

Journal of Chemical Ecology 24: 1173-1186, 1998

CHEMICAL ECOLOGY OF THE DEFENSE OF TWO NYMPHALID BUTTERFLY LARVAE AGAINST ANTS

Frances Osborn¹ and Klaus Jaffe

Depto. Biología de Organismos
Universidad Simón Bolívar
Apartado 89000, Caracas 1080, Venezuela

¹ Present address : Instituto de Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumana, Venezuela, Fax 5893-514023

ABSTRACT

We analyzed the behavioral responses of the ants *Camponotus rufipes* and *Solenopsis geminata* towards all instars of *Dione junio* and *Abanote hylonome* (Lepidoptera: Nymphalidae). We also analyzed ant behavior towards hexane extracts of larvae and extracts of the spines and neck glands of the fifth instars of both species and identified the chemical compounds present. Larvae of both species were repellent to ants from the first instar onward. Later instars survived ant attacks better than earlier instars. The spines and neck glands of the larvae influenced the behavior of *C. rufipes*. The chemical compounds contained in the hexane extracts of whole first and fifth instars, and in the spines and neck glands of fifth instars, were principally carboxylic acids and terpenes. Further bioassays confirmed the repellent effect of some of these acids toward ants.

Key words : Ants, *Camponotus rufipes*, *Solenopsis geminata*, *Dione junio*, *Abanotes hylonome*, Leiodoptera, Nymphalidae, repellency, defense.

Running head : Nymphalid defense against ants

INTRODUCTION

Ants have been cited as important predators of insects both in natural and agricultural ecosystems (Pavis et al., 1992 ; Way and Khoo, 1992). Lepidopteran larvae may be considered an attractive prey, due to their relative immobility and soft cuticle. Nevertheless, many larvae possess an array of chemical defenses effective against both invertebrate and vertebrate predators (Brower et al., 1967 ; Weatherston et al., 1979 ; DeVries, 1987 ; Montllor et al., 1991 ; Attygalle et al., 1993 ; Deml and Dettner, 1993 ; Dyer, 1995). Although traditionally the study of larval chemical defenses has been directed mainly towards vertebrate predators, in recent years, the importance of invertebrate predators (including ants) has been recognized. Several studies demonstrate that the chemistry of the larva (repellent or non-repellent) is an important factor in their defense against invertebrates. (Bernays, 1988 ; Bernays and Cornelius, 1989 ; Dyer and Floyd, 1993 ; Dyer, 1995). Defensive compounds (effective against ants) have been found in the integument of some larvae (Montllor et al., 1991 ; Nickisch-Rosenegk and Wink, 1993), in the hemolymph (Deml and Dettner, 1994 , 1995) and released from specialized glands (Eisner et al., 1972 ; Honda, 1983 ; Deml and Dettner, 1993 , 1994, 1995). The compounds identified in lepidopteran larvae that have an ant defensive function are usually fatty acids (Honda, 1981, 1983 ; Howard et al., 1982), although other substances such as terpenes (Honda, 1983), ketones (Eisner et al., 1972), aldehydes (Deml and Dettner, 1994, 1995), sulphur allelochemicals (Nowbahari and Thibout, 1992), and proteins (Reimann, 1983) have been found.

MATERIALS AND METHODS

Species of Ants and Butterfly Larvae Used in This Study.

Camponotus rufipes (Formicinae) is a common species of ant widely distributed in South America. Although it forms colonies on the ground, it is commonly found in the vegetation, and thus probably regularly encounters lepidopteran larvae. *Solenopsis geminata* (Myrmecinae) is also common in South America. Although this species is found less often foraging in vegetation, it is aggressive, and has been cited as a possible biological control for certain insect pests (Risch and Carroll, 1982a, b ; Perfecto, 1991 ; Jaffe et. al., 1991).

The butterfly larvae studied were the nymphalid species, *Dione junio* and *Abananote hylonome*. Both belong to subfamilies known to be highly cyanogenic (Heliconiinae and Acraeinae, respectively) (Brown and Francini, 1990). Cyanogenic compounds are probably more effective against birds and other vertebrate predators (Brower et al., 1963 ; Nahrstedt and Davis, 1983, 1985 ; Franzl and Naumann, 1985). Both *D. junio* and *A. hylonome* are spiny and possess an eversible neck gland situated ventrally in the prothorax, just in front of the first pair of legs. It is probable that they contain other defensive compounds which are secreted via the spines and/or neck gland when the larva is molested (pers. obs.).

A. hylonome larvae were collected from their host-plants *Verbania* sp. (Compositae), which are commonly found in the surroundings of the Simón Bolívar University, Sartenejas, Caracas, Venezuela. *D. junio* larvae were collected from two passion fruit plantations (*Passiflora edulis*, Passifloraceae), located in Mesa de Urape, Dto. Acevedo and Las Maravillas, Dto. Brión, Miranda State, Venezuela.

