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Alice Ramos de Moraes

"Biologia e ecologia de *Pleuroptya silicalis* (Lepidoptera: Crambidae) e *Urbanus esmeraldus* (Lepidoptera: Hesperiidae): táticas defensivas e interações com formigas em arbustos de *Urera baccifera* (Urticaceae)"

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Orientador: Prof. Dr. Paulo Sérgio Moreira Carvalho de Oliveira

Co-Orientador: Prof. Dr. André Victor Lucci Freitas

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BANCA EXAMINADORA

Prof. Dr. Paulo Sérgio Moreira Carvalho de Oliveira ______________________ Assinatura

Prof. Dr. Ronaldo Bastos Francini ______________________ Assinatura

Profa. Dra . Karina Lucas da Silva-Brandão ______________________ Assinatura

Prof. Dr. Gustavo Quevedo Romero ______________________ Assinatura

Prof. Dr . Luiz Francisco Lembo Duarte ______________________ Assinatura

Dedicatória

Dedico este trabalho a todos aqueles, biólogos ou curiosos, que possam aproveitar as informações aqui contidas de alguma maneira positiva – como contribuição profissional ou mesmo enriquecimento pessoal.

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Resumo

1. O presente trabalho investiga aspectos comportamentais e de história natural de duas espécies de lepidópteros que se alimentam de *Urera baccifera* (Urticaceae), uma planta visitada por 22 espécies de formigas. Ambas as espécies, *Pleuroptya silicalis* (Lepidoptera: Crambidae) e *Urbanus esmeraldus* (Lepidoptera: Hesperiidae), constróem abrigos foliares e apresentam diferentes mecanismos de defesa contra predação. Por exemplo, quando perturbadas, larvas de *P. silicalis* sacodem o corpo violentamente, jogam-se da folha, mordem e regurgitam. Larvas de *U. esmeraldus* mordem e regurgitam, apenas. Ambas as espécies preferem folhas maduras, passam por cinco estádios de desenvolvimento e apresentam características comuns a outros membros de suas famílias.
2. *Pleuroptya silicalis* constrói abrigos foliares em forma de tubo, enchendo-os com seda e fezes, sendo comum encontrar vários indivíduos no mesmo abrigo. Já *Urbanus esmeraldus* constrói dois tipos de abrigos foliares ao longo de seu desenvolvimento e apenas uma larva é encontrada em cada abrigo.
3. Abrigos foliares artificiais, similares aos abrigos de *P. silicalis* (porém sem fezes ou seda dentro) não fornecem proteção a cupins, usados como herbívoros simulados. As fezes também não provocam mudanças de comportamento em formigas no laboratório, não as atraindo aos abrigos ou repelindo dos mesmos. As fezes podem, entretanto, funcionar como barreira mecânica, dificultando o acesso ao interior do abrigo.
4. *Urbanus esmeraldus* lança suas fezes a grandes distâncias. Experimentos demonstraram que fezes no chão induzem formigas a subirem na planta hospedeira. Por outro lado, fezes arremessadas longe da base da planta não produzem o mesmo efeito. Além disso, larvas de 5º estádio cortam o pecíolo das folhas em que descansam, e das quais se alimentam, tornando-as murchas precocemente. Uma vez que formigas conseguem transpor o pecíolo cortado, este comportamento pode estar relacionado à redução de predação por aves, já que estas podem utilizar sinais visuais indicativos de presença

e/ou atividade de lagartas no forrageamento. O corte do pecíolo pode ainda reduzir o parasitismo das larvas (prejudicando a transmissão de vibrações provenientes da lagarta e dificultando a ação de parasitóides que dependam deste tipo de sinal para localização do hospedeiro), ou mesmo acelerar a eliminação de compostos secundários da planta. Tais hipóteses, entretanto, precisariam ser testadas.

5. Durante o ano de 2006, a presença de formigas não foi suficiente para diminuir a infestação por todas as espécies de lepidópteros de *Urera baccifera*, ao contrário do observado em anos anteriores (2003 e 2004). Esta variação temporal pode ser explicada por uma diferença na abundância dos herbívoros (mais abundantes em 2006), determinando assim o nível de sucesso das formigas na proteção à planta.

Abstract

1. This work investigates the biology and behaviour of two lepidopteran species that feed on the nettle *Urera baccifera* (Urticaceae). The plant is visited by 22 ant species, which are attracted by the nettle's fleshy fruits and pearl bodies. Larvae of both species build leaf shelters: *Pleuroptya silicalis* (Crambidae: Pyraustinae) makes leaf rolls, and *Urbanus esmeraldus* (Hesperiidae: Pyrginae) builds two different kinds of shelters (peaked-roof shelters and leaf folds). Both species have 5 instars of development and present morphological and behavioural similarities to other members in each of their families.
2. Larvae of *P. silicalis* fill the leaf rolls with silk and faeces (frass). Artificial rolls, very similar in shape and size, but without silk or frass, did not prevent termite workers glued on the inside from being preyed by ants. Although frass did not alter the behaviour of ant foragers in the laboratory, faecal pellets could play an important role against predators and parasitoids by mechanically preventing them from entering the roll.
3. Larvae of *Urbanus esmeraldus* throw their faecal pellets at great distances. We experimentally demonstrated that frass located near the base of an artificial shrub induce foraging ants to climb on the plant in greater numbers than faecal pellets 30 cm away from the plant. Thus frass ejection influences directly larval vulnerability to ants.
4. Fifth-instar larvae of *U. esmeraldus* cut the petiole of the leaves they rest and feed. Ants, however, are not deterred by the cut petiole and it is suggested that this larval behaviour could be related with avian predation pressure. Because the cut leaves soon wither, the visual effect can be deceptive for insectivorous birds that tend to forage more often on healthy leaves. Alternatively, cutting the leaf could reduce the plant's

secondary compounds, or decrease attack by parasitoids that use leaf-borne vibrations to locate their hosts.

5. In 2006 ant presence did not affect infestation by lepidopteran larvae on *U. baccifera* shrubs. Although ants have been reported by other authors to decrease caterpillar infestation in previous years, at increased herbivore abundance ant visitation may not be sufficient to suppress caterpillars on host plants.

Sumário

Introdução Geral

Dentre os vários tipos de associações entre organismos observadas, os mutualismos destacam-se pela sua ubiqüidade, diversidade e abundância e são caracterizados pelo fato das espécies participantes (geralmente duas) proporcionarem benefícios uma a outra (Begon *et al.* 1996). Tais associações são de grande interesse científico e inúmeros trabalhos abrangem diversos aspectos deste tipo de interação. Mutualismos podem variar ao longo de um gradiente contínuo, indo desde interações difusas e facultativas até obrigatórias e altamente especializadas. Estas variações se devem a vários fatores, tais como as espécies envolvidas e suas abundâncias, e a fatores ambientais diversos (e.g., espaço e tempo, estação do ano), conferindo um caráter de "condicionalidade" a tais interações (Bronstein 1994, 1998; Thompson & Cunningham 2002).

