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RESEARCH ARTICLE

Exposure to creosote bush phenolic resin causes avoidance in the leaf-cutting ant *Acromyrmex lobicornis* (Formicidae: Attini)

La exposición a la resina fenólica de jarilla causa deterrencia en la hormiga cortadora de hojas *Acromyrmex lobicornis* (Formicidae: Attini)

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ABSTRACT

We focused our study on the effects of *Larrea cuneifolia* phenolic resin on leaf-cutting ants from two populations (Sierra de las Quijadas National Park and San Roque) of *Acromyrmex lobicornis* in San Luis, Argentina. We conducted two bioassays of food choice (field and laboratory) to compare the effects of phenolic resin on ant workers from these two populations. Results of the field experiment indicated that there were no differences in preference for either leaves treated with resin or untreated leaves among colonies from both localities. However, results of the laboratory experiments with individual ants indicated a significant effect of population and treatment on the time spent in different treatments. While leaf-cutting individual workers from Quijadas preferred the phenolic resin, workers from San Roque avoided it. These results evidence that ants respond according to time of exposure to chemicals from plants (presence or absence) and that the effects of resin among a population can be observed and measured on individual ant workers, even in the absence of fungus garden influences in the nest.

Key words: *Acromyrmex lobicornis*, avoidance, choice food, preference, San Luis-Argentina.

RESUMEN

Este estudio se ha centrado en los efectos que produce la resina fenólica de *Larrea cuneifolia* sobre dos poblaciones distintas (Parque Nacional de Sierra de las Quijadas y la localidad de San Roque) de *Acromyrmex lobicornis* en San Luis, Argentina. Se diseñaron dos tipos de bioensayos (a campo y en laboratorio) para comparar los efectos de la resina fenólica sobre las hormigas obreras de estas dos poblaciones. Los resultados de los experimentos de elección de la oferta alimentaria en el campo, indicaron que no hubo ninguna diferencia de preferencia entre las colonias, ni por las hojas tratadas con resina ni por las hojas sin tratar para ambas localidades. Sin embargo, los resultados de los experimentos de laboratorio con las hormigas obreras individualmente indicaron efectos significativos entre las poblaciones y entre los tratamientos. Mientras que las hormigas obreras de Quijadas prefirieron la resina fenólica, las obreras de San Roque la rechazaron. Estos resultados evidencian que las hormigas responden de acuerdo al tiempo de exposición a los productos naturales de las plantas (presencia y ausencia) y que los efectos de la resina entre poblaciones, puede ser observada y medida en las hormigas obreras aun en ausencia de la influencia del jardín de hongos de la colonia.

Palabras clave: *Acromyrmex lobicornis*, deterrencia, elección alimentaria, preferencia, San Luis-Argentina.

INTRODUCTION

Attini ants (Hymenoptera: Formicidae) are the dominant herbivores in the neotropics, consuming far more vegetation than any other group of animals including mammals, homopterans and lepidopterans (Hölldobler & Wilson 1990, Wirth et al. 2003). Leaf-cutting ants selectively cut vegetation into small fragments that they transport to the nest, where the plant material is degraded by a symbiotic fungus (Cherret 1989). The fungus garden represents the sole food source of the developing brood. Adult workers obtain

a large proportion (more than 90 %) of their energy requirements from the plant sap of the harvested material (Roces 2009). These insects and the fungus they feed on are sensitive to the presence of secondary compounds (Hebling et al. 2000, Bigi et al. 2004), playing the most important role in the acceptance or avoidance of potential foraging plants (Howard 1987, 1988).

The tight relationship between time of exposure over evolutionary and ecological time scales and natural selection has been shown to be responsible for habituation (Szentesi & Bernays 1984, Papaj & Prokopy 1989) or local adaptation in insects (Fox & Morrow 1981, Mayhew 1997), however little is known about different responses of leaf-cutting ants to chemical compounds from various habitats (Saverschek et al. 2010). Another intriguing aspect of the natural history of leaf-cutting ants is that the mechanisms behind the transfer of information from one worker to another are unclear. Workers recognize plants with harmful compounds to the fungus garden and this information is passed onto other workers (Ridley et al. 1996, North et al. 1999), therefore individual foraging decisions are vital for the survival of the colony.