The larvae were transported to the laboratory in plastic containers in a cool box. The two species were maintained separately in glass containers (20 cm x 20 cm x 50 cm) at a constant temperature of 25⁰C and a relative humidity of 70-80%. They were fed daily with fresh leaves from their respective host-plants.

Three colonies of each of the two ant species, *C. rufipes* and *S. geminata*, were collected in the surroundings of the University and maintained in the laboratory in plastic bowls of 1 m diameter and 50 cm depth, at the same temperature and humidity as the butterfly larvae. A metal tripod 40 cm high was put in each bowl on which we placed a glass platform of 20 cm² (the foraging area). Two plastic lids of 5 cm diameter and 1 cm depth were placed on the glass platforms. In these, we put pieces of soft netting, one soaked in water and the other soaked in a 1:1 ratio of water and honey. Every two days a larva of *Tenebrio molitor* (Coleoptera:Tenebrionidae) was placed on the foraging area. These beetles were raised in the laboratory in an eight-liter bucket, on a diet of oat and wheat-germ.

Bioassays with Live Larvae (Bioassay I).

The objective of this bioassay was to discover whether or not the live larvae possess chemical defenses against ants, to evaluate the effect of these defenses on each species of ant, and to investigate the development of the defenses in the different larval stages. We placed a larva (*D. junio* or *A. hylonome*) on the foraging area of one of the colonies of each species of ant and observed the behavior of the ants during the first 20 interactions. We compared the responses of the ants towards the butterfly larva with their responses towards a larva of *T. molitor* (control). We also noted whether the butterfly larva survived the bioassay. If the larva died during the bioassay, we noted whether the ants carried it to the nest or left it on the glass platform. The butterfly larva and the control larvae could not be placed simultaneously on the foraging area due to the difficulty of distinguishing which of the two was causing the behavior of the ants. For this reason, the butterfly larva and the control were tested separately, one after the other (the first to be tested was chosen randomly), with each pair of bioassays being considered an experiment. Five replicates were performed for each species of larva, and for each instar, with alternation between colonies of ants so that no ant colony participated in more than two experiments per day.

The results of the five replicates were summed and analysed with a chi-square test (Sokal and Rohlf, 1981). These data were further analyzed to obtain a Spearman correlation for each species of ant. In each case, the following variables were correlated: the responses of alarm, inspection, repellency, and attack by the ants in the presence of the larva, and the survival of the larva. The indices used for each variable are explained in greater detail in the results.

Bioassays with Hexane Extracts (Bioassay II).

In order to characterize the chemical nature of the defense systems of *D. junio* and *A. hylonome*, and to evaluate the contribution of the spines and neck glands to their chemical defenses, the behavior experiments were repeated with hexane washes of whole larvae and hexane extracts of the spines and the neck glands of each species. Preliminary tests showed that hexane washes produced better behavioral results compared to methanol washes, probably because they also extracted a lower number

of compounds compared to methanol. The washes (or extracts) of whole larvae were prepared in the following way: ten individuals of each instar of the two species were introduced into glass vials (1.5 x 3 cm) and 1 ml of solvent was added to each. The larvae were then removed and the solution was concentrated to 100 μ l before each bioassay. The extracts of the spines were prepared by placing the spines from three individuals and the neck glands from five individuals in a glass vial, and adding 1 ml of hexane, and were subjected to ultrasonic waves in an ultrasonic bath for seven minutes in order to improve extraction, which were later evaporated with N₂ gas to 100 μ l. The bioassays were similar to those already described for Bioassay I, but in this case, the extracts of *D. junio* and *A. hylonome* were applied to a *T. molitor* larva. Each *T. molitor* larva received 10 μ l of the extract to be evaluated and the control *T. molitor* larva received the same quantity of pure hexane. The behavior of the ants towards the treated or control *T. molitor* larva was compared. The extracts and the control were tested separately. The order in which the extracts were assayed was random, and 30 sec was allowed for the solvent to evaporate before presenting the treated or control larvae to the ants.

The behavior of the ants was noted as for Bioassay I until they carried the larvae to the nest, or for a maximum period of four minutes. If at the end of the four minutes, the larvae was not carried to the nest, it was left on the foraging tray until it was carried to the nest or for a maximum period of 30 minutes. Each bioassay was repeated six times, and the results were analyzed with a Friedman test (Siegel and Castellan, 1988).

Confirmation of the Repellent Effect of the Compounds Identified (Bioassay III).

The five acids used in the experiments were obtained commercially. Solutions of each (10%) were prepared in 1 ml of hexane in clean glass vials. The solutions were tested as described for Bioassays II, except that the two test materials were placed simultaneously on the foraging area, and experiments were undertaken only with *S. geminata*. Each bioassay was repeated 10 times, and the results analyzed with a Wilcoxon test for each behavior response.