Grande parte de nosso conhecimento acerca de mutualismos entre plantas e animais foi adquirida a partir dos estudos de interações entre formigas e plantas (Beattie 1985; Bronstein 1998). Durante a maior de parte de sua história, formigas e angiospermas estiveram associadas. Formigas são muito abundantes e parecem especialmente propensas a desenvolver mutualismos com plantas (Hölldobler & Wilson 1990; Bronstein 1998). Especialmente nos trópicos, muitas espécies de formigas usam plantas como substrato para forrageamento em busca de presas (vivas ou mortas), ou de alimentos produzidos pela própria planta (Carroll & Janzen 1973). Recursos como néctar produzido e liberado por nectários extraflorais, corpos alimentícios, exsudato liberado por hemípteros, e locais para nidificação levam formigas a explorarem as plantas (Heads & Lawton 1985; Hölldobler & Wilson 1990; Vasconcelos 1991; Whitman 1994; Oliveira 1997; Oliveira & Del-Claro 2005). O forrageamento intenso de formigas na vegetação pode resultar em diversos tipos de benefícios para as plantas. As formigas podem, por exemplo, fornecer nutrientes às plantas, dispersar suas sementes, promover sua polinização, predar ou perturbar herbívoros reduzindo a herbivoria e, com isso, promover aumento da produção de frutos (Horvitz & Schemske 1984; Beattie 1985; Oliveira 1997; Oliveira *et al.* 1999).

A presença e a atividade intensa de formigas na vegetação resultaram em um grande número de interações entre plantas, herbívoros e formigas, que podem ser de natureza facultativa ou obrigatória. Quando uma terceira espécie (como um herbívoro) está envolvida, é mais provável inclusive que a associação mutualística apresente maior variação em seu resultado (Bronstein 1998). Em relação à planta, o resultado final da interação vai depender do modo pelo qual o comportamento das formigas afeta o desempenho dos herbívoros, e seu conseqüente dano à planta hospedeira (Oliveira & Del-Claro 2005).

Em resposta à pressão de predação por formigas, herbívoros em geral desenvolveram diversas estratégias defensivas que, através da diminuição dos riscos de predação, permitem seu estabelecimento e permanência em plantas visitadas por formigas (Heads & Lawton 1985; Ito & Higashi 1991; Loeffler 1996; Oliveira & Freitas 2004). Lagartas de lepidópteros possuem inúmeros inimigos naturais, vertebrados e invertebrados, e ilustram bem essa resposta à predação (Salazar & Whitman 2001).

A ordem Lepidoptera, cuja classificação se baseia principalmente em estudos envolvendo adultos (Scoble 1995; Freitas & Brown 2004), é extremamente abundante e de biologia complexa. O potencial de contribuição dos estudos de biologia e desenvolvimento de imaturos ainda é pouco explorado, especialmente no que diz respeito a estudos de sistemática (Brown & Freitas 1994).

Lepidópteros exercem um grande impacto sobre as plantas que consomem (no caso de larvas) ou polinizam (adultos), bem como sobre seus predadores e parasitóides. O número de espécies fitófagas dentro desta ordem é aproximadamente o mesmo de Coleoptera, o que sugere um grande impacto de lepidópteros como consumidores primários (Scoble 1995). Aves e formigas são seus mais importantes predadores, atuando como principal causa de mortalidade em diferentes fases de seu desenvolvimento: aves são importantes predadoras de larvas grandes, pupas e também adultos, enquanto que formigas são geralmente o principal fator de mortalidade de larvas jovens, especialmente durante seu estabelecimento na planta (Laine & Niemelä 1980; Smiley 1985; Scoble 1995; Freitas & Oliveira 1996).

Ao longo de seu desenvolvimento, larvas de lepidópteros enfrentam diferentes tipos de inimigos naturais, tendo muitas vezes de mudar suas estratégias defensivas. As lagartas possuem uma grande variedade de estratégias defensivas que, de acordo com Salazar & Whitman (2001), podem ser morfológicas (cor, cutícula espessa, pêlos e espinhos), comportamentais (construção de abrigos, remoção de fezes, proteção por fio de seda), fisiológicas, mutualísticas (associação com formigas), químicas (toxinas internas, glândulas, regurgitar), ou ainda uma combinação destas. Entretanto, resta ainda a necessidade de se demonstrar a eficácia de algumas características larvais contra inimigos naturais (Dyer 1995).

O sistema abordado neste estudo envolve três espécies de lepidópteros cujas larvas se alimentam de arbustos de *Urera baccifera* (Urticaceae) em uma reserva florestal nos arredores de Campinas (SP). Esta planta é visitada por 22 espécies de formigas distribuídas em 11 gêneros: *Camponotus* (7 espécies), *Pheidole* (4 espécies), *Crematogaster* (2 espécies), *Pseudomyrmex* (2 espécies), e *Acromyrmex*, *Atta*, *Cephalotes*, *Linepithema*, *Pachycondyla*, *Solenopsis* e *Tapinoma* (1 espécie cada). As formigas visitam as plantas em busca de seus frutos, ricos em carboidratos e proteínas, e dos corpos alimentícios (corpos perolados) produzidos em suas folhas, pecíolos e pedicelos de inflorescência e frutos (Dutra *et al.* 2006).

Todas as espécies de lepidópteros que utilizam *U. baccifera* como planta hospedeira apresentam algum tipo de adaptação contra predação. Larvas de 1º. a 3º. estádio de *Smyrna blomfildia* (Fruhstorfer) (Nymphalidae: Coloburini) constroem pontes de fezes, estruturas finas e alongadas feitas com pelotas fecais e seda, sobre as quais as larvas descansam (Machado & Freitas 2001). Lagartas de *Urbanus esmeraldus* (Butler) (Hesperiidae: Pyrginae) e *Pleuroptya silicalis* (Guenée) (Crambidae: Pyraustinae) constróem diferentes tipos de abrigos foliares.

Os objetivos principais deste trabalho são: (1) Descrever o comportamento e a história natural de *Urbanus esmeraldus* e de *Pleuroptya silicalis*, com ênfase nas interações com formigas sobre a planta hospedeira; (2) investigar, por meio de observações e experimentos de campo e laboratório, o impacto de formigas sobre as populações destes lepidópteros.

