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Influence of natural fragrances on recruitment olfactory conditioning and acceptance of forage material in the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae)

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ABSTRACT. *Influence of natural fragrances on recruitment olfactory conditioning and acceptance of forage material in the leaf-cutting ant* Atta sexdens rubropilosa *(Hymenoptera: Formicidae).-* We examined the effect of natural fragrances on olfactory conditioning of recruitment to new food sources and the effects of concentrations of natural fragrances on pick-up responses of the leaf cuttinmg ant, *Atta sexdens rubropilosa*, in laboratory and field colonies. 0.01% concentrations of eugeniol and eucalyptol, applied to corn flakes, were compared with untreated corn flakes. We were unable to demonstrate olfactory conditioning by previously exposing scouts to new food odors, unlike recorded examples in *Acromyrmex lundi.* Furthermore, using serial dilutions (1.0, 0.1, 0.01 and 0.001%) of these fragrances, acceptability of fragment impregnated corn flakes was inversely related to concentration, but increased over time as fragrances volatilized. These results may be interpreted as indicating different types of recruitment processes, probably in response to different ecological uses by these leaf-cutters in their natural environment. These results may also suggest why leaf-cutters often do not carry cut vegetation during the same day - they await dissipation of inhibitory volatiles.

KEY WORDS. *Atta sexdens*, Conditioning, Acceptability, Behavior, Foraging, Recruitment

Introduction

Leaf-cutting ants (*Atta* and *Acromyrmex*) cultivate their food fungus gardens on a number of vegetable sources which are harvested by the ants in the Neotropics. However, little is known of the sources of information used to organize and regulate recruitment to food sources, or as to the factors that determine if a vegetable source will be cut and carried to the nest (Fowler, et al., 1991). Recently, Roces (1990) suggested that olfactory conditioning is present in *Acromyrmex lundi* (Guérin) that allows scout ants to transmit information of resources to recruited ants, which find the resource patch by following a pheromonal trail. This process was interpreted as a regulator of load-size selection (Roces & Nuñez, in press). However, due to major ecological and behavioral differences between species, it does not follow that this system should be present in all leaf-cutters. For example, *A. lundi* specializes on flower fall and softer vegetation,

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while other species, such as *A. sexdens* (L.) actively cut vegetation (Fowler & Stiles, 1980). The mass recruitment system of *A. lundi* is more restricted. Also, *A. sexdens* specializes on longer-lived resources than does *A. lundi* (Fowler, et al., 1991). In this paper, we examine the presence of olfactory conditioning to natural fragrances on recruitment in *A. sexdens rubropilosa* (Forel) under laboratory and field conditions. We further examine the effects of fragrance concentration on acceptability, and examine how the dissipation of fragrances over time affects acceptability.

Materials and Methods

Four small, approximately 1 year-old, colonies of *A. sexdens rubropilosa*, were used for laboratory bioassays, and 4 approximately 3 year-old colonies were used for field bioassays. Laboratory colonies were housed in 2 l plastic containers in Teflon@ coated plastic rectangular trays, 40x30 cm. These trays were connected by plastic tubes to other trays of the same size which were used as foraging chambers. Bioassays consisted of drenching corn-flakes with varying concentrations (w/w) of the commercial fragrances eugeniol and eucalyptol.

To test olfactory conditioning in the laboratory, we placed a 0.01% w/w concentration on food-colored corn-flakes. For each bioassay, the foraging tray of the colony was cleared, and after 6 hrs we okaced one treated corn flake in the foraging tray. After disacovery and pick-up by the first ant, we used the 20 of same-corn flakes as a test and used different food-colored non-treated flakes as controls, and recorded pick-up response. Pick-up bioassays were terminated when the first 20 flakes were removed. Control bioassays were run by first presenting an untreated flake, and when this was taken to the nest by the first worker, we repeated the experiment as before. We used the probability distribution of binomial choices (z statistic) to test deviations from random pick-up by comparing the cumulative frequency of treatment flakes with control flakes (Sokal & Rohlf, 1981) and the non-parametric Wald-Wolfowitz runs test (Siegel, 1956) to examine sequences of of treatment and control flake removal.

In the field, bioassays were run by placing a treated corn-flake in an area of ground-cutting activity (Fowler & Robinson, 1979). After this was carried to the physical foraging trail and deposited, we repeated experiments as in the laboratory by placing 20 of each control and treatment flakes in a row in the same area, with sequences determined from a table of random numbers. We used the same statistical tests as before.

