

# $\beta$ -eudesmol-induced aggression in the leaf-cutting ant *Atta sexdens rubropilosa*

C. G. S. Marinho, T. M. C. Della Lucia\*, R. N. C. Guedes, M. M. R. Ribeiro & E. R. Lima

Departamento de Biologia Animal, Universidade Federal de Viçosa, 36571-000, Viçosa, MG, Brazil

Accepted: 14 June 2005

**Key words:** *Eucalyptus maculata*, aggressive behavior, ant behavioral changes, Hymenoptera, Formicidae, recognition system, chemical communication

## Abstract

Leaf-cutting ants are highly polyphagous insects, but some plants escape their attack due to the presence of secondary metabolites that are toxic to the ant–fungus symbiosis. Previous studies have demonstrated that the terpenoid  $\beta$ -eudesmol extracted from *Eucalyptus* species (Myrtaceae) is responsible for the deleterious behavior in colonies of leaf-cutting ant species. The objective of this study was to evaluate the effect of  $\beta$ -eudesmol on workers of the leaf-cutting ant *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). This chemical caused behavioral modification in the colonies, leading to mutilation and death of workers. It is suggested that  $\beta$ -eudesmol interferes with colony nestmate recognition. As a consequence, colony cohesion may be disrupted by  $\beta$ -eudesmol what could be used as an additional control tactic against this important pest ant.

## Introduction

Leaf-cutting ants, as social insects, use recognition systems to distinguish nestmates (Lenoir et al., 1999). This complex recognition behavior is based on individual ant and colony odor, and is indispensable for colony maintenance (Crozier & Dix, 1979). These odors or cues are of a chemical nature, and are characteristic for each colony.

Jutsum et al. (1979) demonstrated the importance of environmental cues, such as leaf substrate, in the recognition process. According to Jaffé (1983), the head is the body part where substances responsible for nestmate recognition accumulate; the alarm pheromone, also produced in the head, could be involved in the process. In *Atta laevigata* (F. Smith) (Hymenoptera: Formicidae), nestmate recognition is based on odors from the secretion of mandibular glands and these odors are dispersed throughout the ant cuticle (Hernández et al., 2002). When recognition does not occur, aggressive behavior ensues (Whitehouse & Jaffé, 1995; Della Lucia et al., 2001; Hernández et al., 2002) and several attack strategies result.

Interference with recognition is a form of disruption of the society and could have extreme effects on colonies, including colony death (Marsaro et al., 2004). This might be very effective in the control of leaf-cutting ants,

which are considered severe pests throughout Central and South America (Della Lucia et al., 2001).

The leaf-cutting ants *Atta laevigata* and *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) exhibit a peculiar behavioral modification among nestmates when in contact with leaves of *Eucalyptus maculata* Hook (Myrtaceae) (Anjos & Santana, 1994). When supplied with *E. maculata* leaves, the workers of *A. laevigata* and *A. sexdens rubropilosa* bit and mutilated body parts of their own nestmates (Anjos & Santana, 1994). Recently, Marsaro et al. (2004) discovered that  $\beta$ -eudesmol and elemol were the substances present in *E. maculata* that interfered with nestmate recognition. When workers were exposed to these sesquiterpenes they began exhibiting aggressive behavior towards each other. This study aimed at testing various concentrations of synthetic  $\beta$ -eudesmol on colonies of *A. sexdens rubropilosa* to determine their effect on workers' behavior.

## Materials and methods

### Colony collection and maintenance

A total of six colonies were used in the bioassays. Three colonies had 25 L of fungus garden (large size colonies) and were 7 years old. The other three had 1.5 l of fungus (small size colonies) and were 2 years old. The colonies were maintained at  $24 \pm 5^\circ\text{C}$ ,  $75 \pm 5\%$  r.h., and L12:D12 photoperiod in the laboratory according to Della Lucia et al. (1993). They were supplied daily with water and

\*Correspondence: E-mail: tdlucia@ufv.br

leaves of several plant species such as *Ligustrum japonicum*, *Tecoma stans*, *Acalypha wilkesiana*, *Rosa spec.*, and cereals.