Leaf-cutting ants appear to be well suited to explore the rules underlying the organization of collective foraging. At the individual level, research has only focused on the rules workers use to decide about the size of leaf fragment to be cut (Roces 2002) and most of the literature on foraging behavior has focused on plant selection at the colony level (field and laboratory) (Roces 2009). Many of them have demonstrated that foraging individuals' decisions workers are learnt and mediated by the condition of the symbiotic fungus (North et al. 1999, Herz et al. 2008, Saverschek et al. 2010). The multiple factors affecting foraging decision are difficult to evaluate in natural conditions, so it is not surprising that sophisticated experimental designs had to be developed in the laboratory to explore the mechanisms underlying individual decision making and social communication (Roces 2009).

Acromyrmex lobicornis (Emery) is a generalist species which behaves mainly as a cutter during spring and as a picker during winter (Claver 2000). Widely distributed in the central part of Argentina this species reaches from subtropical areas in southern Brazil and Bolivia (23° S) to Patagonia (44° S) (Farji-Brener & Ruggiero 1994, Claver 2000). Claver (2000) found that colonies of the leaf-cutting ant *A. lobicornis* forage on *Larrea cuneifolia* Cav. (Zygophyllaceae) in the Monte Desert in Argentina. Both species share most of their distribution area, mainly in the central arid and semiarid regions of Argentina (Hunziker et al. 1977).

Larrea cuneifolia (also called creosote bush) is a plant known for the toxic phenolic resin that covers their leaves. Both mature and immature leaves of creosote bush contain this phenolic resin in a proportion of 10 % and 25 % dry mass, respectivelly (Meyer & Karasov 1991). The resin is composed of a complex mixture of partially O-methylated flavones and flavonols. Its major component (40 % dry mass) is nordihydroguaiaretic acid (NDGA) (Mabry et al. 1977, Rhoades 1977). Its main biological activities are anti-fungic, antiviral and phytotoxic (Mabry et al. 1977, Elakovich & Stevens 1985, Arteaga et al. 2005, Vargas-Arispuro et al. 2005).

In this study, we examined preference/avoidance responses of individual ant workers (laboratory studies) and ant workers in their field colonies (field studies) of *A. lobicornis* from two different habitats, to *L. cuneifolia* phenolic resin. The habitats considered here were (i) Sierra de las Quijadas, San Luis Argentina (hereafter called Quijadas ants) where *L. cuneifolia* is present and (ii) San Roque, San Luis Argentina (hereafter called San Roque ants) where *L. cuneifolia* is absent. We tested the hypothesis that Quijadas ants will show preference to creosote bush phenolic resin, while San Roque ants will show avoidance to creosote bush phenolic resin. We also propose that individual workers, even when isolated from the colony, are capable of distinguishing creosote bush phenolic resin.

METHODS

Study sites

Sierra de las Quijadas National Park is located in the west-central part of Argentina (32°47' S, 67°10' W at 800 masl). Its environment constitutes an ecotone between the biogeographical provinces of El Chaco (characterized by thick profuse forests with large-sized species) and El Monte (characterized by xerophilous vegetation and by resinous and prickly shrubs) (Cabrera & Willink 1980). San Roque is located in the west-central

part of Argentina (33°16'20" S and 66°16' W at 906 masl), 105 km from Sierra de las Quijadas. Its environment corresponds to the biogeographical province of El Espinal (characterized by xerophilous woods) (Cabrera & Willink 1980).

Experimental design

In order to test our hypothesis we designed two experiments: (i) a two-choice field experiment, aimed to test the effects of the phenolic resin of *L. cuneifolia* on the foraging behavior of forager workers of *A. lobicornis* on an active foraging trail; and (ii) a contact Petri dish bioassay aimed to measure the avoidance response of individual worker *A. lobicornis* when the phenolic resin is present. Both tests were performed on forager ant workers from Quijadas and San Roque populations.