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CAPÍTULO 1:

"Biology and defence mechanisms of *Pleuroptya silicalis* (Lepidoptera: Crambidae) and *Urbanus esmeraldus* (Lepidoptera: Hesperiidae) on the host plant *Urera baccifera* (Urticaceae)"

INTRODUCTION

Even though studies on the biology and development of Neotropical Lepidoptera have increased in quantity during the last years, there are still many groups lacking general information such as the majority of moth families and butterfly families Hesperiidae, Lycaenidae and Riodinidae (DeVries 1987, 1997). The full potential of information about immature biology and development is still unknown, especially concerning its contribution to systematic studies of the Lepidoptera (Brown & Freitas 1994). Due to the lack of adequate material for immature comparison studies and because adults are easier to collect and store (Scoble 1995), lepidopteran larval stages have not been as extensively researched as adults. Lepidopteran classification has therefore developed mainly as a result of studies of the adults, but still larvae have been extremely important in solving problems, either supporting or demonstrating weaknesses in adult classification (Kitching 1985; Scoble 1995 and references therein; Freitas & Brown 2004). Larval studies and their complementary value to adult studies should therefore be more emphasized.

The head and body of lepidopteran larvae present various setae and there is often a difference between the number of setae found on the 1st and on subsequent instars (Scoble 1995). Setae occurring on 1st instar larvae are named primary setae and their number and position are relatively constant throughout the order. However, there are small but significant variations both in their distribution and occurrence, so they can and have been used in studies for systematic purposes (Hinton 1946; Kitching 1985; Freitas 2003). Later instars present secondary setae, which often obscure primary ones, and are not regular in position and cannot be homologized individually. On the other hand, primary setae can be named in a consistent way because of their regularity of position. The nomenclature currently used follows Hinton (1946). Each primary seta is numbered and the number is prefixed by a letter to indicate the kind of seta and its approximate location (for example, the letters D and SD stand for to the dorsal and subdorsal groups of setae on the body, respectively). A setal map is a stylized figure used to represent the position of primary setae on the thorax and abdomen.

The ecological and environmental importance of the Lepidoptera is due to their impact as primary consumers – they about equal Coleoptera in number of phytophagous species, although they present other feeding habits as well (Scoble 1995). According to the environmental conditions, some species develop the status of pests and are of great concern for humans, such as the genus *Spodoptera* (Noctuidae). Finally, their importance is also due to the fact they are preyed upon and parasitized at all stages of their development.

Birds and ants are considered to be the most important predators of lepidopteran larvae (Laine & Niemelä 1980, Salazar & Whitman 2001). Avian predation is an important cause of mortality of lepidopteran larvae and adults (Scoble 1995). On the other hand, ant predation on the host plant is usually considered the main factor of mortality of lepidopteran juveniles, specially during the period of larval establishment on the plant (Smiley 1985). In fact, some adult butterflies even use visual cues to evaluate the plant before ovipositing, and respond negatively to ant density on the host plant (Freitas & Oliveira 1996; Oliveira & Freitas 2004).

Nonetheless, lepidopteran larvae have numerous other natural enemies from a wide variety of taxa such as bugs, spiders, scorpions, frogs, marsupials, rodents, bats and primates (Scoble 1995; Salazar & Whitman 2001). Due to such a diversity of predators, caterpillars present many different defence strategies that can be classified into the following categories: A) morphological: presence of hairs, spines, warning coloration, cuticle thickness; B) mutualistic: association with ants; C) chemical: internal toxins, glands, regurgitation; D) physiological: encapsulation, and E) behavioural (Salazar & Whitman 2001). The defence suite of a caterpillar can also be a combination of these tactics since predators of different guilds are likely to be deterred by different defence mechanisms - although some of them may deter predators of different guilds (Dyer 1997). Moreover, caterpillars face different sets of natural enemies as they grow and often must switch defensive strategies during their development (Salazar & Whitman 2001).

Behavioural defences include diverse mechanisms such as hanging by a silk thread, dropping off the leaf, feeding at night, biting, thrashing (to move the body violently), removing frass from the vicinity, building leaf shelters (Brower 1984; Heads & Lawton 1985; Freitas & Oliveira 1992; Potting *et al.* 1999; Weiss 2003), as well as mimicry. These mechanisms usually enable caterpillars to overcome ant attacks, depending on the situation (Heads & Lawton 1985; Freitas 1991; Potting *et al.* 1999). Although behaviour is a predictor of caterpillar rejection by ants, most studies have not been able to differentiate which kinds of behaviour are more effective against ant predation (Dyer 1995).

Leaf shelter construction is a behavioural defence strategy exhibited by many different lepidopteran species (Damman 1987; Loeffler 1996; Eubanks *et al.* 1997; Salazar & Whitman 2001; Jones *et al.* 2002). Species from at least 18 families of Lepidoptera build external shelters on their host plants by folding, rolling, tying, or joining plant structures with silk (Scoble 1995). Despite the relatively common occurrence of this life-history trait, the real function of leaf shelters has been little studied (Jones *et al.* 2002). Some authors have ascribed different functions to leaf shelters, such as modification of microclimate, adaptation for feeding on phototoxic plants (Sandberg & Berenbaum 1989), increased leaf nutritional quality (Sagers 1992), and protection against natural enemies (Damman 1987; Ruehlmann *et al.* 1988; Loeffler 1996; Jones *et al.* 2002).

In this chapter we investigate the system involving three lepidopteran species whose larvae are the main herbivores of the nettle *Urera baccifera* (L.) Gaudich (Urticaceae). *Urera baccifera* (Plate 1A) is a thin-stemmed shrub, generally about 1 – 2 m high, covered with many sharp, stinging spines all over the stem, leaves and reproductive parts (including fruits) (Francis 2000). It is a typical pioneer species and moderately shade-intolerant. This plant is visited by 22 different ant species from 11 genera: 7 species of *Camponotus*, 4 species of *Pheidole*, 2 species of *Crematogaster,* 2 species of *Pseudomyrmex* and 1 species of *Acromyrmex*, *Atta*, *Cephalotes*, *Linepithema*, *Pachycondyla*, *Solenopsis* and

Tapinoma (Dutra *et al.* 2006). Capuchin monkeys (*Cebus apella*) and seven birds species are the main seed dispersers of *U. baccifera* in the study area (Galetti & Pedroni 1994; Galetti & Pizo 1996), but ants climb onto the plant to collect the fleshy fruits produced during the fruiting period (which varies from April to June) and also the food bodies produced on leaves and stems (Plate 1B).

Larvae from all three lepidopteran herbivores have some kind of defence mechanism against predation. Individuals from 1st to 3rd instar of *Smyrna blomfildia* (Fruhstorfer) (Nymphalidae: Coloburini) build frass chains, which are stick-like structures made of faecal pellets (frass) and silk, on the tip of which they rest (Machado & Freitas 2001). The moth *Pleuroptya silicalis* (Guenée) (Crambidae: Pyraustinae) builds leaf rolls and fills them with silk and frass. Larvae are bright green and feed inside the shelters, leaving it only to build a new one. More than one larva can be found in a single shelter. The adult is shown in Plate 2F. The skipper *Urbanus esmeraldus* (Butler) (Hesperiidae: Pyrginae) builds two types of leaf shelters during its ontogeny, and leaves the shelters to feed. Larvae live singly in the shelter and exhibit house cleaning behaviour, that is, they do not keep their frass inside the shelter. The adult is shown in Plate 3G.