Chemical Analysis.

Hexane extracts of 20 individuals of the first and 5 individuals of the fifth instar of *D. junio* and *A. hylonome* were prepared in 1 ml in clean glass vials. Extracts of spines cut from five individuals of each species (approximately 50 spines per individual) and 20 neck glands were extracted in clean glass vials with 1 ml of hexane per vial. The dissections of spines and neck glands were made in distilled water with the aid of a stereoscopic microscope. The extracts were evaporated to 100 μ l by blowing a gentle stream of N₂ over the extract, and 1 μ l was used for analysis.

The standards used for co-injections were: acetic, oleic, linoleic, palmitic and stearic acids at 2.5 ppm. One μ l of extract and/or standard was analyzed with gas chromatography (GC) coupled to mass spectrometry (MS). GC was performed with a Hewlett Packard 5 and a Perkin Elmer Autosystem, both with splitless injector, a fused silica capillary column (25 m x 0.18 mm ID, stationary phase 0.15 μ m of 5% phenylmethylsilicone). The carrier gas was helium at a flow rate of 1 ml/min, and the oven was held at 40⁰C for 4 min, raised to 280⁰C at 6⁰C/min and held for 30 min. Compounds were detected by the Perkin Elmer mass selective detector, QMASS-900. Mass spectra were identified by comparison of the

retention times and mass spectra of synthetic standards and by means of the NIST library. The parameters for the mass spectrometer were the following: Scanning of masses, 20-450 units of atomic mass ; electron ionization at 70 eV ; electro-multiplier voltage, 1100 V ; interface temperature, 170⁰C.

RESULTS

Definition of the Behavioral Responses of the Ants.

Preliminary experiments with live larva allowed us to define the following behavioral responses of the ants towards the larvae:

Antennation: ants wave their antennae in the direction of the larva but maintain a distance of >1 mm from it.

Explore: ants touch the larva with the tips of their antenna and immediately retire (≤ 1 sec).

For statistical purposes these two categories were lumped together as "Repellency".

Inspection: ants touch the larva with their antenna for a period of > 1 sec.

Attack: ants bite the larva with their mandibles or sting the larva, sometimes dragging it across the foraging tray. The stinging behavior is only applicable to *S. geminata*. *C. rufipes* does not possess a stinging apparatus, although the ants curve the abdomen ventrally until the apex touches the body of the larva.

Ignore: ants do not respond to the presence of the larva.

Alarmed Circling: ants open their mandibles (*C. rufipes*) or raise their abdomens (*S. geminata*) while running in irregular circles around the foraging area with a marked increase in the speed of their movements.

Alarmed trembling: ants vibrate rapidly with their mandibles open. They may be stationary or run in circles in the same way as for Alarmed Circling. This behavior was observed only in *C. rufipes*.

For statistical purposes, alarmed circling and alarmed trembling were placed in the category "Alarm".

Cleaning of the antennae or the first pair of legs: ants repeatedly pass their antenna from the basal to the distal part of the first pair of legs or pass their legs through the mandibles.

Behavior Against Chemical Defenses.

Chi-square analysis of Bioassay I data (tests with live larvae) are shown in Table 1. Both *D. junio* and *A. hylonome* were repellent to *C. rufipes*. Ants were more likely to become alarmed with later instars, and the survival rate of later instars was also higher. A similar pattern was observed when *S. geminata* confronted *D. junio* except that the third and fourth instars, although repellent to ants, were attacked as much as the control. The larvae generally caused alarm in the ants, and although the larvae rarely survived encounters with *S. geminata*, later instars were generally not taken to the nest when killed. Fifth instars were left on the tray if killed and the ants proceeded to cover them with bits of earth. First and second instars of *A. hylonome* were more repellent to *S. geminata* than third and fourth instars which were more inspected. Although the butterfly larvae were always attacked less than the control, they seldom survived, except for the first instar.

For each type of behavior the ants showed towards larvae (repellency, alarm, inspection and attack), we calculated the difference (d_1) between the number of ants that showed a given behavioral

response to the butterfly larva and the number that responded to the control. We constructed an index in which a value of 3 indicated that the larva survived, a value of 2 was given if it was killed but left on the tray, and 1 was assigned if it was killed and taken to the nest. We calculated the difference (d_2) between the survival values of the control and the larva for each experiment. The values of d were then correlated with each other for each species of ant by a Spearman correlation (Table 2). The correlations show that for *C. rufipes*, the survival of the larva correlates positively with the state of alarm in the ants, and that the attack and inspection behaviors are negatively correlated with repellency. For *S. geminata*, the attack rate is positively correlated with their state of alarm, and the survival of the larvae is negatively correlated with the attack rate. The inspection response is also negatively correlated with the repellency.