L. cuneifolia material used in each experiment was obtained from Sierra de las Quijadas National Park. Leaves and branches from six plants with the same phenological characteristics and with a minimum distance of 50 m among them (to avoid collecting material from clones) were collected.

Two-choice field test: field experiments were performed during the wet season in both locations: during December 2004 in Sierra de las Quijadas National Park and during March 2007 in San Roque. Three mature ant colonies separated by more than 1000 m, were randomly chosen at each locality. An active foraging trail from each nest of *A. lobicornis* was used as the test arena. Two types of plant material were offered along each foraging trail (Howard 1988): (i) untreated leaves with resin (hereafter called LR) and (ii) leaves without resin (hereafter called LWR), obtained by treating leaves with ethyl ether for three hours (50 g of leaves / 300 ml of ethylic ether) to remove the resin following Mangione et al. (2000). This resin extraction method allows the maximum level of extraction without altering other chemical properties of the leaves. Twenty grams of fresh plant material (10 g type^{-1}) was placed along a trail. Trials were conducted for three hours and were performed over two consecutive days once a day. Remains were oven dried to constant mass. Dry matter removed by ants, over the three hours period, was taken as an indicator of preference.

Two-choice contact bioassays: laboratory tests were carried out during December 2005 and April and May 2006 to compare the avoidance by previous experience of workers of *A. lobicornis* to leaves either treated or untreated with ether and resin. We used *A. lobicornis* workers, collected from an active foraging trail in each environment: (a) two mature colonies from the Sierra de las Quijadas National Park (with *L. cuneifolia)* and (b) two mature colonies from San Roque (no *L. cuneifolia)*. Ant workers were collected manually and kept in boxes with some ice to reduce their activity until the experiment began. We isolated the foragers for the forty eight hours before the experiment. A series of Petri dish experiments were undertaken to test avoidance of *A. lobicornis* to the following treatments levels: untreated (U); ether (E); leaves with resin (LR); leaves without resin (LWR) and resin extracted with ether (EER). The combinations of treatments resulted in 12 different experiments: U vs. U; E vs. E; E vs. U; EER vs. E. E vs. LR; LR vs. U; LWR vs. U; LWR vs. LR; LWR vs. E; EER vs. U; EER vs. LR; EER vs. LWR. The first three experiments were used as controls. Treatments including E were used as positive controls as ether had been used as the extraction solvent.

The experiment consisted in wiping the halves of a 9-cm diameter Petri dish (lid and base) with leaves or chemical compounds (0.1 ml), following the combinations described above. Treatment of Petri dishes with liquid material (E and EER) was performed using a small paint brush. EER is the remaining extract after washing *L. cuneifolia* leaves out with ether. The extraction was carried out as described in the field experiment. The resin concentration of EER in one half of the Petri dish was 0.37 µg mm^{-2}, which is 52 times lower than the amount of resin present in each single leave of *L. cuneifolia* (19.72 µg mm^{-2}). LR was obtained by wiping one gram of fresh leaves or *L. cuneifolia* in one half of the Petri dish. The average amount of resin measured after wiping one gram of leaves on the Petri dish was 0.21 ± 0.04 µg mm^{-2} (n = 10). To determine the amount of resin in the Petri dish, the dish was rinsed with ether, collected in a vial, weighted and oven dry to constant weight. The same procedure using *L. cuneifolia* leaves without resin was followed to obtain LWR. A new Petri dish was used for each trial. Each Petri dish on the external surface (lid and base) was marked where combinations of treatment sides met (lid and base). The halves were aligned so each side (up and down)