As preliminary studies had indicated that concentrated fragrances were apparently repellent, we used serial dilutions (w/w), 1.0, 0.1, 0.01, and 0.001%, of eugeniol and eucalyptol on colored corn flakes as before. Using four laboratory colonies, we ran ten bioassys per colony, but retained corn-flakes for varying time periods after the application of fragrances. Corn-flakes were held at ambient temperature in a separate room after and between bioassays. Data were analyzed by examining the pick-up ratio, or the number of treated per control flakes carried to the nest during the bioassay. Means were compared with analysis of variance, after ratios were transformed to the natural logarithm (Sokal & Rohlf, 1981).

Results and Discussion

Using our experimental procedure, we were unable to document any evidence of olfactory conditioning to natural fragrances in either laboratory or field colonies of *A. sexdens rubropilosa* (fig. 1). All observed pick-up responses did not vary from random expectations, nor did the

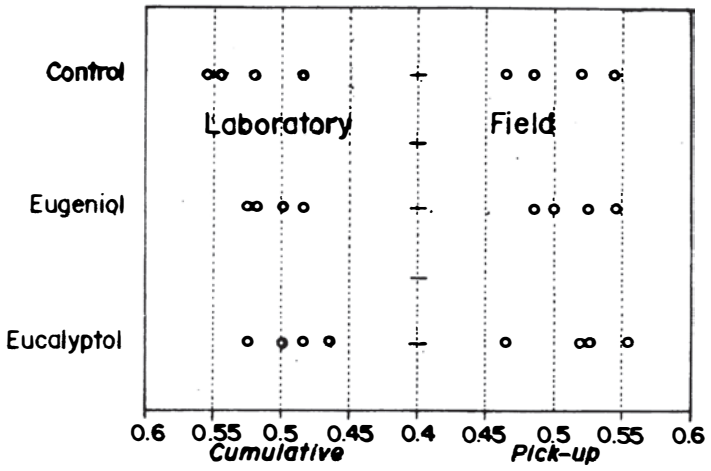

FIGURE 1. Pick-up response of 4 laboratory and 4 field colonies of *A. sexdens rubropilosa* to fragrance impregnated corn-flakes compared with corn flakes. Circles represent the cumulative frecquency of the positive removal of the first 10 particles of 20 offered per nest in conditioning and control bioassays. Control assays provided non-treated corn-flakes first, which were compared with 0.01% eucalyptol impregnated flakes. Critical two-tailed values are 0.3 and 0.7. All values are non-significant.

[El comportamiento de recoger cereales impregnados con fragancias en 4 colonias de laboratorio y 4 colonias en el campo de *A. sexdens rubropilosa*. Los circulos representan la frecuencia de cereales de las primeras 10 de 20 ofrecidos por nido en bioensayos de control y condicionamiento. En los ensayos de control, los cereales ofrecidos primeramente no fueron impregnados con fragancias, y después fueron comparados con cereales impregnados con 0.01% eucaliptol. Los valores críticos son 0.3 y 0.7. Todos los valores no son significativos.]

sequence of pick-ups ($p>0.05$ for all combinations). These results are in direct contrast to the data of Roces (1990) for *A. lundi*. However, Roces (1990) used a different experimental design, by presenting food reward only during the conditioning, but not learning sequence, while we offer food throughout our tests. Additonally, we tested treatment or control flakes with their converse, while Roces (1990) used two types of treatment flakes. However, these differences are real under natural conditions. *A. lundi* is a specialist on flowers and ephemeral resources, and those fragments cut are carried by those ants which cut them to the nest. In contrast, *A. sexdens rubropilosa* cuts long-lasting concentrated resources, and those workers which cut fragments do not carry them to the nest, but rather cut fragments are taken to the nest by another sub-set of foragers (Fowler & Stiles, 1980). For conditioning to occur in *A. sexdens rubropilosa*, both the cutting foragers and the pick-up foragers would have to be conditioned simultaneously. Also, given the mass effects of the pheromonal recruitment system (Jaffe & Howse, 1988), combined with a strongly developed physical foraging trail system (Fowler & Stiles, 1980), the selective advantage of olfactory conditioning in *A. sexdens rubropilosa* would be minimal.

We also found that the concentration of natural fragrances influenced pick-up response (fig. 2). For both fragrances tested, immediate pick-up response was inversely proportional to concentration, but increased over time ($F=23.68$, $p<0.05$). The lowered pick-up response with increasing concentrations of