This work had the following objectives: (1) to describe the larval stages and development of two of three lepidopteran species that feed on *U. baccifera*, *U. esmeraldus* and *P. silicalis*, and (2) to record the putative defence mechanisms of both species.

MATERIALS AND METHODS

Study area

Field work was carried out in the Santa Genebra Reserve, in the city of Campinas, São Paulo state, southeast Brazil (22°49'S, 47°06'W). The reserve is predominantly covered by semi-deciduous mesophytic forest and the climate is defined as warm and wet, with dry winter and wet summer. The mean annual temperature is 21.6 °C and average rainfall is 1381 mm (Morellato & Leitão-FIlho

1995). The shrubs of *Urera baccifera* are predominantly located along the main trail in the forest, about 1000 m long, and at the south border of the reserve.

Methods

Larvae of both species, as well as eggs of *Urbanus esmeraldus* and leaves of *Urera baccifera*, were collected in the study area and maintained in controlled temperature in the laboratory. Each larva was individually placed on a plastic container (6 cm height x 5 cm diameter), together with a fresh *Urera baccifera* leaf and a piece of toilet paper to absorb the moisture excess. The leaves had their petioles involved with cotton which was regularly moistened. The containers were cleaned and the toilet paper was changed daily. Leaves were replaced every two or three days (following Freitas 1991).

We described the stages of both species according to the following aspects (based on Freitas & Oliveira 1992; Aiello 1993):

a) egg: colour, shape, mean size in mm (diameter and height), surface texture and sculpturing, mean time to emergence of the larva (in days);
b) larval instars: head colour, ornaments, texture, mean width of head capsule, body colour, presence of setae or other projections, maximum length in mm, behavioural aspects and mean duration of each instar, in days.
c) pupa: colour, mean length in mm, general appearance, mean duration (in days).

Larvae of *P. silicalis* were collected and reared between February and April 2003, whereas larvae of *U. esmeraldus* were collected and reared in May and December 2005. Dry head capsules were kept separately for posterior measurement with a microscope fitted with a calibrated micrometric ocular. Some first-instar individuals of both species were also fixed in Kahle solution for body chaetotaxy studies.

We reared one 4th instar and one 5th instar *U. esmeraldus* larvae on potted plants in the laboratory (one larva per plant and one at a time). The pots containing the plants were placed, one at a time, over a white paper (80 cm x 168 cm) on which the faecal pellets of *U. esmeraldus* fell. We marked the exact spots where the pellets fell with a pen, in order to visualize the deposition pattern on the ground. We also marked the caterpillar's position relative to the ground. This procedure was repeated everyday until the larva abandoned the plant to pupate.

RESULTS

Description of early stages *Pleuroptya silicalis*

First instar: Head capsule light beige, see-through, with long setae. Head capsule width 0.50-0.58 mm (mean = 0.55 mm, SD = 0.04, n = 3). Body covered with thin long setae, beige, dorsally light green, bright, see-through (with visible dark green intestinal content), legs and prolegs of the same coloration of the body. Body chaetotaxy illustrated in Figure 1. Maximum length: 7 mm. Duration: 3 days. See Plate 2B.

Second instar: Head capsule light beige. Head capsule width 0.72-0.82 mm (mean = 0.79 mm, SD = 0.03, n = 8). Body covered with thin long setae, light green, bright with visible dark green intestinal content. Legs and prolegs light green and bright. Maximum length: 12 mm. Duration: 1-4 days (mean = 2.17 days, SD = 0.83, n = 12).

Third instar: Head capsule light beige. Some individuals may present brown spots on top of the head. Head capsule width 0.86-0.98 mm (mean = 0.90 mm, SD = 0.05, n = 8). Body covered with thin long setae, green, bright, with strongly visible dark green intestinal content. Some individuals present a pair of dark brown plates on the prothorax. Legs and prolegs green, bright. Maximum length: 18 mm. Duration: 1-5 days (mean = 2.6 days, SD = 0.99, n = 20).

Fourth instar: Head capsule darker than the previous instar, with more conspicuous dark brown spots. Head capsule width 1.08-1.32 mm (mean = 1.18 mm, SD = 0.07, n = 15). Body darker than the previous instar, bright, dark green dorsally and lighter green ventrally, covered with thin long setae and with a pair of thin lateral white stripes. Dark brown plates on the prothorax also more conspicuous than previously. Legs and prolegs of the same colour of the ventral portion of the body. Maximum length: 26 mm. Duration: 2-6 days (mean = 3.35 days, SD = 0.98, n = 23).

Fifth instar: Head capsule light brown with conspicuous dark brown spots. Head capsule width 1.6-2.2 mm (mean = 1.97 mm, SD = 0.14, n = 17). Body covered with thin long setae, dark green dorsally and lighter green ventrally, bright, with a pair of thin lateral white stripes. Conspicuous dark brown plates on the prothorax. Legs and prolegs of the same colour of the ventral portion of the body. Two days before pupation the body becomes opaque, light green, and the white stripes are no longer visible. Maximum length: 32 mm. Duration: 5-7 days (mean = 6.15 days, SD = 0.9, n = 13). Prepupa is fixed to the substrate by the anal prolegs. See Plate 2C, D.

Pupa: Entirely brown, elongated, with mobile abdominal segments. Total length: 13-17 mm (mean = 15.96 mm, SD = 1.02, n = 27). Duration: 8-10 days (mean = 8.91 days, SD = 0.67, n = 23). See Plate 2E.

Ecological and behavioural notes

Feeding habits: Larvae of 1st and 2nd instars do not consume the leaf epidermis, feeding only on the internal tissues. From the 3rd instar on, larvae eat all tissues, except large leaf veins. Larvae of all instars feed inside the shelters, leaving only to build a new one.

Shelter building and occupation: Larvae of all instars build leaf shelters. They usually roll the side of the leaf, parallel to its axis, and fasten it with silk – see Plate 2A. Smaller larvae can also roll the leaf from its apex, so that the roll is transverse to the axis. They fix numerous silk threads inside the shelter and this results in a dense array of silk and faeces (frass). It is very common to find many larvae living in the same shelter. Aggregations are usually formed by many small larvae (1st to 3rd instar) and one big larva (4th or 5th instar). We recorded all larvae inside 21 shelters we collected in March 2006. We found that two thirds (14 shelters) had more than one larva inside, of which 12 shelters (85.71%) contained at least one larva of 4th/5th instar together with smaller ones, and only 2 (14.29%) contained only larvae of 1st, 2nd and/or 3rd instars.