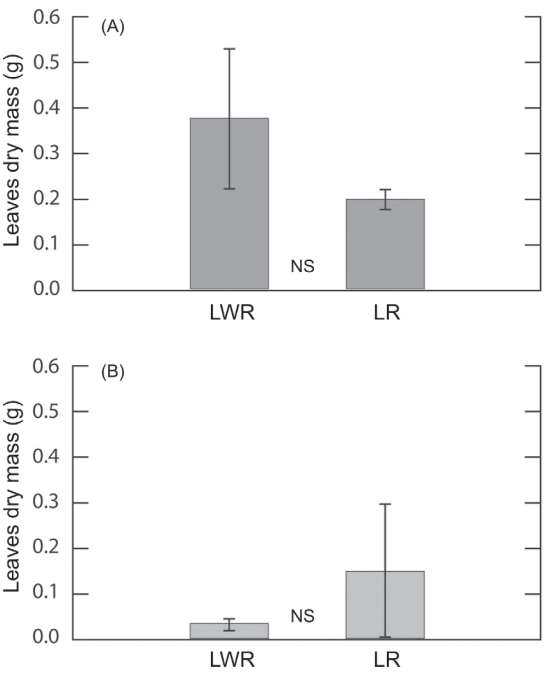

Fig. 1: Comparison of dry matter (mean ± SE) of *Larrea cuneifolia* leaves removed by *Acromyrmex lobicornis* ants from three different nests from populations at (A) Sierra de las Quijadas National Park and (B) San Roque, in the two-choice field test. LWR: leaves without resin, LR: leaves with resin. NS means no significant difference.

Comparación de la masa seca removida (media ± EE) de *Larrea cuneifolia* por las hormigas de *Acromyrmex lobicornis* de tres diferentes nidos en: (A) Parque Nacional de Sierra de las Quijadas y (B) San Roque. Para el test de doble elección a campo con LWR: hojas sin resina y LR: hojas con resina. NS indica diferencia no significativa.

matched, after which one ant foragers was placed in the centre of the Petri dish and given a 20 s acclimatization period. Treatments were assigned randomly to each side of the Petri dish. Time spent in each treatment by one ant foragers was recorded over a 300 s observation period. Mid-way through the observation period (at 150 s) the Petri dish was flipped over to use the other part of the dish as the arena. Avoidance was assessed by considering the difference in time (seconds) spent between treatments during each experiment (Ghazoul 2001). Each experimental treatment was replicated 28 to 30 times (Raffa et al. 2002). The experiment was recorded on a Sony 8 mm TR 54 digital camera and then transferred to a DVD to facilitate the scoring of the time spent at each treatment.

Statistical analyses

We used a two-factor repeated measurement ANOVA to test for the effect of population, leaf treatment (i.e. LWR vs. LR) and time (i.e. day 1 vs. day 2) on the amount of biomass consumed by foragers in the field experiment. For the laboratory experiments, we used a two factor nested ANOVA to test for the effect of population, treatment (twelve different levels) and colonies within population on the difference in time (seconds) spent between treatments during each assay. To facilitate the calculations and the interpretation of the difference among the treatments they were ordered left and the right, always following the same order for 706 total experiments. Values close to zero mean that workers spent approximately the same time either side of the Petri dish. Negative values mean that workers avoid what was applied in the left side of the Petri dish and positive values mean that workers avoid what was applied in the right side of the Petri dish (see Table 2 and Fig. 2 for experiment notations). Independent contrasts were used to test differences between populations for each treatment level, considering the mean square error from the factorial ANOVA but without nesting colonies within population. Within each population, we used Tukey's multiple comparison tests

TABLE 1

Field and laboratory two-choice bioassays, for San Roque and Quijadas ants workers of *A. lobicornis*. Removed biomass in field was statistically measured using a two factor repeated measurement ANOVA; with population (Quijadas and San Roque) and leaf condition (leaves with resin and leaves without resin) as factors. Differences between treatments in the Petri dish bioassay were analyzed with a two factor nested ANOVA. Note: Population are both Quijadas and San Roque localities, treatment are the twelve different combinations of levels or type of exposure, colonies are each ant nests. The asterisk means significant difference.