Experiments with Hexane Extracts.

The results of experiments with Bioassay II are presented in Table 3. For *A. hylonome*, whole larva extracts repel *C. rufipes* significantly more than the solvent, and the ants attacked the control more than whole larva and extracts of spines. There was no significant difference between the extracts and the control with respect to the inspecting behavior of *C. rufipes*. The behavior of *S. geminata* towards the extracts of *A. hylonome* showed that whole larvae repelled the ants more than the control and were inspected less. Hexane extracts of *D. junio* larva, spines, and neck gland repelled *C. rufipes* more than the control, and the ants attacked the extracts of whole larvae and the neck gland less.

Chemical analyses revealed that the hexane extracts of both larvae contained mainly carboxylic acids. The compounds identified from the first and fifth instar, and from the spines and neck glands of *D. junio* and *A. hylonome* are presented in Table 4. The chemical composition of the first and fifth instars of both species are quantitatively and qualitatively different.

Repellency of the Acids Tested.

Using Bioassay III, we found that all the acids tested were repellent for *S. geminata*. The ants rarely approached the *Tenebrio* larvae treated with these compounds and always showed alarm behavior (Table 5). Although both oleic and linoleic acid and the combination of the two caused alarm in the ants, there was no significant difference between number of ants attacking the treated *Tenebrio* larva and those attacking the control. The combination of linoleic and oleic acid tended to be less attacked than the separate acids. Acetic acid was the only one which did not cause alarm behavior in the ants, and it is notable that this was the only acid attacked significantly less than the control.

DISCUSSION

Both *D. junio* and *A. hylonome* repel and cause alarm in the ants. When *C. rufipes* workers are alarmed, the larvae are more likely to survive, whereas an increased alarm in *S. geminata* results in an increased level of aggression of workers, at least if close to the nest. The bioassays were carried out close to the ant colonies in an area that may be territorial for the ants. It is likely then that the chemical defenses of these larvae will be more effective against ants foraging relatively far from their nest outside their nest territory.

The fact that hexane extracts of both species produced repellency demonstrates that the

repellent nature of the *D. junio* and *A. hylonome* has a chemical basis. Although extracts of whole larva produced a more marked reaction than extracts of the spines and neck glands in both species of ant, the latter too had an effect. The extracts of the spines and neck glands were less concentrated (equivalent to 0.3 and 0.5 individuals, respectively) than those of whole larvae, due to the methodology used. This difference in concentration could explain the different results.

The repellent properties of *A. hylonome* and *D. junio* may be due to at least three of the acids: acetic, linoleic, and oleic acid (Table 5). These compounds are repellent to different species of ants (Howard et al., 1982 ; Honda, 1983). Dani et al. (1996) investigated the sternal gland secretion of adult females of *Polistes dominulus* and *P. sulcifer* (Vespidae) and discovered several long chain carboxylic acids. Of these, oleic, linoleic, and palmitoleic acid (all unsaturated) had a repellent effect on the ants *Crematogaster scutellaris*, *Formica cunicularia*, and *Lasius* sp., while lauric, myristic, palmitic, and stearic acid (saturated) did not. Howard et al. (1982) found that oleic acid present in the eggs of *Gastrophysa cyanea* (Coleoptera: Chrysomelidae) was repellent to several species of ants including *Camponotus pennsylvanicus* and *Solenopsis invicta*. Workers of the ant, *Iridomyrmex humilis*, rapidly consumed controls but covered *Tenebrio* larvae treated with oleic acid with small pieces of earth. Honda (1983) investigated the effect on the ants *Lasius niger* and *Crematogaster matsumurai* of several compounds present in the defensive secretions of larvae in the genera *Papilio* and *Graphium*. Among the compounds tested, acetic acid was found to be repellent and extremely toxic, producing 100% mortality in both species tested.

Among the sesquiterpenes present in the extracts of whole larvae of *A. hylonome*, only α -farnesene has been reported as a component of the defensive secretions of butterfly larvae of *Papilio* spp. (Papilionidae) (Honda, 1981). Honda (1983) reports that α -farnesene has a limited effect on *L. niger* and *C. matsumurai*. This compound was repellent to both species of ant in the present study. The compound, β -farnesene, which differs from α -farnesene only in the relative position of the substituent groups, has been reported as an alarm pheromone in three subfamilies of aphids (Dettner and Leipert, 1994) as well as being the principal component in the defensive secretion of *Atta* spp. (Hernández, 1996). It is possible that α -farnesene forms part of the defense system of *A. hylonome*.

Chemical Defense of Dione junio.