Defence strategies: Besides building shelters, larvae also present other behavioural defensive tactics when disturbed. Thrashing (moving the body violently) and dropping off the leaf are the behaviours most frequently observed, but they can also bite, regurgitate, or jump small distances. Larval response to shelter disturbance is quick: the larva moves towards the opposite edge of the roll and, if disturbance persists, moves the body violently and jumps off the leaf.

Description of early stages *Urbanus esmeraldus*

Egg: Spherical, white, slightly bright, with 13 longitudinal ridges and 3 well-defined transverse ridges. One day before larval eclosion it becomes dark in the top (head capsule of the growing larva). Height and diameter 1.1 mm (n = 2). See Plate 3A, B.

First instar: Head capsule black, without visible projections. Head capsule width 0.68-0.70 mm (mean = 0.70 mm, SD = 0.01, n = 4). Body light yellow after eclosion, becoming greener and slightly bright with time. Green intestinal content visible, legs and prolegs of the same colour of the body, and last two abdominal segments light yellow. Pair of black plates on the prothorax. Body chaetotaxy illustrated in Figure 3. Maximum length: 8 mm. Duration: 6-7 days (mean = 6.67 days, SD = 0.58, n = 3).

Second instar: Head capsule black. Head capsule width 1.00-1.10 mm (mean = 1.05 mm, SD = 0.04, n = 9). Body dark green, bright and see-through. Green intestinal content visible, legs and prolegs of the same colour of the body and last two abdominal segments yellow. Pair of prothoracic black plates more conspicuous. Maximum length: 11 mm. Duration: 3-4 days (mean = 3.33 days, SD = 0.58, n = 3). See Plate 3C.

Third instar: Head capsule black. Head capsule width 1.54-1.78 mm (mean = 1.68 mm, SD = 0.09, n = 10). Body dark green, less bright than the previous instar, with a pair of thin lateral light-coloured stripes, ill-defined. Thoracic segments reddish on the ventral portion and see-through prolegs of the same colour of the body. Pair of conspicuous yellow spots on the last abdominal segments. Pair of black plates on the prothorax. Maximum length: 17 mm. Duration: 3-7 days (mean = 5 days, SD = 1.15, n = 7).

Fourth instar: Head capsule black, hairy, with a pair of opaque orange spots next to the stemmata. Head capsule width 2.67-3.00 mm (mean = 2.79 mm, SD = 0.11, n = 12). Body dark green, covered with short hairs, with a pair of thin lateral light green stripes. Thoracic segments reddish on the ventral portion and see-through prolegs of the same colour of the body. Pair of conspicuous well-defined yellow spots on segments A8 and A10. Pair of black plates on the prothorax. Pair of dorsal yellow glands between segments A5 and A6. Legs black. Maximum length: 32 mm. Duration: 5-8 days (mean = 1.04 days, SD = 6.25, n = 8).

Fifth instar: Head capsule black, hairy, with a pair of well-defined orange spots next to the stemmata. Head capsule width 3.89-4.75 mm (mean = 4.32 mm, SD = 0.26, n = 15). Body dark green, covered with short hairs, with a pair of thin lateral light green stripes. Thoracic segments reddish on the ventral portion and see-through and prolegs also reddish. Pair of conspicuous well-defined yellow spots on segments A8 and A10. Pair of black plates on the prothorax. Pair of dorsal yellow glands between segments A5 and A6. Legs black. Two or three days before pupation, the body becomes brownish purple, with black conspicuous spiracles and dorsal artery. The lateral stripes become more inconspicuous. Maximum length: 49 mm. Duration: 9-13 days (mean = 11 days, SD = 1.51, n = 8). See Plate 3D, E.

Pupa: Entirely brown, elongated, without projections. White wax covers the entire pupa. Pupates in the soil. Total length: 20-26 mm (mean = 22.33 mm, SD = 1.59, n = 15). Duration: 10-16 days (mean = 13.50 days, SD = 2.20, n = 8). See Plate 3F.

Ecological and behavioural notes

Feeding habits: Unlike *P. silicalis*, larvae of *U. esmeraldus* do not feed inside the shelters, and consume all leaf tissues. Full-grown larvae (5th instar) generally feed on a leaf different from the one they build their shelter.

Shelter building and occupation: Larvae of all instars build shelters, and only one larva is found in a shelter. *Urbanus esmeraldus* builds two different kinds of shelters during its development, and these structures differ in shape from the rolls built by *P. silicalis*. Small larvae (1st – 3rd instar) construct a peaked-roof, cone-shaped shelter by making two cuts on the leaf, folding the flap towards the centre of the leaf and securing it to the surface with silken "guy-wires" (Lind *et al.* 2001). They rest on the cone "ceiling" (Plate 4C, E, F). Larvae of 4th and 5th instars simply fold one side of the leaf, big enough for them to rest underneath (Plate 4D). They do not roll the leaf as tightly or as many times as does *P. silicalis*.

From the larvae reared on the plants in the laboratory, we were able to observe that fifth-instar larvae chew out totally (or almost totally) the petiole of the leaf where they build the shelter, and deposit silk on the incision (Plate 4B). The leaf hangs as if its petiole was broken and, a few days later, the larva makes another incision above the first one, at the junction of the petiole with the stem, depositing silk on it. Then the leaf soon withers, and becomes unattractive. Interestingly, the larvae also cut the petiole of the leaf they feed (Plate 4A). This was observed at night.

We also noticed that *U. esmeraldus* throws its faecal pellets at great distances from the plant base, in a scattered pattern around the trunk (Figure 3). The pellets were found as far as 88.4 cm from the plant base.

Defence strategies: Besides building shelters, *U. esmeraldus* exhibits other behavioural defence tactics, such as biting and, more rarely, regurgitating. Larvae of *U. esmeraldus* do not thrash, move rather slowly and remain attached to a silk mat laid down on the surface of the leaf.

DISCUSSION

Larvae of *Pleuroptya silicalis* have an external morphology very similar to other members of the family Crambidae. Also, the behaviour of shelter building by rolling the host plant leaves occurs among other species of the same family as well. Crambids, especially members of the subfamily Pyraustinae, are generally concealed feeders - leaf rollers, tiers or borers (Romanowski 1991; Scoble 1995). Other species within the genus *Pleuroptya* use species of Urticaceae (including the genus *Urera*) as host plants, such as *Urtica dioica, Urera caracasana* and *Urera elata* (http://janzen.sas.upenn.edu/index.html; Romanowski 1991). We were not able to find eggs of *P. silicalis* in the field, nor obtain them in the laboratory.