Bioensayos de doble elección de campo y laboratorio, para las hormigas obreras de *A. lobicornis* de Quijadas y San Roque. La biomasa removida en los ensayos a campo fue medida estadísticamente usando un ANOVA de medidas repetidas a dos factores; por un lado las poblaciones de Quijadas y San Roque y por el otro la condición de las hojas: hojas con resina y hojas sin resina. Las diferencias entre los tratamientos de los bioensayos en las cajas de Petri fueron analizadas con un ANOVA anidado a dos factores. Nota: Las poblaciones son ambas localidades de Quijadas y San Roque, los tratamientos son las doce diferentes combinaciones de niveles o tipos de exposición, las colonias son cada uno de los nidos de las hormigas. El asterisco indica diferencia significativa.

Experiments/Source	F	d.f.	P
Two-choice field test (repeated measures ANOVA)			
Population	5.9	1	0.041
Leaf condition	0.13	1	0.72
Leaf condition x Population	3.32	1	0.1
Error		8	
Two-choice contact bioassays (nested analysis)			
Population	63.07	1	< 0.001*
Treatment	5.3	11	< 0.001*
Colonies (Population)	1.24	2	0.29
Treatment x Population	14.11	11	< 0.001*
Treatment x Colonies (Population)	2.23	22	0.001*
Error		658	
Two-choice contact bioassays (factorial analysis)			
Population	61.29	1	< 0.001*
Treatment	5.1	11	< 0.001*
Treatment x Population	13.68	11	< 0.001*
Error		682	

to compare the mean difference in time spent in each treatment across treatment combinations. Statistical tests were performed using the software SYSTAT 10 (Wilkinson 2000).

RESULTS

Two-choice field test

We found that Quijadas ants removed more leaves without resin (LWR) than San Roque ants (Fig. 1; Table 1). However, San Roque ants showed a smaller rate of removal of both offers. There was no preference for leaf condition (LR or LWR) and there was no interaction between leaf condition and population (Table 1). There was no effect of time (days) ($F_{1, 8}$ = 0.0002, P-value = 0.98).

Two-choice contact bioassays

There was a significant effect of population and treatment on time spent in different treatments (Table 1). Colonies nested within population did not show significant differences between treatments. Independent contrasts (see Table 1 for the Mean Square Error of the factorial analysis), showed that San Roque ants spent more time in E, LWR, LR or U when the other halve was treated with the mixture of ether and resin (EER).

Independent contrast analysis showed that leaf-cutting ants from the two populations assigned different amounts of time to each treatment. Foragers from San Roque spent only 21 %, 29 %, 28 % and 24 % of the total time in EER