In *D. junio*, acetic and linoleic acid were present in all extracts, while oleic acid was found only in extracts of whole larvae. *C. rufipes* was repelled by all the extracts of *D. junio*, and ants attacked the whole larvae and the neck gland less than the control. Acetic acid, present in the spines and neck gland of *D. junio*, is probably the most important component in the repellency of this species. The first instar was not repellent to either ant species. The lack of the methylesters of linoleic and oleic acids in this instar could be the cause of the lower repellency. However, these methyl esters were also absent from the extracts of the spines and neck gland, which were repellent to *C. rufipes*.

Chemical Defense of Abananote hylonome.

In *A. hylonome*, we identified palmitic, linoleic, and stearic acid, and the methyl esters of palmitic and stearic acid in all extracts, except in the neck glands where oleic acid was absent. Live *A. hylonome*

larvae were repellent and survived several of the bioassays, especially with *C. rufipes*, although these larvae do not contain acetic acid in their defensive secretion. The extracts of the spines of *A. hylonome* were attacked less by *C. rufipes* than the control, whereas the neck gland extracts were attacked similarly to the control. The combination of linoleic and oleic acid caused less attack than the separate acids. In the experiments with *S. geminata*, there was no significant difference between the control and extracts of *D. junio*, while in the experiments with *A. hylonome*, only washes of the whole larva showed significant differences with the control. This indicates that the aggressiveness of *S. geminata* can annul the repellent effect of the larval defensive compounds and that the combination of linoleic and oleic acid in *A. hylonome* represents an effective defense against these ants. This suggests that in *A. hylonome*, with no acetic acid, the combination of linoleic and oleic acid may be at least partially responsible for the chemical defense.

In *A. hylonome*, sesquiterpenes were absent in first instars but present in 4th and 5th, which also alarmed more *C. rufipes* workers. With *C. rufipes*, alarm of the ants is related to survival of the larvae, suggesting that the terpenes contribute to this survival.

First instars of *A. hylonome* did not induce aggression from *S. geminata* workers. The absence of sesquiterpenes could contribute to this lack of aggression. These terpenes are also absent from spines and neck gland extracts of *A. hylonome*. It was not possible, however, to prove the separate effect of the sesquiterpenes, but it is likely that they induce alarm response in *S. geminata* as well as in *C. rufipes*. In *S. geminata*, the alarm response results in increased aggression towards the larvae. Thus, the lack of terpenes in the first instar could account for the lack of aggression. This result emphasizes the fact that a chemical defense effective against one species of ant is not necessarily effective against other species.

There is more of each compound in fifth than in first instars, and there was a tendency for later instars to survive more often than early ones. Thus, it is probable that in the first instars, the quantity and concentration of the defensive compounds in a single larva is not enough to be effective against ants. One bite by the ants can kill a first instar, whereas later instars are more resistant. Larvae of both species of butterfly are gregarious until the fifth instar. In the experiments undertaken, only one larva was presented to the ants. The chemical defenses employed by the larvae may be more effective when they are aggregated. The benefits of gregarious behavior in insect defenses has been discussed by Vulinec (1990). Codella and Raffa (1995) found that groups of 40 larvae of Diprionidae (Hymenoptera) showed higher survival rates than groups of five. The survival of larvae of *Hemileuca lucina* (Saturnidae: Lepidoptera) was greater in groups of 20 individuals (80% survived) than in groups of five (35% survived).

The first and fifth instars of both species share many of the compounds identified and seem to be protected by chemical defenses from the start of the larval stage. This has been reported also for *Diaprepes abbreviatus* (Coleoptera), in which first instars are repellent to *S. geminata* (Pavis et al., 1992). First instars of *Lymantria dispar* (Lepidoptera) which had not yet eaten survived more attacks from *Formica* spp. than first instars that had eaten (Weseloh, 1989).

The defensive carboxylic acids identified from *D. junio* and *A. hylonome* are common products of metabolism and widely distributed among insects (Rockstein, 1978). Attygalle et al. (1993), proposed that small quantities of acetic acid may be common in insect defensive secretions. This acid has been

found in the secretion of the beetle, *Helluomorphoides clairvillei* (Attygalle et al., 1992) and in the defensive spray of the ant, *Camponotus floridanus* (Attygalle et al., 1993). It is also present as a major compound in the defensive secretion of ants of the genus *Atta* (Hernández, 1996). In Lepidoptera larvae, acetic acid forms part of the defensive secretion from the osmeteria of *Papilio helenus* (Papilionidae) (Honda, 1983) and the neck gland of *Schizura unicornis* and *S. badia* larvae (Notodontidae) (Weatherston et al 1979).

Acknowledgments : We thank Estación Experimental Miranda, Fonaiap, for access to their field sites, and referees and editors for valuable corrections. This work was partially supported by grant BID-CONICIT QF-36.

