

# The Metapleural Secretion of *Acromyrmex laticeps* (Forel) does not have Fungicide Effect on the Entomopathogenic Fungus *Beauveria bassiana* (Bals.) Vuill.

Luciane Kern Junqueira<sup>1</sup> & Elena Diehl<sup>2</sup>

1. Pontifícia Universidade Católica de Campinas, e-mail: [lkjunque@yahoo.com.br](mailto:lkjunque@yahoo.com.br) (Autor para correspondência<sup>✉</sup>). 2. Sem vinculação institucional, e-mail: [elena.diehl@pq.cnpq.br](mailto:elena.diehl@pq.cnpq.br).

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**Abstract.** The metapleural gland (MPG) is an exocrine structure, exclusive to formicids, being more developed in females and workers. This structure is stunted or nonexistent in males. Several possible functions for the secretion produced by this gland have been suggested, but a protection against entomopathogens is the most accepted hypothesis. To test this, we applied a suspension of spores ( $1.30 \times 10^6$ ) of *Beauveria bassiana* (Bals.) Vuill. on the MPG and the antero-superior portion of the metathorax (ASM) of winged females of *Acromyrmex laticeps* (Forel). We only applied the suspension in the ASM of males, since they do not have the atrium characteristic in the MPG. Our results indicated that the metapleural secretion of females has no fungicidal effect on *B. bassiana*, and therefore both males and winged females are sensitive to this entomopathogenic fungus.

**Keywords:** Attini; entomopathogenic fungus; intracolony assepsy.

## Ausência de Efeito Fungicida da Secreção Metapleural de *Acromyrmex laticeps* (Forel) Contra *Beauveria bassiana* (Bals.) Vuill.

**Resumo.** A glândula metapleural (GMP) é uma estrutura exógena, encontrada apenas nos formicídeos, sendo mais desenvolvida nas fêmeas e operárias, enquanto nos machos ou está atrofiada ou não existe. Tem sido sugerido várias possíveis funções para a secreção produzida por esta glândula, sendo a hipótese de efeito protetor contra entomopatógenos a mais estudada. Para testar esta hipótese, foi aplicada uma suspensão de conídios ( $1,30 \times 10^6$ ) de *Beauveria bassiana* (Bals.) Vuill. nas GMP e na porção ântero-superior do metatórax (ASM) de fêmeas aladas de *Acromyrmex laticeps* (Forel), enquanto nos machos a suspensão foi aplicada apenas na porção ASM, pois estes não apresentam o átrio característico da GMP. Os resultados obtidos indicaram que a secreção metapleural das fêmeas desta espécie não possui efeito fungicida sobre *B. bassiana*, e que tanto os machos como as fêmeas aladas são sensíveis a este fungo entomopatogênico.

**Palavras-chave:** Assepsia intracolônia; Attini; fungo entomopatogênico.

Ants have many natural enemies, including viruses (VALLES *et al.* 2005), bacteria (PINTO *et al.* 2003; DEDEINE *et al.* 2005), microparasites and parasitoids (SCHMIDT-HEMPEL 1998; BROWN 1999), invertebrates (TONHASCA *et al.* 2001; ROLFF & SIVA-JOTHY 2003), and vertebrates (JAFFÉ 1993; DIEHL-FLEIG 1995a; 1995b). Accordingly, they also display numerous defense mechanisms, including behavior (DIEHL-FLEIG & LUCCHESI 1991; JAFFÉ 1993), increased genetic diversity, either by polyandry or polygyny (SCHMID-HEMPEL 1994; CROZIER & FJERDINGSTAD 2001) immunological mechanisms (SCHMID-HEMPEL 2005), especially secretions of metapleural glands (HÖLLDOBLER & WILSON 1990; SCHMID-HEMPEL 1998; SCHLÜNS & CROZIER 2009). Despite these defense mechanisms, the occurrence of entomopathogenic fungi in ants is relatively common (PEREIRA & STIMAC 1992; DIEHL-FLEIG *et al.* 1993; OI *et al.* 1994; DIEHL & JUNQUEIRA 2001).

Formicids are the only insects that share a unique defense mechanism consisting of a pair of exocrine glands located in the posterolateral portion at the end of the mesosome (BOR *et al.* 2002). Such glands are rare in males and arboreal species (HÖLLDOBLER & WILSON 1990; JAFFÉ 1993). Although not mutually exclusive, four hypotheses about the function of these metapleural glands (MPG) have been proposed, it would function as: 1) intracolony or intraspecific recognition, 2) determinant of territory either/or nest entrance, 3) chemical defense, or 4) antiseptic action.

However, only the last two have not receive severe critics (YEK & MUELLER 2010).