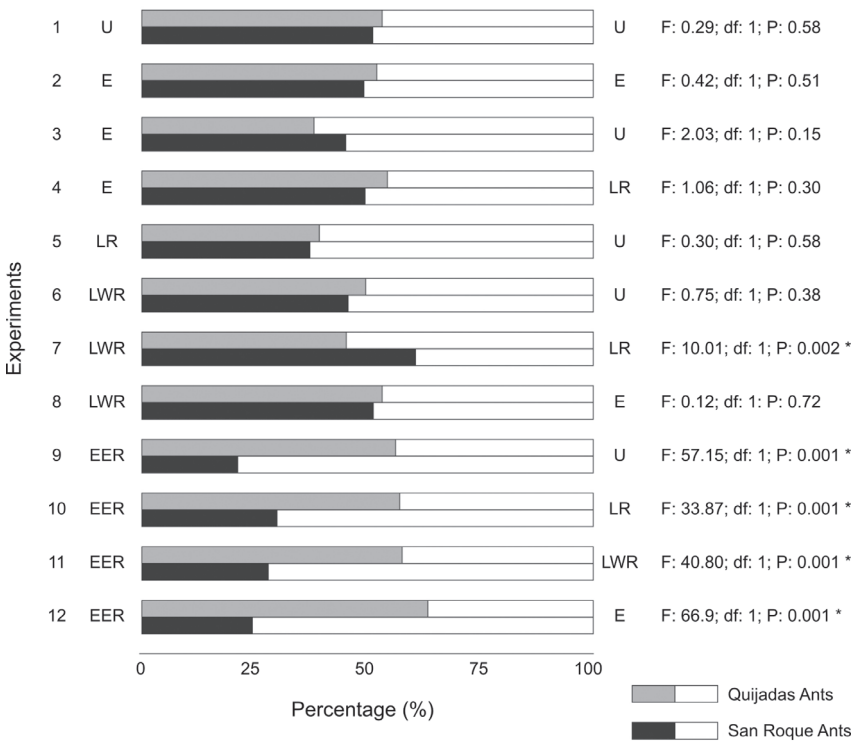

Fig. 2: Percentage of time spent in each treatment for each experiment for Quijadas ants (gray and white combination) and San Roque ants (black and white combination). Each experiment is the combination of the treatments, for example: experiment E vs. LR, means that the left side of the Petri dish contained ether (E) and the right side contained resin from fresh leaves (LR). Ants were exposed for 300 s. F and P-values come from independent contrast analyses between populations for each treatment. Each experiment was carried out with N = 28 to 30 ants.

Porcentaje del tiempo que pasaron las hormigas de Quijadas (combinación gris y blanco) y las de San Roque (combinación negro y blanco) para cada tratamiento y para cada experimento. Cada experimento es la combinación de los tratamientos, por ejemplo: el experimento E vs. LR, significa que el lado izquierdo de la caja de Petri contiene éter (E) y el lado derecho contiene hojas frescas con resina (LR). Las hormigas fueron expuestas durante 300 s. Los valores de F y P provienen de análisis de contraste independiente entre las poblaciones para cada tratamiento. Cada experimento fue realizado con N = 28 a 30 hormigas.

for experiments 9, 10, 11 and 12, respectively. Moreover, while San Roque ants spent 40 % of the time in the LR side, Quijadas ants, spent 55 % (experiment 7) (Fig. 2).

Tukey's multiple comparison tests indicated that San Roque ants spent more time in the treatments without resin. These results suggest that San Roque ants show avoidance to EER. In contrast, Quijadas ants showed a tendency to spend more time in EER for experiments 9 to 12 compared to experiment 3 (Fig. 2 and Table 2). Quijadas ants also showed significant differences between experiments involving EER and the experiment involving LR vs. U (experiment 5). There was no effect of ether on any of the experiments of this study, neither there were differences among the control experiments (1 to 3). Experiments involving one side with diethyl ether (E) and the other side of the Petri dish with another substance did not differed from experiments 1 to 3 (Table 2).

DISCUSSION

Differential response of ant foragers to creosote phenolic resin

Acromyrmex lobicornis from both populations did not show preferences for LR or LWR during field experiments. However, during laboratory experiments individual workers from San Roque consistently avoided the side of the Petri dish treated with resin. In general, San Roque ants spent only about a quarter of time on treatments where creosote bush resin was present, whereas they spent about 60 % of their time on treatments without resin. In contrast, Quijadas ants showed the exact opposite. They spent more time in treatments where creosote bush resin was present, even when they could chose the side not treated with resin (see Fig. 2). Taken together, this suggests that Quijadas ants and San Roque ants behave differently when they are exposed to the same natural compound. While San Roque ants showed an extreme avoidance to resin, Quijadas ant's responses were moderate without rejecting the resin. It is then in the frame offered by laboratory assays that we accepted both hypotheses: (1) there is a differential response between populations, and (2) individual ants, when isolated from the colony, are capable of distinguishing creosote bush phenolic resin. These laboratory results constitute a new evidence of differential individual ant responses between populations when exposed to the same natural products, even in the absence of the influence of the fungus garden in the nest.