REFERENCES

- ATTYGALLE, A. B., MEINWALD, J., and EISNER, T. 1992. Defensive secretion of the carabid beetle, *Helluomorphoides clairvillei*. *J. Chem. Ecol.* 18: 489-498.
- ATTYGALLE, A. B., SMEDLEY, S. R., MEINWALD, J., and EISNER, T. 1993. Defensive secretion of two notodontid caterpillars (*Schizura unicornis*, *S. badia*). *J. Chem. Ecol.* 19 : 2089-2104.
- BERNAYS, E.A. 1988. Host specificity in phytophagous insects: selection pressure from generalist predators. *Entomol. Exp. Appl.* 49: 131-140.
- BERNAYS, E.A., and CORNELIUS, M. L. 1989. Generalist caterpillar prey are more palatable than specialists for the generalist predator *Iridomyrmex humulis*. *Oecologia* 79: 427-430.
- BROWER, L.P., BROWER, J.V.Z., and COLLINS, C. T. 1963. Experimental studies on mimicry. 7. Relative palatability and Müllerian mimicry among neotropical butterflies of the subfamily Heliconiinae. *Zoologica N.Y.* 48: 65-84.
- BROWER, L.P., BROWER J.V.Z., and CORVINO, J.M. 1967. Plant poisons in a terrestrial food chain. *Proc. Natl. Acad. Sci. U.S.A.* 57: 893-898.
- BROWN, K. S., and FRANCINI, R.B. 1990. Evolutionary strategies of chemical defense in aposematic butterflies: cyanogenesis in Asteraceae-feeding American Acraeinae. *Chemoecology.* 1:52-56.
- CODELLA, S.G., and RAFFA, K.F. 1995. Contributions of female oviposition patterns and larval behavior to group defense in conifer sawflies (Hymenoptera: Diprionidae). *Oecologia* 103: 24-33.
- DANI, F.R, CANNONI, S., TURILLAZZI, S., and MORGAN, E. D. 1996. Ant repellent effect of the sternal gland secretion of *Polistes dominulus* (Christ) and *P. sulcifer* (Zimmerman) (Hymenoptera: Vespidae). *J. Chem. Ecol.* 22 : 37-48.
- DEML, R., and DETTNER, K. 1993. Biogenic amines and phenolics characterize the defensive secretion of saturnid caterpillars (Lepidoptera: Saturniidae): a comparative study. *J. Comp. Physiol. B.* 163:123-132.
- DEML, R., and DETTNER, K. 1994. *Attacus atlas* caterpillars (Lep., Saturniidae) spray an irritant secretion from defensive glands. *J. Chem. Ecol.* 20 :2127-2138.
- DEML, R., and DETTNER, K. 1995. Effects of emperor moth larval secretions, hemolymph, and

components on microorganisms and predators. *Entomol. Exp. Appl.* 76: 287-293.

DETTNER, K., and LEIPERT, C. 1994. Chemical mimicry and camouflage. *Ann. Rev. Entomol.* 39: 129-154.

DEVRIES, P.J. 1987. *The Butterflies of Costa Rica and Their Natural History*. Princeton University Press. Princeton

DYER, L. A. 1995. Tasty generalists and nasty specialists? Antipredator mechanisms in tropical lepidopteran larvae. *Ecology* 76 : 1483-1496.

DYER, L. A., and FLOYD, T. 1993. Determinants of predation on phytophagous insects: the importance of diet breadth. *Oecologia* 96: 575-582.

EISNER, T.F., KLUGE, K.F., CARREL, J.C., and MEINWALD, J. 1972. Defence mechanisms of arthropods. XXXIV. Formic acid and acyclic ketones in the spray of a caterpillar. *Ann. Entomol. Soc. Am.* 65:765-766.

FRANZL, S., and NAUMANN, C.M. 1985. Cuticular cavities: storage chambers for cyanoglucoside containing cavities in larvae of a zygaenid moth. *Tissue Cell.* 17:267-278.

HERNANDEZ, J.V. 1996. Ecología química del comportamiento agnóstico de *Atta laevigata* (Hymenoptera: Formicidae). PhD thesis, Universidad Simon Bolivar, Caracas.

HONDA, K. 1981. Larval osmeterial secretions of the swallowtails (*Papilio*). *J. Chem. Ecol.* 7 : 1089-1113.

HONDA, K. 1983. Defensive potential of components of the larval osmeterial secretion of papilionid butterflies against ants. *Physiol. Entomol.* 8:173-179.

HOWARD, D.F, BLUM, M.S., JONES, T.H., and PHILLIPS, D.W. 1982. Defensive adaptations of eggs and adults of *Gastrophysa cyanea*. (Coleoptera: Chrysomelidae). *J. Chem. Ecol.* 8 : 453-462.