Urbanus esmeraldus is also morphologically similar to other members of the family Hesperiidae (Scoble 1995). Shelter building and frass ejection are behaviours found in other members of this family (Scoble 1995; Jones *et al.* 2002). Most of the known hesperiid larvae live singly in a shelter, and shelter type may vary not only among species, but also through larval ontogeny in the same species (Lind *et al.* 2001; Greeney & Jones 2003). According to Greeney & Jones (2003), this family probably contains the greatest diversity of larval shelters within the Lepidoptera, as they may range from a simple resting spot secured by some strands of silk on the base of a leaf, to elaborate peaked and perforated structures. Larvae of the hesperiid *Epargyreus clarus* (Cramer) are known to undergo ontogenetic changes in leaf shelter construction – they build 4 different types of shelters during their development (Lind *et al.* 2001). It is very likely that *U. esmeraldus* also undergoes such ontogenetic changes. Actually, both kinds of shelters built by *U. esmeraldus* are very similar to 2 of the shelters built by *E. clarus* (two-cut fold and leaf roll – see Lind *et al.* 2001 for more details). The pyralid moth *Herpetogramma aeglealis* (Walker) also changes its shelters with growth, and inhabits approximately 5 shelters of 3 different types during its development (Ruehlmann *et al.* 1988). Ontogenetic changes in shelter size and style may be explained by biological needs and/or physical capabilities of the larva, which change as it grows. As suggested by Lind *et al.* (2001), large larvae are able to manipulate large pieces of leaves and cutting may become unnecessary – larvae of 4th and 5th instar of *U. esmeraldus* in fact only fold the leaves.

Lepidopteran larvae are usually able to overcome ant attacks through a number of behavioural mechanisms (Heads & Lawton 1985; Freitas 1991). When disturbed, *P. silicalis* exhibited behaviours that may be related to defence against predation or parasitism such as dropping off the leaf, biting, and especially thrashing (moving the body violently) and regurgitating (very rare – considered chemical defence by Salazar & Whitman 2001). The numerous strands of silk laid inside the shelter could also help the caterpillar detect the presence of enemies once they are inside, since the larva promptly responds to any disturbance (A. R. Moraes, pers. obs.). Larvae of *U. esmeraldus* also present such behaviours, including biting in response to disturbance. Potting *et al.* (1999) demonstrated that behaviours such as biting or thrashing can significantly reduce parasitism rates. They also regurgitate, although rarely. Regurgitating in response to successive attacks is common among the Lepidoptera, and it is often associated with the presence of dissuasive substances in the regurgitated fluid (Brower 1984; Salazar & Whitman 2001). In a study trying to link larval traits with defence against predators, Dyer (1995) determined that behaviour, chemistry, diet breadth, morphology and developmental stage are significant predictors of caterpillar rejection by the ant species of *Paraponera clavata*. No caterpillar behaviour stood out as being the best defensive response - however, thrashing alone appeared to be an ineffective defence and possibly attracted other ants (Malicky 1970; Dyer 1995). Prey chemistry and diet breadth were the best predictors of rejection – caterpillars with unpalatable extracts and specialist caterpillars were more rejected not only by the ants, but also by wasps (*Polistes instabilis*) and predatory bugs (*Apiomerus pictipes*) (see also Dyer 1997). Predators of different guilds are deterred by different defence mechanisms, although some defensive traits may work against different guilds.

Shelter building can be an effective defence against predation in certain situations (Damman 1987; Loeffler 1996; Eubanks *et al.* 1997; Jones *et al.* 2002). However, in some occasions leaf shelters may actually increase its inhabitant's vulnerability. Factors such as prey density, type of predator (including its learning ability and experience level) may determine the effectiveness of leaf shelters as an efficient device against predators (Jones *et al.* 2002; Weiss *et al.* 2004). Additionally, Gentry & Dyer (2002) argue that although leaf shelter construction has been proved effective against predation, it is not necessarily the same when it comes to parasitism. In fact, concealed larvae may become even more vulnerable, as they have a rather sedentary life (Hawkins 1994) and the chemical cues from its frass or silk are static and relatively easy to locate (Agelopoulos *et al.* 1995). This can be particularly true in the case of *P. silicalis* because its shelters are filled with frass and silk. Larvae of *U. esmeraldus*, however, may not be severely affected because they exhibit frass ejection behaviour, so that frass does not remain inside the shelters or in the vicinity.

Weiss (2003) states that many lepidopteran species that build some kind of external shelter on their host plant (folding, rolling or tying some of the plant's structures) also present frass ejection behaviour. Based on direct reports of frass ejection and on the presence of associated anal structures (such as a sclerotized comb generally found in caterpillars that eject frass), it was determined that this behaviour occurs in at least 17 lepidopteran families. Interestingly, within some families, shelter-building larvae eject their frass, whereas non-shelter-dwelling species generally do not (Scoble 1995; Weiss 2003). According to some authors, frass may act as chemical and visual cue to natural enemies (Stamp & Wilkens 1993; Müller & Hilker 1999; Weiss 2003, 2006). In the case of the hesperiid *Epargyreus clarus*, its frass ejection behaviour is positively related to defence against predation by the wasp *Polistes fuscatus* – wasps attacked significantly more larvae that were in close proximity to frass (Weiss 2003).

Because proximity to its own frass is likely to make the larva vulnerable, we inferred that ejected frass near the base of the host plant could provide cues to potential predators like ants (similar to hemipteran exudates acting as chemical cues to ants – see Del-Claro & Oliveira 1996). An irregular distribution of the faecal pellets on the ground, as observed for 5th-instar larvae of *U. esmeraldus*, could therefore make it difficult for ants to locate the larva. Thus an experiment to test if ants are somehow attracted to frass of *U. esmeraldus* is needed (please refer to Chapter 2).

The behaviour of chewing out the petiole of the leaf exhibited by 5th-instar larvae of *U. esmeraldus* is also reported for grass feeding hesperiines that cut along the mid vein of the leaf, which is subsequently silked in order to firmly hold the shelter in a different plane of the leaf blade (Greeney & Jones 2003). However, its purpose remains yet to be discovered. Firstly, it could be interpreted as a strategy to eliminate some toxic compounds of the plant. The cut in the petiole can lead to the elimination of the plant's secondary compounds together with the sap and/or may cause the internal flux of those compounds to diminish (Dussourd 1993; Lewinsohn & Vasconcellos-Neto 2000). We do not know, however, if *U. baccifera* produces secondary compounds. Moreover, if this behaviour was really shaped by such feeding constraints, it would be expected that caterpillars presented other means of avoiding toxic compounds throughout their development (Lewinsohn & Vasconcellos-Neto 2000). In addition, feeding constraints do not explain why they chew out the petiole of the leaf where they rest. We suggest that it may be a defence mechanism against natural enemies. The cut petiole may deter crawling predators (such as ants) from reaching the surface of the leaf, and consequently minimizing caterpillar exposure to them. Caterpillars may also be less exposed to avian predators, which would not search for prey on withered leaves (Heinrich & Collins 1983). Birds are important predators of older larvae and pupae, whereas arthropods are probably more important predators of eggs and early larvae (Scoble 1995). Finally, vibrations may be the main stimuli used by parasitoids when their hosts are concealed feeders (Djemai *et al.* 2004), and an incision on the petiole could reduce the transmission of substrate-borne vibrations to other parts of the plant.