The role of ant foragers

Our study revealed the presence of intraspecific variability in food choice by leaf-cutting ants from different habitats. Saverschek et al. (2010) showed for *Atta colombica* (Guérin) that workers' foraging experience is a key factor in decision making, especially in environments with intra-annual variation in leaf quantity and quality. Dussutour et al. (2009) demonstrated that individual workers of *Atta colombica* make individual decisions on load size having experienced a restriction to loading only once, for a short period of time and without having information from workers in the nest. In our study, at the individual level, the workers associated the chemical cues from their habitat depending on colony origin and consequently San Roque ants avoided the unknown natural products. Even though it is clear that individual foragers have enough information to make a decision to search for the experienced plant species (Röschard & Roces 2011), it is not known why workers foraging in field assays responded differently.

In the laboratory assays of our study the environmental cue was the contact or odor. Roces (1990) demonstrated that the conditioning to odor cues from chemicals for a scout of *Acromyrmex lundi* (Guérin) under controlled laboratory conditions may differ from the set of complex cues ants integrate when they are exposed to food offers in the field. However, San Roque ants not only avoided EER, but also preferred LWR over LR (see Fig. 2, experiment 7). In short, they avoided anything containing phenolic resin. However, we do not know which of the resin's secondary compounds were responsible for the effect observed in our study. Creosote bush phenolic resin is composed, besides phenolics, of many other compounds such as terpenes and waxes (Mabry et al. 1977). These compounds in combination may shape the entire set of complex cues that ants are able to detect and integrate in the field. For

TABLE 2

Results of Tukey's post hoc tests to compare experiments within each population. Values shown are: sample size N, mean time difference spent in each treatment in seconds, standard error. Mean difference (seconds) was calculated as the average of the difference in time spent between treatments. Values close to zero indicate that ants spent approximately the same time on either side of the Petri dish. Negative values indicate that ants avoid the treatment in the left side of the Petri dish and positive values indicate that ants avoid the treatment in the right side of the Petri dish. Fifth and ninth column indicate significant difference among experiments carry out for Quijadas ants and San Roque ants respectively. Note: The asterisks indicate a significant difference: * P-value < 0.05; ** P-value < 0.01 and *** P-value < 0.001.

Resultados del test Tukey's post hoc para comparar los experimentos entre cada población. Los valores mostrados son: el tamaño de la muestra N, la diferencia del tiempo medio en segundos que pasan las obreras en cada tratamiento, el error estándar. La diferencia media del tiempo (segundos) fue calculada como el promedio de las diferencias de tiempo pasado entre los tratamientos. Los valores cercanos a cero significan que las obreras pasaron aproximadamente el mismo tiempo en ambos lados de la caja de Petri. Valores negativos significan que las hormigas rechazaron el tratamiento del lado izquierdo de la caja de Petri y los valores positivos significan que las hormigas rechazaron el tratamiento del lado derecho de la caja de Petri. La quinta y novena columna indican las diferencias significativas entre los experimentos realizados para las hormigas de Quijadas y las de San Roque respectivamente. Nota: Los asteriscos indican diferencias significativas: * P < 0.05; ** P < 0.01 and *** P < 0.001.

Experiments	Quijadas ants			Tukey Honestly Significant	San Roque ants			Tukey Honestly Significant
	N	Mean difference in time (s)	SE	Difference	N	Mean difference in time (s)	SE	Difference
1-U vs. U	30	16.6	29.1		29	1.1	15.4	
2-E vs. E	30	10.8	18.3		30	-7.5	15.1	9***, 10***, 11**, 12***
3-E vs. U	29	-72.8	20.2	10***, 9**, 11**, 12**	29	-31.8	16.7	8*, 9***, 10**, 11*, 12*
4-E vs. LR	30	23.9	19.6	3*	28	-5.7	22.3	9***, 10***, 11**, 12***
5-LR vs. U	30	-63.8	19.7		30	79.3	21.1	
6- LWR vs. U	29	-4.1	19.5		28	-29.1	17.1	
7-LWR vs. LR	30	-30.5	18.5		29	60.7	14.3	5***
8-LWR vs. E	30	16	22.1		29	5.8	19.2	
9-EER vs. U	29	41.3	20.1	5*	30	-174.2	20.2	1***, 5*, 6***, 7***, 8***
10-EER vs. LR	30	43.1	20.4	5**	29	-122.8	22.2	1**, 6*, 7***, 8***
11-EER vs. LWR	30	46.3	19.4	5*	30	-134.3	23.6	1***, 6**, 7***, 8***
12-EER vs. E	29	78.5	18.8	5***, 8*	29	-154.8	24.4	1***, 5***, 6***, 7***, 8***