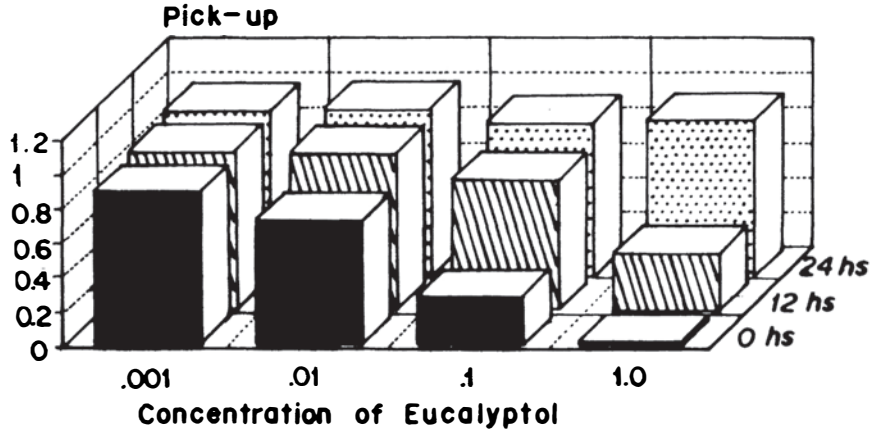

FIGURE 2. Mean pick-up response of laboratory colonies of *Atta sexdens rubropilosa* (number of treated flakes taken/number of untreated flakes taken) of corn-flakes with various concentrations (%) of eugeniol or eucalyptol, compared with untreated flakes over time.

[La respuesta media de recoger cereales por colonias de *Atta sexdens rubropilosa* en el laboratorio (número de cereales tratados recogidos/número de cereales no tratados recogidos) con varias concentraciones (%) de eugeniol o eucaliptol, comparados con cereales no tratados con el decorrer del tiempo.]

natural fragrances in otherwise acceptable forage substrate found in our bioassys is intriguing. *A. sexdens* is known to cut both native species of *Eugenia* (Myrtaceae), the source of eugeniol, and exotic, cultivated species of *Eucalyptus*, the source of eucalyptol, in Brazil (Fowler, et al., 1991). *Eucalyptus* extracts are also known to have repellent properties for other ant species (Jahn, 1991).

Based upon previous studies (Fowler & Robinson, 1979) and newer studies (Schlindwein, 1991), a marked characteristic of *A. sexdens* is to forage on previously cut *Eucalyptus* leaves, which are left in the field until either dry or wilted. These leaves and leaf fragments are then carried into the nest. Our results suggest that the pick-up behavior found in both field and laboratory colonies for substrates after a time period in which fragrances would volatilize may be characteristic of the foraging ecology of this species, and may explain the recorded variability in the selection of forage substrate (Littledyke & Cherrett, 1978). Indeed, temporal dependence has not been examined in the chemical aspects of substrate selection by these ants (Cherrett, 1978). Such behavior had beforehand been interpreted as inefficient (Fowler & Robinson, 1979), but may indeed be selective. As *A. sexdens* cuts large quantities of leaves in trees which are then dropped to the ground and are subsequently found by other foragers, cut and transported to the foraging trail, where they are picked-up and transported to the nest by another worker subset, this behavior may permit the volatilization of inhibitory fragrances over time. Indeed, if such is the case, we predict that species using this foraging strategy, *A. sexdens*, *A. saltensis* (Forel), *A. robusta* Borgmeier and *A. laevigata* (Fr. Smith), may indeed be preparing acceptable forage substrate for later collection. Thus, simple, yet plausible, hypotheses are easily tested in

the field. Perhaps for cut-and-drop foragers, as described above, their behavior is in response to repellent concentrations of natural fragrances more so than an energetic return for production-line division of labor (Fowler & Robinson, 1979; Hubbell et al., 1980).

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Resumen

Influencia de fragancias naturales sobre el condicionamiento olfatorio de reclutamiento y aceptabilidad de material vegetal en la hormiga cortadora Atta sexdens rubropilosa *(Hymenoptera: Formicidae).*

Se estudiaron los efectos de fragancias naturales sobre el condicionamento olfatorio del reclutamiento a recursos alimenticios nuevos y los efectos de concentraciones de fragancias naturales sobre la respuesta de recoger recursos alimentares en la hormiga cortadora, *Atta sexdens rubropilosa*, en colonias en el laboratorio y en el campo. Concentraciones de 0.01% de eugeniol y eucaliptol fueron adicionadas a cereal de maíz y fueron comparadas con cereales normales como controles. No se encontró evidencia de acondicionamento olfatorio en el reclutamento después de exponer hormigas exploradoras a olores alimentarios nuevos. La aceptabilidad de cereales de maíz impregados con concentraciones seriales de 1.0, 0.1, 0.01 y 0.001% de eugeniol y eucaliptol fue inversamente proporcional a la concentración, pero aumentó con el tiempo debido a la volatilización de las fragancias. Los resultados pueden indicar tipos divergentes de procesos de reclutamiento en hormigas cortadoras, probablemente como respuesta a sus usos ecológicos en su ambiente natural. Estos resultados también sugieren porqué las hormigas cortadoras frecuentemente no llevan la vegetación cortada en el mismo día - esperan la evaporación de volatiles inhibidores.

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