### Bioassays

The synthetic  $\beta$ -eudesmol was obtained from Sigma-Aldrich Quimica Brazil (São Paulo, Brazil). All leaves on the foraging arena of the colonies were removed 6 h prior to the tests in order to avoid interference with the results. Filter paper (Whatman no. 1) fragments of 6 cm<sup>2</sup> in shapes of rectangles, triangles, and squares (Marsaro et al., 2004) were used for the assays. As *A. sexdens rubropilosa* does not show shape preferences among the fragments, the different shapes were used for the individual chemical concentrations to facilitate their recognition during the bioassays (Santana et al., 1990). Triangular fragments were impregnated with 100  $\mu$ l of  $\beta$ -eudesmol diluted in the solvent hexane (the concentration treatments), square fragments were impregnated with 100  $\mu$ l of hexane alone (control), and rectangular fragments were neither impregnated with the solvent nor with  $\beta$ -eudesmol diluted in the solvent (serving as an additional control against a possible effect of the solvent).  $\beta$ -eudesmol was tested in six concentrations previously diluted in hexane: 0, 10, 100, 500, 750, and 1000  $\mu$ g ml<sup>-1</sup>. The quantity of solution necessary to achieve complete impregnation of any filter paper fragment was always 100  $\mu$ l, regardless of the treatment ( $\beta$ -eudesmol + hexane in each one of the concentrations) or the control (hexane). The chemicals for the control and chemical treatments were pipetted onto their respective filter paper fragments. Five minutes after solvent evaporation, all fragments were simultaneously offered to the workers at the foraging arenas of the colonies. Each bioassay lasted 30 min and ant worker behavior was recorded. An aggression group was defined as two or more nestmates fighting, mutilated, or dead. At the end of each bioassay, the aggression groups were removed and each placed individually in 10-ml vials. The numbers of aggression groups formed, of ants fighting, and of dead or mutilated ants in each group were counted at the end of each bioassay.

All bioassays were conducted in three replications. The  $\beta$ -eudesmol was tested once on each of three large colonies and on each of three small colonies. Only one series of concentrations was applied to each colony to avoid colony conditioning. The bioassays were performed from the lowest to the highest product concentration, with a 1-week interval between each test. The positioning of the filter paper fragments was altered for the various  $\beta$ -eudesmol concentrations to avoid ant conditioning.

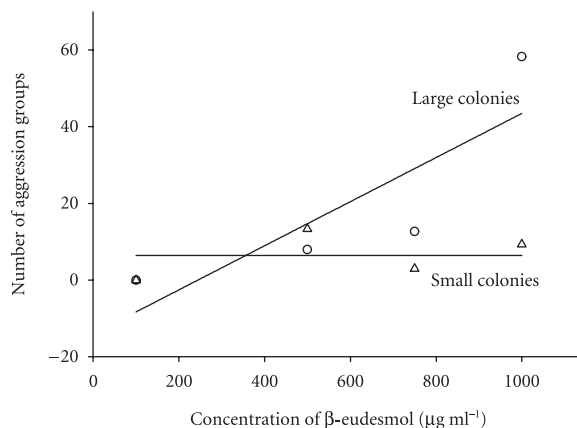
The data were subjected to analysis of covariance with ant colony size as the independent variable and  $\beta$ -eudesmol concentration as covariable (PROC GLM), and subsequently subjected to regression analyses (PROC

REG; SAS Institute, 1989). As the  $\beta$ -eudesmol concentrations of 0, 10, and 100  $\mu$ g ml<sup>-1</sup> did not elicit a response, only the concentration of 100  $\mu$ g ml<sup>-1</sup> was included in these analyses together with the higher concentrations of  $\beta$ -eudesmol tested (i.e., 500, 750, and 1000  $\mu$ g ml<sup>-1</sup>). Only the number of mutilated ants had to be transformed to  $\log(x + 1)$  to stabilize variance and to satisfy normality assumptions (PROC UNIVARIATE; SAS Institute, 1989).

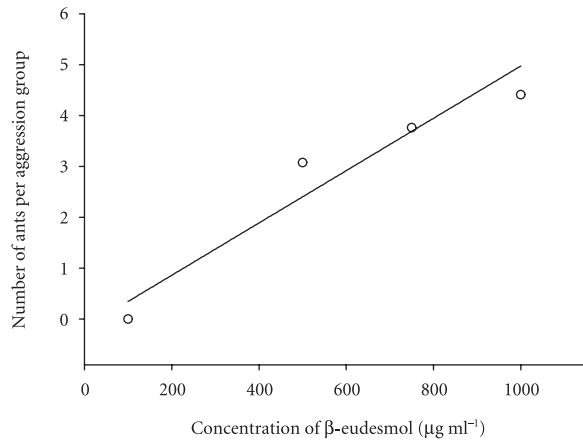
### Results

When colony workers contacted filter paper fragments impregnated with  $\beta$ -eudesmol at 500  $\mu$ g ml<sup>-1</sup> or higher, aggression groups formed (Figures 1–4). This did not occur when the colonies were exposed to the control treatment, even when the fragments were carried into the fungus garden. The behavioral sequence exhibited by workers in contact with  $\beta$ -eudesmol was individual excitement, alarm (open mandibles), self-grooming (legs and antennae), and when in contact with a nestmate, antennal inspection followed by an attack. These attacks consisted of leg, antenna, and abdomen biting. Biting of other body parts occurred, but at a less frequent rate. Immediately after the formation of the first aggression group, new groups formed and the number of ants in close proximity to the  $\beta$ -eudesmol-impregnated filter paper increased.