example, the alkaloid ricinine from *Ricinus communis* L. (Euphorbiaceae) did not elicit lethal effects when administered alone to *Atta sexdens rubropilosa* (Forel), but recinine in combination with other secondary compounds of this plant did increase adverse effect on these ants (Bigi et al. 2004). Therefore, single metabolites will not necessarily elicit adverse effects on workers, unless they are mixed with others or at high concentrations (Hubbell et al. 1984, Howard 1987, 1988). However, for other species, *Astroma* spp., (Rhoades 1977) and Rockland mice (Rios et al. 2008), NDGA was the main component of the phenolic fraction of the resin that proved to be the responsible for toxicity.

A key question is whether ants forage on known or unknown food items. Howard et al. (1996) suggested that ants prefer to forage on known food items rather than on unknown ones. In our two choice field tests, *A. lobicornis* showed indifference to a new food resource. It has been demonstrated for *Atta colombica* that novelty may delay but not prevent foraging on a new food resource. Acceptance may be delayed by more than 48 h (Howard et al. 1996), although Vasconcelos & Cherrett (1996) measured leaf preference in field assays on an active column of *Atta laevigata* (Fr. Smith) successfully in only 30 minutes. However, it is known that leaf-cutting ants receive information concerning the nutritional status of the fungus garden and then can then delay rejection of a food item via avoidance learning within two to three days (Herz et al. 2008, Saverscheck et al. 2010).

Other subjects of interest are the time required for individual ants to learn environmental cues, the time to respond to environmental stimuli or the time individual ants retain information (Dussutour et al. 2009, Saverschek et al. 2010). Some decisions are taken in a short period of time, as it was shown in our laboratory assays. Where the information and individual ant has come from and how long it will last? Some laboratory experiments have demonstrated long-term memory of individual workers in leaf-cutting ant species. For example, Ridley et al. (1996) found that foragers of *Atta cephalotes* (Linnaeus) retained information from the colony over a 30 week period, and experiments with *Acromyrmex lundi* testing a fungicide chemical (Herz et al. 2008) showed that rejection behavior persisted for up to nine weeks. In field colony experiments with *Atta colombica* (Saverschek et al. 2010), it took up to 18 weeks for foragers to harvest a previously avoided plant. This difference seems to be species specific. We suggest that individual ant foragers of *A. lobicornis* retain information from their colonies and therefore can use this information in decision making processes. Our laboratory experiment results indirectly support that the workers ants learn, probably through earlier experience with the characteristics of its habitat (Fowler 1982), and this may have allowed ant foragers to retain enough information to make a decision on food resource based on long term memory (Ridley 1996, Herz et al. 2008, Saverschek et al. 2010).

Therefore the role of foragers play in a leaf-cutting ants well organized society may need more attention since it might have ecological implications regarding foraging area, food resource use and competition. Roces (2002) analyzed the interplay between food-collection behavior and how individual complexity contributes to the emergence of collective foraging patterns in leaf-cutting ants. Probably, as suggested by Saverschek et al. (2010), that the colony-wide memory may be represented by the sum of individuals' foraging experiences. In our study at the individual level leaf-cutting ants from Quijadas are habituated to the chemical cues from creosote bush phenolic resin and that *A. lobicornis* appears to be a promising natural system to study the developmental and behavioral factors underlying habituation to plant natural products. Our findings though, highlight the vital role individual workers of *A. lobicornis* may play for the colony.

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