The function of the MPG has been subjected to study since the 1970s, but the results are often conflicting, especially involving a possible antiseptic effect of its secretion (YEK & MUELLER 2010). Antibiotic substances produced by MPG and possibly other glands have an essential significance for fungus-growing ants (Myrmicinae: Attini), since these ants must protect the symbiotic fungus which they cultivate and feed upon, besides the maintenance of the assepsy of the ants themselves. This secretion has also been suggested as responsible for the characteristic odor of each colony required for the recognition of its individuals, but the absence of this secretion in males may indicate that this behavior may be mediated by other odor sources (HÖLLDOBLER & ENGEL-SIEGEL 1984).

Here, we tested if the metapleural secretion of reproductive individuals of fungus-growing ants (Myrmicinae: Attini) have fungicidal effect on the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill.. For this, we used both females and winged males of *Acromyrmex laticeps* (Forel) collected soon after two nuptial swarming to: 1) determine the frequency of individuals

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with a pair of MPG containing a secretion in its reservoirs and 2) investigate possible sex differences in the resistance to that pathogen.

## MATERIAL AND METHODS

We collected 109 winged females after a nuptial flight in early summer. After the removal of the wings, each female was immobilized with fine-tipped tweezers. Then, we drilled the metapleural reservoirs in their upper-middle portion with micro needle under stereomicroscope (40x). We recorded the presence of a secretion in the reservoir in either left or right gland of each female. We also collected 35 males from the same swarm, to evaluate the occurrence of MPG under stereomicroscope (40x).

We conducted a bioassay to determine sex differences in pathogen resistance with 20 males and 120 females of *A. laticeps* collected after another nuptial flight. This test consisted of a suspension of *B. bassiana* ( $1.30 \times 10^6$ ) being applied with a microcapillary in the MPG of 40 females and in the ASM of another 40 females in a sterile laminar flow chamber. As control we applied a sterile saline solution in 40 other females. Since males do not have MPG, the suspension was applied only to the ASM of 10 males. As a control, we applied a sterile saline solution to the ASM in an equal number of males.

Each ant remained in sterilized jars, with a disk of filter paper at the bottom soaked in 0.05 mL of 25% aqueous sugar solution. Jars remained in a sterile, moist chamber at  $27 \pm 1$  °C, at 80% RH, and a 12:12 hours light: dark regime. We recorded the number of dead and infected individuals daily during 15 days. The results in each group were compared using a chi-squared test (ZAR 1999).

## RESULTS AND DISCUSSION

Social insects are particularly vulnerable to parasites and pathogens due to high individual density, high levels of interaction and intracolony kinship (HÖLLEDOBLER & WILSON 1990; JAFFÉ 1993; BOT *et al.* 2002). These insects have developed numerous defense mechanisms to deal with this situation, such as avoidance behavior, hygiene of both individuals and nest (DIEHL-FLEIG & LUCCHESI 1991; FRITSH & DIEHL-FLEIG 1996), immune defenses (HUGGES & BOOMSMA 2004; BAER *et al.* 2005), age and gender of individuals that may affect immune function (ROLFF 2001), and specifically in the case of formicids, antiseptic substances produced by MPG (BILLEN & VAN BOVEN 1987; HÖLLEDOBLER & WILSON 1990). The effects of antiseptics metapleural secretion have been studied more in length in Attini ants (DIEHL-FLEIG *et al.* 1993; DO NASCIMENTO *et al.* 1996; DIEHL & JUNQUEIRA 2001; BOT *et al.* 2002; BAER *et al.* 2005) and in some *Solenopsis* species (PEREIRA & STIMAC 1992; OI *et al.* 1994; BEXTINE & THORVILSON 2002).

We found that the MPG reservoirs of ten (8.33%) out of 120 females of *A. laticeps* were empty, both reservoirs were filled with metapleural secretion in 109 individuals (90.83%), and only one individual (0.84%) had secretion in only the right reservoir. Thus, 91.7% of females contained a secretion in the metapleural reservoirs (Table 1). The differential susceptibility to infection in individuals of the same colony had already been reported (SCHMID-HEMPER 1994) and could explain the differences of infection found in winged females of *A. laticeps*.

The frequency of infection by *B. bassiana* in females was 72.5% when the fungus was inoculated in the MPG and 85% when applied to the ASM (Table 1). However, this difference was not significant ( $\chi^2 = 3.00$ ,  $df = 1$ , NS), which indicates that the metapleural secretion did not provide higher resistance to the pathogen when females were inoculated in the MPG than the ASM. But when we compare these frequencies of infection with that of the females control, the differences are highly significant ( $\chi^2 = 44.252$ ,  $df = 3$ ,  $P < 0.0001$ ).

Only one male inoculated in the ASM was not infected, while *B. bassiana* was leaving the joints in 90% of them (Table 1). This difference ( $\chi^2 = 31.0$ ,  $df = 1$ ,  $P < 0.001$ ) was highly significant, but when these data are compared with that obtained in the male control group the differences are not significant ( $\chi^2 = 2.22$ ,  $df = 1$ , NS). However these results could have been biased by our small sample size of the males group. However, since males lack MPG, they would not produce the secretion with possible antiseptic function (BAER *et al.* 2005), thus explaining the high frequency of infection.