JAFFE, K., MAULEON, H., and KERMARREC, A. 1991. Qualitative evaluation of ants as biological control agents with special reference to predators on *Diaprepes* spp (Coleoptera: Curculionidae) in citrus groves in Martinique and Guadeloupe, pp. 405-416, in C. Pavis & A. Kermarrec (eds.). *Rencontres Caraibes en Lutte Biologique*, INRA, Paris,

MONTLLOR, C.B., BERNAYS, E.A., and CORNELIUS, M.L. 1991. Responses of two hymenopteran predators to surface chemistry of their prey: Significance for an alkaloid-sequestering caterpillar. *J.*

Chem. Ecol. 17 : 391-399.

NAHRSTEDT, A., and DAVIS, R.H. 1983. Occurrence, variation and biosynthesis of the cyanogenic glucosides linamarin and lotaustralin in species of the Heliconiini (Insecta: Lepidoptera). *Comp. Biochem. Physiol.* 75B : 65-73.

NAHRSTEDT, A., and DAVIS, R.H. 1985. Biosynthesis and quantitative relationships of the cyanogenic glucosides linamarin and lotaustralin in genera of the Heliconiini (Insecta: Lepidoptera). *Comp. Biochem. Physiol.* 82B : 745-749.

NICKISCH-ROSENEGK, E. von, and WINK, M. 1993. Sequestration of pyrrolizidine alkaloids in several arctiid moths (Lepidoptera: Arctiidae). *J. Chem. Ecol.* 19: 1889-1903.

NOWBAHARI, B., and THIBOUT, E. 1992. Defensive role of *Allium* sulfur compounds for leek moth *Acrolepiopsis assectella* Z. (Lepidoptera) against generalist predators. *J. Chem. Ecol.* 18 : 1991-2002.

PAVIS, C, MALOSSE, C., DUCROT, F., HOWSE, P.E., JAFFE, K., and DESCOINS, C. 1992. Defensive secretion of first-instar larvae of rootstalk borer weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae), to the fire-ant *Solenopsis geminata* (F.) (Hymenoptera: Formicidae). *J. Chem. Ecol.* 18 : 2055-2067.

PERFECTO, I. 1991. Ants (Hymenoptera: Formicidae) as natural control agents of pests in irrigated maize in Nicaragua. *Environ. Entomol.* 84 : 65-70.

REIMANN, H. 1983. Chemische Zusammensetzung und Wirksamkeit des Wehrsekretes der Larven von *Zygaena trifoloo* (Esper. 1793). Lepidoptera. Staatsexamensarbeit. University of Bielefeld.

RISCH, S.J., and CARROLL, C.R. 1982a. The ecological role of ants in two Mexican agroecosystems. *Oecologia* 55: 114-119.

RISCH, S.J., and CARROLL, C.R. 1982b. Effect of a keystone predaceous ant, *Solenopsis geminata*, on arthropods in a tropical agroecosystem. *Ecology* 63: 1979-1983.

ROCKSTEIN, M. 1978. Biochemistry of Insects. Academic Press 649pp.

SIEGEL, S., and CASTELLAN, N.J. 1988. Nonparametric Statistics for the Behavioral Sciences. 2nd edition. McGraw-Hill Book Company. New York.

SOKAL, R. R., and ROHLF, F.J. 1981. Biometry. W.H. Freeman and Company, San Francisco.

VULINEC, K. 1990. Collective security: aggregation by insects as a defense, pp. 251-288, *in* D.L. Evans, and J.O. Schmidt (eds.). *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. State University of New York Press. Albany,

WAY, M.J., and KHOO, K.C. 1992. Role of ants in pest management. *Annu. Rev. Entomol.* 37: 479-503.

WEATHERSTON, J., PERCY, J.E., MACDONALD, L.M., and MACDONALD, J.A. 1979. Morphology of the prothoracic defensive gland of *Schizura concinna* (Lepidoptera: Notodontidae) and the nature of its secretion. *J. Chem. Ecol.* 5:165-178.

WESELOH, R.M. 1989. Simulation of predation by ants based on direct observations of attacks on Gypsy Moth larvae. *Can. Entomol.* 121: 1069-1076.

Table 1 Behavioral responses of the ants, *Camponotus rufipes* and *Solenopsis geminata* towards instars of *Dione junio* and *Abananote hylonome*.

Ant spp	Instar	<i>Dione junio</i>				<i>Abananote hylonome</i>			
		Behavior		Survival index		Behavior		Survival index	
		Repel	Attack	Median	Range	Repel	Attack	Median	Range
<i>C. rufipes</i>	1		+	2	1-3		+	2	1-3
<i>C. rufipes</i>	2	-	+	1	1-3		+	2	2-3
<i>C. rufipes</i>	3		+	2	1-3		+	3	3-3
<i>C. rufipes</i>	4	-	+	3	2-3	-	+	3	2-3
<i>C. rufipes</i>	5	-	+	3	2-3	-	+	3	3-3
ANOVA (<i>P</i>)			<0.05	<0.05			<0.05	<0.05	
<i>S. geminata</i>	1		+	1	1-2	-	+	3	1-3
<i>S. geminata</i>	2	-	+	2	1-3		+	1	1-3
<i>S. geminata</i>	3	-		2	1-3	-	+	1	1-2
<i>S. geminata</i>	4	-		2	2-0		+	2	1-2
<i>S. geminata</i>	5	-	+	2.5	2-3		+	2	2-2
ANOVA (<i>P</i>)		<0.05		<0.05		<0.05	<0.05		

Legend: Repel: the larva was repellent to the ants.