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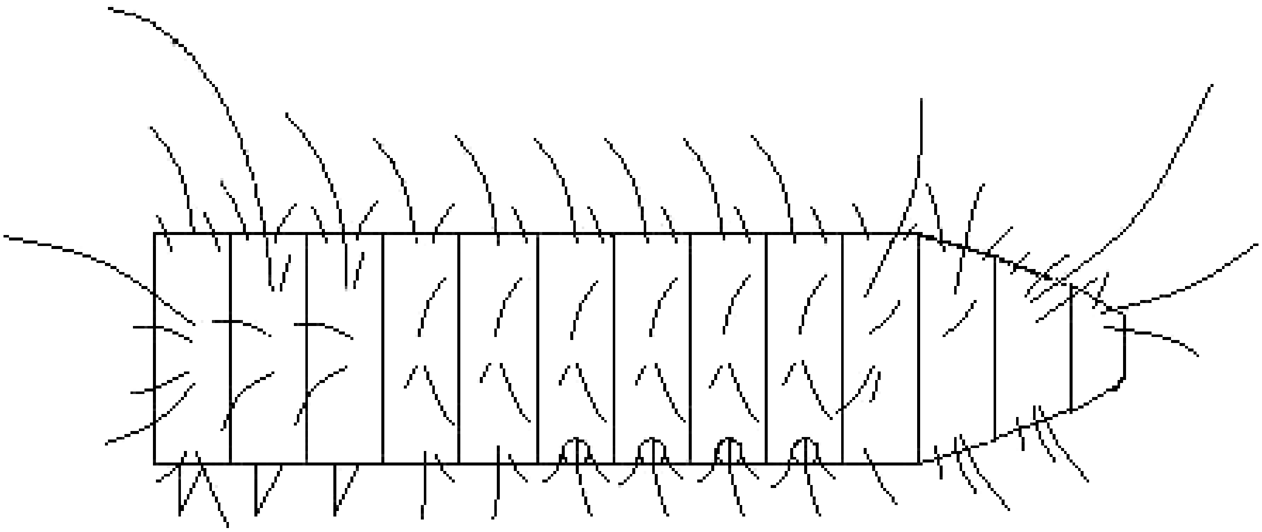

Figure 1. Body chaetotaxy of first instar larva of *Pleuroptya silicalis.*

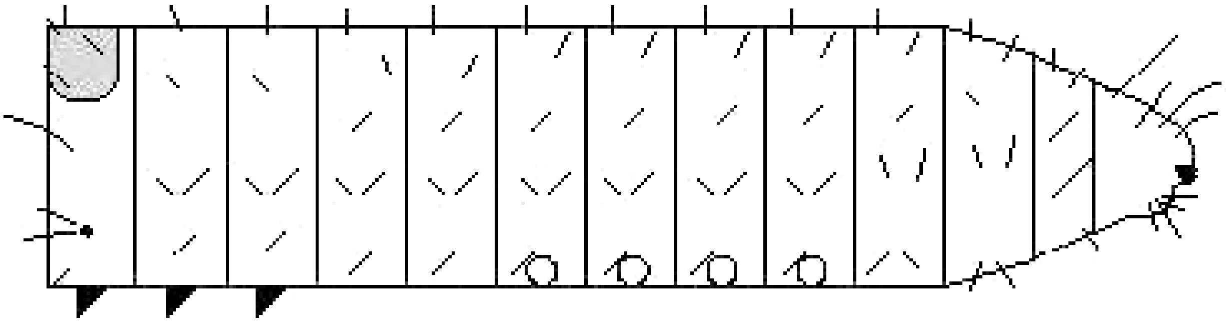

Figure 2. Body chaetotaxy of first instar larva of *Urbanus esmeraldus.*

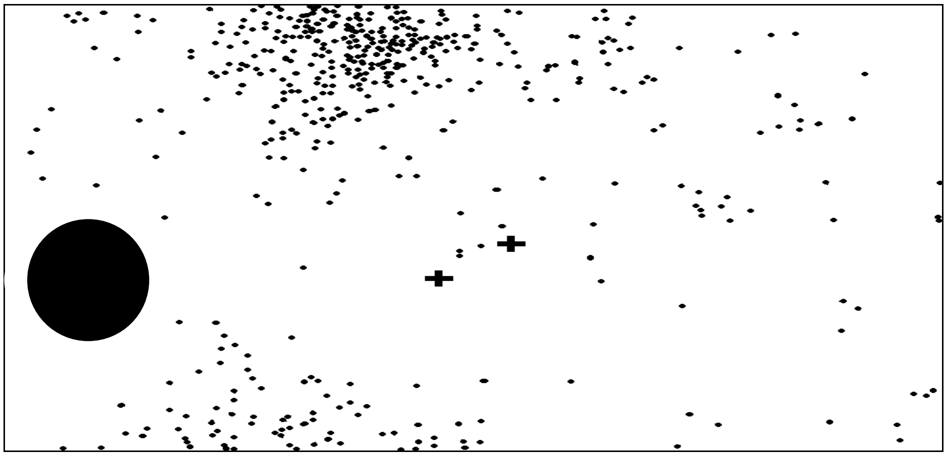

Figure 3. Deposition pattern of frass from a fifth instar larva of *Urbanus esmeraldus* on an 80 cm x 168 cm white surface, after a five-day period. The black spots mark where the faecal pellets hit the ground. The black circle is the base of the plant's vase and the two crosses represent the projection of larval location (on the plant).

CAPÍTULO 2:

"Interactions between lepidopteran larvae and ants on shrubs of *Urera baccifera* (Urticaceae)"

INTRODUCTION

Ants are extremely abundant and comprise in many habitats the majority of the arthropod fauna found on vegetation. They interact with plants in many different ways (Hölldobler & Wilson 1990). Different factors are responsible for ant activity on foliage, such as the existence of predictable and/or renewable food sources, as well as nesting sites. These food sources can be, for example, extrafloral nectar, food bodies, honeydew from phloem-feeding hemipterans and secretions from lepidopteran larvae (Hölldobler & Wilson 1990; Vasconcelos 1991; Whitman 1994; Oliveira 1997; Oliveira & Del-Claro 2005). Intense ant activity on foliage resulted in a wide array of interactions between ants, plants and their herbivores that can range from very loose and facultative, to obligate and highly specialized associations (reviews in Beattie 1985; Bronstein 1998). Ant patrolling activity on foliage affects herbivores in different ways and this may result in positive, negative and neutral consequences for plants (Bronstein 1994). Ants can, for instance, decrease herbivore damage and increase fruit set (Horvitz & Schemske 1984; Beattie 1985; Oliveira 1997; Oliveira *et al.* 1999).