We detected *B. bassiana* in 10% of control males and 12.5% control winged females ( $\chi^2 = 1.0$ ,  $df = 3$ ,  $P < 0.01$ ), possibly due to a contamination during the bioassays (Table 1).

The primary function, architecture, and relative size of MPG may vary (YEK & MUELLER 2010). The size of this gland can vary from reduced or absent in several species of parasitic ants, to hypertrophied in species that use it as a defense, such those in the subgenus *Physocrema* of the genus *Crematogaster*. Meanwhile, this gland is more developed in females than workers, while it is very small, with the atrium closed or absent in males (HÖLLEDOBLER & ENGEL-SIEGEL 1984; HÖLLEDOBLER & WILSON 1990; JAFFÉ 1993; BAER *et al.* 2005). However, HÖLLEDOBLER & ENGEL-SIEGEL (1984) reported that males of some species, such as *Temnothorax allardycei* (Mann), *Novomessor cockerelli* (André), *Formica perpilosa* (Wheeler), *Myrmecocystus mimicus* (Wheeler) and *Iridomyrmex purpureus* (Smith), have these glands.

We had already found previously that males of *Atta sexdens piriventris* (Santschi) and *Acromyrmex heyeri* (Forel) did not have the characteristic atrium externally to the metapleural gland. This is the most peculiar part of this gland (BILLEN & VAN BOVEN 1987). The females and males of the Dorylinae and Ecitoninae subfamilies are larger than the workers, with males lacking metapleural glands (JAFFÉ 1993). The absence of MPG is common in males, and species in which only males possess this gland are unknown (YEK & MUELLER 2010). Males are little active in the colony and their essential function is fertilization (HÖLLEDOBLER & WILSON 1990; DIEHL-FLEIG 1995b). Taken these features together, we would not expected the MPG in males *A. laticeps*, since they die after fertilizing females and such specific structure would be useless.

HUGGES & BOOMSMA (2004) investigated the interaction between the entomopathogenic fungus *Metarhizium anisopliae* (Metschn.) Sorokin and the opportunistic, avirulent fungus *Aspergillus flavus* (Link) in *Acromyrmex echinaior* (Wheeler). Their results indicated that the pathogenic fungus broke the resistance of ants, allowing the negative influence of the non-pathogenic fungus. This situation is similar in immune deficient vertebrates when

Table 1: Number of winged males and females of *Acromyrmex laticeps*, local application of a conidia solution ( $1.30 \times 10^6$ ) of *Beauveria bassiana* and frequency of infected individuals.

Application region	Tested winged females	Number and frequency (%) of infected females	Tested Males	Number and frequency (%) of infected males
Metapleural gland	40	29(72.5)	--	--
Antero-superior portion of the metathorax	40	32(80.5)	10	9(90.0)
Control	40	5(12.5)	10	1(10.0)
<b>Total</b>	<b>120</b>		<b>20</b>	

infected with an opportunistic parasite.

A previous study (BAER *et al.* 2005) compared the immune responses of workers and males of *A. echinator* to a virulent parasite. Since males had lower immune responses than workers have, the authors suggested that this difference could be due to the lower genetic diversity due to haploidy or the reduced investment in immunity by males. It could also be due to the fact that the diploid workers have higher and more genetic diversity, and variable ages, since they are continually produced. Conversely, males are born only in the reproductive period, and would have virtually no variation in age. Nonetheless, when workers were exposed to *M. anisopliae*, they showed a reduced immune response after four days, suggesting that the immune system was destroyed by the virulent parasite.

In this study, the high frequency of males and winged females infected with *B. bassiana* could be due to the metapleural secretion that has reduced fungicidal effect, but also to an inefficient immune system (BAER *et al.* 2005). The contamination in our control group somewhat reinforces this hypothesis.

Our results indicate that the metapleural secretion of winged females of *A. laticeps* do not have an effective fungicidal effect on *B. bassiana*. However, we suggest that other chemical and immunological mechanisms associated or not with behavioral strategies complement and are even more effective than the secretion against entomopathogenic infections in natural environments. Similarly, the metapleural secretion of *A. laticeps* could be other functions, such as marking trails or territory, and recognition of individuals from the same colony.

Only the chemical functions of the MPG in workers several species have been investigated so far, especially in Attini ants and the genus *Solenopsis*. In contrast, there are no studies on males and winged females. The morphology of the MPG in ant males remains completely unknown (YEK & MUELLER 2010). Chemical analysis of the MPG of males, as well as its functional morphology may provide important hypotheses about the evolution of this gland. Especially because its loss in males appears to have occurred more often than in reproductive females and workers.

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