The number of aggression groups formed varied significantly with colony size,  $\beta$ -eudesmol concentration, and their interaction (Table 1). Small colonies did not respond to increasing concentrations of  $\beta$ -eudesmol (Figure 1).



**Figure 1** Number of aggression groups formed in large (circle) and small (triangle) colonies of *Atta sexdens rubropilosa* when workers were exposed to varying concentrations of  $\beta$ -eudesmol. Each symbol represents the mean of three replicates. (Small colonies:  $y = 6.42$ ;  $P > 0.05$ ) (Large colonies:  $y = -4.05 + 0.06x$ ;  $gl_{\text{error}} = 10$ ,  $F = 14.13$ ,  $P = 0.004$ ,  $R^2 = 0.59$ ).

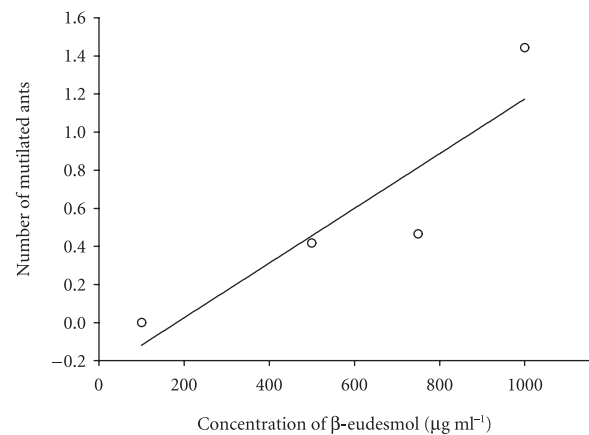


**Figure 2** Number of ants per aggression group formed when *Atta sexdens rubropilosa* nestmates were exposed to varying concentrations of  $\beta$ -eudesmol. Each symbol represents the mean of six replicates. ( $y = -0.17 + 0.0051x$ ;  $g_{\text{error}} = 22$ ,  $F = 14.74$ ,  $P = 0.004$ ,  $R^2 = 0.40$ ).

However, there was an increase in the number of aggression groups formed in the large colonies with increasing  $\beta$ -eudesmol concentrations (Figure 1). Nevertheless, the interaction between colony size and  $\beta$ -eudesmol concentration did not affect significantly the number of ants per aggression group (Table 1). The number of ants per aggression group was therefore pooled together to test the isolated effect of colony size, which was not significant, and of  $\beta$ -eudesmol concentration, which was significant (Table 1). There was an increase in the number of ants per group with increased concentrations of  $\beta$ -eudesmol (Figure 2). These findings indicate that higher concentrations of  $\beta$ -eudesmol increased disruption in the colony.

The number of mutilated ants showed the same trend as observed for the number of ants per aggression group (Table 1). The interaction between colony size and  $\beta$ -eudesmol concentration was not significant and the pooled data indicated significant differences only for  $\beta$ -eudesmol

concentration, not for colony size (Table 1). The fitted curve indicated an increase in the number of mutilated ants with increased concentrations of  $\beta$ -eudesmol (Figure 3). In contrast, the number of dead ants followed a trend similar to the number of aggression groups formed where the interaction between colony size and  $\beta$ -eudesmol concentration was significant (Table 1). However, small ant colonies did not respond to the chemical, unlike the larger colonies, which showed increased response with increased  $\beta$ -eudesmol concentrations (Figure 4).



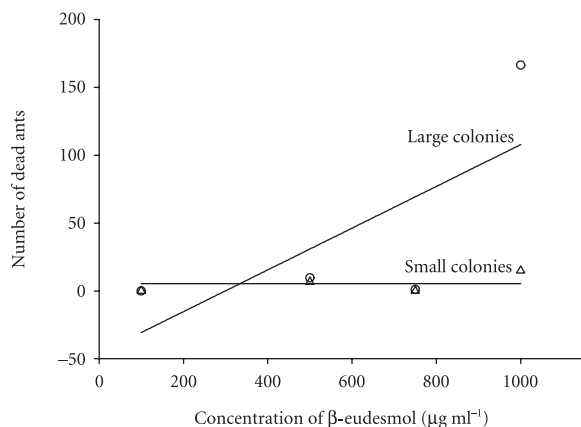
**Figure 3** Number of mutilated ants [transformed to  $\log_{10}(x + 1)$ ] when *Atta sexdens rubropilosa* nestmates were exposed to varying concentrations of  $\beta$ -eudesmol. Each symbol represents the mean of six replicates. ( $y = -0.26 + 0.0014x$ ;  $g_{\text{error}} = 22$ ,  $F = 26.20$ ,  $P < 0.0001$ ,  $R^2 = 0.54$ ).

