an old field, the establishment, growth, and survival of *Inga vera* were strongly dependent on only two ant species, *Camponotus fastigatus* and *Crematogaster quadriformis*. Under natural sun conditions, plants from which the ants were experimentally excluded ended the experiment with half the height and half the number of leaves and leaflets. During the summer and winter, their mature leaflets exhibited a higher herbivory rate that accumulated throughout time to produce a higher overall herbivory level. Similar effects of ant protection on plant performance have been reported on several ant–plant systems (Janzen 1966, Bentley 1976, 1977, Koptur 1984, Barton 1986, Vasconcelos 1991, Fonseca 1994, de la Fuente and Marquis 1999). However, two additional results should be highlighted.

First, in the sun, the presence of the ants almost doubled their plant partners' dry biomass, an integrated measure that summarizes the net benefit of the plants during the nonreproductive phase. Although most experimental studies evaluate the impact of the ants on vegetative traits and not on the plants' reproduction (but see Barton 1986, Vasconcelos 1991), as far as we know, no previous study has reported the effect of ants on plant biomass, an important component of a plant's fitness. Second, the presence of the ants positively affected *I. vera* survival, a fundamental component of the plant's fitness. Very few experimental studies demonstrated the effect of ants on plant mortality (Janzen 1966, Koptur 1991). Perhaps not coincidentally, one of the few cases was recorded within the genus *Inga* itself. Studies on *I. densiflora* and *I. punctata* in Costa Rica showed that ants were effective in removing caterpillars and other herbivores from the leaves. Herbivory damage doubled in plants from which ants were experimentally excluded. In the sapling stage, mortality rate increased with herbivory level, achieving 80% on totally defoliated plants (Koptur 1984, 1991). Although mutualisms between extrafloral-nectary-bearing plants and their ants are considered to be facultative, our results suggest that, at least in some ecological settings, dependency levels can be higher than previously expected.

Temporal variation

Although our experiment was mainly designed to evaluate the causes underlying spatial variation of the mutualism, the interaction between *Inga vera* plants and their ants changed considerably through time, as evidenced by the significance of the time factor on most repeated-measures analyses of variance. This can be attributed to two main temporal processes: seasonality and ontogeny. Seasonality is a common factor for explaining temporal variation in mutualisms, since costs and benefits of the interaction can change from season to season (Horvitz and Schemske 1990, Alonso 1998, Moya-Ragoza and Larsen 2001, Billick and Tonkel 2003). For instance, seasonal variation in temperature and rainfall modified the intensity of ant–plant mutualisms in several Mexican localities (Rico-Gray et al. 1998). A great seasonal variation was found in the benefits provided to *I. vera* plants by their associated ants. In months of elevated temperature leaf production was higher, and ant visitation was more intense. In colder months leaf production dropped considerably, and ant abundance declined accordingly. The temporal dynamics of the herbivores also exhibited a clear seasonal pattern, although not in synchrony with the mutualists. This phenological asynchrony explains why ant protection and plant mortality rate were more intense during the summer.

Furthermore, over the course of the experiment *I. vera* plants grew from seedlings to small trees containing tens of leaves with fully developed extrafloral nectaries. The significance of the time factor on the repeated-measures analyses of variance certainly can be attributed to this ontogenetic change. Ontogeny was not only responsible for changes in the phenological attributes of the host, but significantly affected the abundance of the associated insects. By accumulating more leaves, the offer of a resource to the herbivores increased substantially. Not surprisingly, the abundance of *Selenothrips rubrocinctus* per plant was higher in the second year of the experiment. Similarly, by increasing the offer of nectar during the ontogeny, the plants induced a higher abundance of ants in the second year. In the Amazon, the ontogeny of the ant-plant *Tachigali* (Caesalpinioideae), from small saplings to emergent trees, not only changed the number of associated ants but also the identity of the ant partner (Fonseca 1993, Fonseca and Benson 2003).

Mutualisms are the product of a complex web of direct and indirect biotic interactions that can change considerably in time and space. By recognizing that abiotic factors represent an important primary source of variation in ant–plant–herbivore interactions, we will improve further the understanding about the ecology and evolution of mutualisms.

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APPENDIX

A table summarizing means for variables related to *Inga vera* size, vegetative phenology, herbivory level, herbivory rate, and dry biomass is available in ESA's Electronic Data Archive: *Ecological Archives* E086-113-A1.