doi:10.12741/ebrasilis.v7i3.449
e-ISSN 1983-0572
Publicação do Projeto Entomologistas do Brasil
www.ebras.bio.br

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

Luciane Kern Junqueira[1]✉ & Elena Diehl[2]

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

EntomoBrasilis 7 (3): 207-210 (2014)

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 x 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; intracolonial 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 x 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 alados 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 & Lucchese 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 (Bot *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) intracolonial 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

Funding Agency: FAPERGS.

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* (1.30×10^6) 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±1 ºC, 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 intracolonial kinship (Hölldobler & 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 & Lucchese 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; Hölldobler & 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-Hempel 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 date 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 (Hölldobler & Engel-Siegel 1984; Hölldobler & Wilson 1990; Jaffé 1993; Baer *et al.* 2005). However, Hölldobler & 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 (Hölldobler & 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 echinatior* (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 x 106) 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)
Total	**120**		**20**	

e-ISSN 1983-0572

infected with an opportunistic parasite.

A previous study (Baer *et al.* 2005) compared the immune responses of workers and males of *A. echinatior* 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.

REFERENCES

Baer, B., A. Krug, J.J. Boomsma & W.O.H. Hughes, 2005. Examination of the immune responses of males and workers of the leaf-cutting ant *Acromyrmex echinatior* and the effect of infection. Insectes Sociaux, 52: 298–303.

Bextine, B.R. & H.G. Thorvilson, 2002. Field applications of bait-formulated *Beauveria bassiana* alginate pellets for biological control of the red imported fire ant (Hymenoptera: Formicidae). Environmental Entomology, 31: 746-752.

Billen, J. & J.K.A. Van Boven, 1987. The metapleural gland in Old World army ants: a morphological and ultrastructural description (Hymenoptera, Formicidae). Revue de Zoologie Africaine, 101: 31-41.

Bot, A.N.M., D. Ortius-Lechner, K. Finster, R. Maile & J.J. Boomsma, 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insectes Sociaux, 49: 363-370.

Brown, B.V., 1999. Differential host use by neotropical phorid flies (Diptera: Phoridae) that are parasitoids of ants (Hymenoptera: Formicidae). Sociobiology, 33: 95–103.

Crozier, R.H. & E.J. Fjerdingstad, 2001. Polyandry in social Hymenoptera – disunity in diversity? Annales Zoologici Fennici, 38: 267-285.

Dedeine, F., M. Ahrens, L. Calcaterra & D.D. Shoemaker, 2005. Social parasitism in fire ants (*Solenopsis* spp.): a potential mechanism for interspecies transfer of *Wolbachia*. Molecular Ecology, 14: 1543–1548.

Diehl, E. & L.K. Junqueira, 2001. Seazonal variations of metapleural secretion in the leaf cutting ant *Atta sexdens piriventris* Santschi (Myrmicinae, Attini), and its effect on the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. Neotropical Entomology, 30: 517-522.

Diehl-Fleig, E. & M.E. de P. Lucchese, 1991. Reações comportamentais de operárias de *Acromyrmex striatus* (Hymenoptera, Formicidae) na presença de fungos entomopatogênicos. Revista Brasileira de Entomologia, 35: 101-107.

Diehl-Fleig, E., 1995a. Sucesso no estabelecimento de colônias de *Acromyrmex striatus* (Roger) (Hymenoptera, Formicidae). Anais da Sociedade Entomológica do Brasil, 24: 625-630.

Diehl-Fleig, E., 1995b. Formigas: organização social e ecologia comportamental. Ed. Unisinos, São Leopoldo, 168p.

Diehl-Fleig, E., M.E. da Silva, A. Specht & M.E.V. Labres, 1993. Efficiency of *Beauveria bassiana* for *Acromyrmex* spp. control (Hymenoptera: Formicidae). Anais da Sociedade Entomológica do Brasil, 22: 281-285.

Do Nascimento, R.E., E. Schöesters, E.D. Morgan, J. Billen & D.J. Stradling, 1996. Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). Journal of Chemical Ecology, 22: 987-1000.

Fritsh, S. & E. Diehl-Fleig, 1996. Reações comportamentais de *Acromyrmex heyeri* e *A. striatus* (Hymenoptera - Formicidae) à fungos filamentosos. Acta Biologica Leopoldensia, 18: 77-92.

Hölldobler, B. & E.O. Wilson, 1990. The ants. Harvard University Press, Cambridge, MA, 732p.

Hölldobler, B. & H. Engel-Siegel, 1984. On the metapleural gland of ants. Psyche, 91: 201–224.

Hughes, W.O.H. & J.J. Boomsma, 2004. Let your enemy do the work: within-host interactions between two fungal parasites of leaf-cutting ants. Proceedings of the Royal Society of London B: Biological Sciences, 271: S104-S106.

Jaffé, K., 1993. El mundo de las hormigas. Editorial Equinócio. Ediciones de La Universidad Simon Bolivar. Venezuela. 186p.

Oi, D.H., R.M. Pereira, J.L. Stimac & L.A. Wood, 1994: Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). Journal of Economic Entomology, 87: 623-630.

Pereira, R. & J. Stimac, 1992. Transmission of *Beauveria bassiana* within nests of *Solenopsis invicta* (Hymenoptera: Formicidae) in the laboratory. Environmental Entomology, 21: 1427-1432.

Pinto, L.M.N., A.O. Azambuja, E. Diehl & L.M. Fiuza, 2003. Pathogenicity of *Bacillus thuringiensis* isolated from two species of *Acromyrmex* (Hymenoptera, Formicidae). Brazilian Journal of Biology, 63: 301-306.

Rolff, J. & M.T. Siva-Jothy, 2003. Invertebrate ecological immunology. Science, 301: 472–475.

Rolff, J., 2001. Effects of age and gender on immune function of dragonflies (Odonata, Lestidae) from a wild population. Canadian Journal of Zoology, 79: 2176–2180.

Schlüns, H. & R.H. Crozier, 2009. Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae). Myrmecological News, 12: 237-249.

Schmid-Hempel, P., 1994. Infection and colony variability in social insects. Philosophical Transactions of the Royal Society B: Biological Sciences, 346: 313-321.

Schmid-Hempel, P., 1998. Parasites in social insects. Princeton University Press, Princeton, NJ, 409p.

Schmid-Hempel, P., 2005. Evolutionary ecology of insect immune defenses. Annual Review of Entomology, 50: 529-551.

Tonhasca Jr., A., M.A.L. Bragança & M. Erthal Jr., 2001. Parasitism and biology of *Myrmosicarius grandicornis* (Diptera, Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera, Formicidae). Insectes Sociaux ,48: 154–158.

Valles, S. M. & C. Strong, 2005. *Solenopsis invicta* virus-1A (SINV-1A): Distinct species or genotype of SINV-1? Journal

of Invertebrate Pathology, 88: 232–237.
Yek, S.H. & U.G. Mueller, 2010. The metapleural gland of ants. Biological Revue, 1-18.
Zar, J. H., 1999. Biostatistical analysis. Prentice Hall Upper Sadlle River, New Jersey. 663p.

Received in: 06/17/2014
Accepted in: 07/30/2014

****

Suggested citation:

Junqueira, L.K. & E. Diehl, 2014. The Metapleural Secretion of *Acromyrmex laticeps* (Forel) does not have Fungicide Effect on the Entomopathogenic Fungus *Beauveria bassiana* (Bals.) Vuill. EntomoBrasilis, 7 (3): 207-210.
Available in: doi:10.12741/ebrasilis.v7i3.449