Attack: the larva was attacked by the ants.

(-) response of the ants greater towards the larva

(+) response of the ants greater towards the control

Table 2. Correlation coefficients of relations between the responses of ants towards live larvae of two nymphalid species and the survival of the latter.

Ant spp.	Behavior	Inspection	Repellent	Alarm	Attack
<i>C. rufipes</i>	Repellent	-0.535**			
<i>C. rufipes</i>	Alarm	0.072	0.016		
<i>C. rufipes</i>	Attack	-0.059	-0.354**	0.057	
<i>C. rufipes</i>	Survival	-0.086	0.125	0.321*	-0.209
<i>S. geminata</i>	Repellent	-0.453**			
<i>S. geminata</i>	Alarm	-0.451**	0.091		
<i>S. geminata</i>	Attack	-0.682**	-0.095	0.447**	
<i>S. geminata</i>	Survival	0.158	0.024	-0.092	-0.271*

* $P \leq 0.05$, ** $P \leq 0.01$, N = 25

Table 3. Frequency of behavioral responses of ants towards extracts of larvae

Extracts	Behavior					
	Inspection		Repellent		Attack	
	Median	Range	Median	Range	Median	Range
<i>C. rufipes</i> vs. <i>A. hylonome</i>						
Whole larva			4*	1-4	1.5*	1-2
Spines	NS		2.25	1-3	1.5*	1-3
Neck gland			2.75	2-4	3	1.5-4
Pure hexane (Control)			1	1-3	4	3-4
<i>S. geminata</i> vs. <i>A. hylonome</i>						
Whole larva	1*	1-1	4*	3-4		
Spines	3	2-4	3	1-3	NS	
Neck gland	3	2-4	2	2-4		
Pure hexane (Control)	3	2-4	1	1-2		
<i>C. rufipes</i> vs. <i>D. junio</i>						
Whole larva			3*	2-4	2.5*	1-3.5
Spines	NS		3*	2-4	3	1-3
Neck gland			3*	2-4	2*	1-2
Pure hexane (Control)			1	1-1	4	3.5-4

* Significant difference between the extract and the control, $P \leq 0.05$, N = 6

Table 4. Relative proportion (in % relative to the most abundant compound)) of compounds found in hexane extracts of larvae of *Dione junio* and *Abananote hylonome*.

Compound	1st instar	5 th instar	Spines	Neck gland
<i>Dione junio</i>				
1. Acetic acid	51	26	14	34
2. Tetradecanoic acid	< 1	< 1	< 1	1
3. Methyl ester palmitic acid	18	< 1	17	1
4. Palmitic acid	14	16	24	42
5. Methyl ester linoleic acid		11		
6. Methyl ester linolenic acid		11		
7. Methyl ester stearic acid	100	< 1	< 1	5
8. Linoleic acid	76	100	100	100
9. Oleic acid	92	100		
10. Stearic acid	7	3	14	12
<i>Abananote hylonome</i>				
2. Tetradecanoic acid	3	< 1	7	< 1
3. Methyl ester palmitic acid	20	< 1	8	2
4. Palmitic acid	40	20	100	5
7. Methyl ester stearic acid	80	4	9	1
8. Linoleic acid	19	100	32	100
9. Oleic acid	100	48	75	
10. Stearic acid	40	2	20	< 1
11. α - farnesene		3		
12. Isomer of α -farnesene		2		
13. Cubenene		17		
14. Cadinene		19		
15. Copaene		31		

Table 5. Behavioral responses of *Solenopsis geminata* towards the listed acids.

Behavior	Acids							
	Acetic		Linoleic +oleic		Linoleic		Oleic	
	%	N	%	N	%	N	%	N
Repellency	98.2**	10	88.2*	10	100**	9	97.2**	8
Inspection	7.3**	10	34.5	10	2.2**	9	67.3	10
Attack	14.5**	10	19.4	8	71.4	6	70	10
Alarm	90	5	100**	8	100*	5	100**	7

% refers to the percentage of replicates where the number of ants that responded to the presence of the acid was greater than the number of ants that responded to the presence of the control.

* and ** indicate statistical significance using a chi-squared test for $P < 0.01$ and 0.001 respectively.