## Discussion

The behavioral sequence observed for synthetic  $\beta$ -eudesmol was also verified for the natural product extracted from *E. maculata* plants by Marsaro et al. (2004). Modification of the behavior of the leaf-cutting ant *Atta cephalotes* upon exposure to concentrations of caryophellene epoxide extracted

**Table 1** Analysis of covariance showing the interactions among colony size of *Atta sexdens rubropilosa* and concentrations of  $\beta$ -eudesmol. Asterisks indicate significant differences at  $P < 0.05$

Sources of variation	d.f.	Number of aggression groups formed		Number of ants per aggression group		Number of mutilated ants		Number of dead ants	
		F	P	F	P	F	P	F	P
Model	7	10.54	<0.0001*	9.95	<0.0001*	9.16	<0.0001*	46.81	<0.0001*
Error	16	—	—	—	—	—	—	—	—
Colony	1	10.36	0.0054*	4.55	0.06	1.35	0.26	42.86	<0.0001*
Concentration	3	12.34	0.0002*	19.53	<0.0001*	17.87	<0.0001*	55.03	<0.0001*
Colony*concentration	3	8.80	0.0011*	2.16	0.13	3.06	0.0584	39.90	<0.0001*



**Figure 4** Number of dead ants of *Atta sexdens rubropilosa* nestmate workers from large (circle) and small (triangle) colonies exposed to varying concentrations of  $\beta$ -eudesmol. Each symbol represents the mean of three replicates. (Small colonies:  $y = 4.42$ ;  $P > 0.05$ ) (Large colonies:  $y = -46.06 + 0.15x$ ;  $g_{\text{error}} = 10$ ,  $F = 10.08$ ,  $P = 0.009$ ,  $R^2 = 0.50$ ).

from *Hymenaea courbaril* was also observed by Hubbell et al. (1983). According to these authors, ants jumped or reared back into the arena and began intense grooming.

Our results demonstrate that ant workers in contact with  $\beta$ -eudesmol develop aggressive behavior. In addition to a simple aggressive behavior, the response to this compound could be that it interferes with nestmate recognition in such a way as to elicit aggression. We believe this second possibility is the most correct, based on the fact that aggression started when one worker contacted the product and other workers contacted that worker. Thereafter, a chain of aggressive behavior followed.

We verified that only ants in the foraging arena engaged in fights. Those that worked in the fungus garden did not come in contact with the impregnated filter paper, and did not exhibit behavioral changes. Our results indicate that synthetic  $\beta$ -eudesmol induced aggressiveness in workers of *A. sexdens rubropilosa*. The workers of this ant species avoid cutting *E. maculata* leaves in the field (Anjos & Santana, 1994) probably due to the presence of sesquiterpenes, among which  $\beta$ -eudesmol is predominant.

The aggressive behavior between nestmates in contact with  $\beta$ -eudesmol seemed to result from the lack of or interference with the recognition ability of these ants. Ant nestmate recognition has been widely studied and reviewed (Carlin & Hölldobler, 1987; Lahav et al., 1999; Lenoir et al., 1999; Howard & Blomquist, 2004) and is very important for colony success. According to Crozier & Dix (1979), nestmate recognition occurs by means of odors originated from a mixture of genetic factors (endogenous) and environmental factors (exogenous). The latter can vary

according to habitat, food, seasons, and climatic changes. The relative importance of each factor varies with species, although little is known about it in leaf-cutting ants (Jutson et al., 1979; Viana et al., 2001; Hernández et al., 2002). The compound  $\beta$ -eudesmol might interfere with nestmate recognition by changing hydrocarbon profiles or by blocking olfaction. This certainly requires further investigation.

The number of dead ants varied with colony size but the number of mutilated live ants was similar in small and large colonies. These results are probably due to a greater capacity of large colonies to fight, thus resulting in a higher number of dead ants. In smaller colonies, fighting capacity was less developed so combat was weaker.

In conclusion,  $\beta$ -eudesmol brings about a behavioral alteration in ant workers of *A. sexdens rubropilosa* when they are exposed to increasing concentrations of this compound, particularly for large ant colonies and at concentrations equal to or higher than  $500 \mu\text{g ml}^{-1}$ . In the presence of such  $\beta$ -eudesmol concentrations, nestmates seem not to recognize each other. Because of the disruption caused on ant colonies,  $\beta$ -eudesmol warrants further study as a potential control tool against leaf-cutting ants.

## Acknowledgements

The authors thank the Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for scholarships and support. The authors express their gratitude to Dr Rosa Muchovej, University of Florida, Southwest Florida Research and Education Center and Stephen Muchovej, Columbia University, for valuable comments and revision of this manuscript.

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