Lepidopteran larvae have numerous natural enemies, vertebrate and invertebrate, and have to deal with different sets of natural enemies as they grow, often having to switch defensive strategies during their development (Salazar & Whitman 2001). Birds are significant predators of older larvae and pupae - as well as adults - and ants are major predators of eggs and early larvae. Thus predation by birds and ants is probably the most important cause of caterpillar mortality. (Laine & Niemelä 1980; Scoble 1995; Salazar & Whitman 2001). In response to predation pressure, caterpillars have evolved many defensive tactics that allow them to exploit ant-visited plants (Heads & Lawton 1985; Ito & Higashi 1991; Loeffler 1996; Oliveira & Freitas 2004). Caterpillars present many different defence strategies, which can be subdivided into the following categories, according to Salazar & Whitman (2001): a) morphological: hairs, spines, coloration, cuticle thickness; b) behavioural: shelter/frass chain construction, removal of frass, startle displays, hiding evidence of feeding, thrashing, dropping; c) mutualistic: association with ants; d) chemical: internal toxins, glands, regurgitation; e) physiological: encapsulation. A caterpillar's defensive suite can also be a combination of any of the tactics.

Behavioural defence comprises a wide variety of mechanisms, many of which are related to feeding behaviour, such as feeding inside plant tissues, hiding evidence of feeding, disperse feeding throughout the plant, nocturnal feeding, and others (revised by Salazar & Whitman 2001). Some feeding behaviours that impose trade-offs to the caterpillar's growth and development can also be associated with predation avoidance (Heinrich 1979; Damman 1987; Montllor & Bernays 1993). For instance, the moth *Omphalocera munroei* (Martin) feeds on old leaves of the genus *Asimina* (Annonaceae), although these are nutritionally poorer than the young ones (Damman 1987). This imposes a larval developmental rate 20% slower and can be explained by the fact that old leaves provide caterpillars more resistant shelters, which protect them more efficiently against predation.

Leaf shelter construction is a behavioural defence strategy exhibited by different lepidopteran species (Damman 1987; Loeffler 1996; Eubanks *et al.* 1997; Salazar & Whitman 2001; Jones *et al.* 2002). Larvae from at least 18 families build external structures in their host plant by folding, rolling, tying or joining plant structures with silk. Some authors have ascribed different functions to leaf shelters. Sandberg and Berenbaum (1989) demonstrated that leaf tying by *Platynota flavedana* (Tortricidae) allows them to feed on a phototoxic plant. By feeding inside the ties, larvae are shielded from light and therefore protected from phototoxic compounds. Sagers (1992) describes the effects on leaf nutritional quality by leaf-rolling behaviour of moths from different families. They make a roll of leaves around expanding buds of *Psychotria horizontalis* (Rubiaceae) that significantly reduces their toughness and tannin concentration. Some studies have also shown that leaf shelters can be effective against predation, specially by ants and wasps (Damman 1987; Loeffler 1996; Eubanks *et al.* 1997; Jones *et al.* 2002; Weiss 2003).

Frass ejection occurs in at least 17 lepidopteran families (Weiss 2003) and can also act as an anti-predatory strategy. The skipper *Epargyreus clarus*

(Cramer) (Lepidoptera: Hesperiidae) throws its fecal pellets long distances away from its shelter and it was demonstrated that caterpillars, in the presence of frass, are more attacked by the predatory wasp *Polistes fuscatus* (Weiss 2003). Many lepidopteran species that build some kind of external shelter on their host plant also present frass ejection behaviour (Weiss 2003), but this is not a strict rule. Many lepidopteran larvae live in close association with their own frass to no apparent ill effect and even make use of it as a defensive device (Müller & Hilker 1999; Weiss 2006). Keeping frass inside the shelter may be an effective barrier against natural enemies, as described by Vasconcelos (1991) for two species of lepidopteran caterpillars that feed on a myrmecophytic (i. e., ant-inhabited) plant. Both species are able to overcome ant predation by building ant-proof shelters.

However, the presence of frass inside leaf shelters can be risky for the larva, since it may provide chemical cues to natural enemies (Agelopoulos *et al.* 1995; Weiss 2003). When searching for hosts, parasitoids make use of a variety of means, including visual, tactile and chemical signals (Vinson 1976; Godfray 1994; Djemai *et al.* 2004). The latter include volatiles associated with the host itself, its habitat, its frass, or even its host plant (Vinson 1976; Weseloh 1993; Quicke 1997). Any signal that betrays the presence of an organism is extremely disadvantageous.

Gentry & Dyer (2002) state that, although leaf shelter construction may be effective against predation, it may not be against parasitism– in fact, it can even make the inhabitant more susceptible to parasitoid attack. This is because sheltered caterpillars are rather sedentary (Hawkins 1994), and cues from the silk or accumulated frass are static and easy to locate (Agelopoulos *et al.* 1995). In response to parasitoid contact, larvae may drop off the leaf, remain still, regurgitate, or behave aggressively by moving the body vigorously and biting (Gross 1993; Godfray 1994; Quicke 1997; Potting *et al.* 1999; Gentry & Dyer 2002). In spite of the wealth of information on parasitoid response to frass, only a few studies have assessed the response of predatory invertebrates to frass of potential prey (Weiss 2006), and this number is even smaller if the predators are ants. Müller and Hilker (1999) have examined the response of the generalist ant *Myrmica rubra* to faecal shields of three species of cassidine larvae, and found out that ants were actually attracted to the shields. This result contrasted with other studies that demonstrated that shields of chrysomelid larvae act as defensive mechanisms against generalist predators such as ants (Nogueira-de-Sá & Trigo 2002).

The present study investigates the system involving three lepidopteran species whose larvae are the main herbivores of the nettle *Urera baccifera* (L.) Gaudich (Urticaceae), which in turn is constantly visited by ants. *Urera baccifera* is a thin-stemmed shrub about 1 – 2 m high (Plate 1A). It is covered with many sharp, stinging spines all over the stem, leaves and reproductive parts (including fruits) (Francis 2000). Its distribution ranges from Mexico to Brazil and it is a typical pioneer species, moderately shade-intolerant (Francis 2000, Martins & Rodrigues 2002). There is a period of leaf fall from April/May to July and in August plants sprout again. *Urera baccifera* produces two kinds of ant attractants, pearl bodies and fruits, and is visited by 22 ant species, including 7 species of the genus *Camponotus*, 4 of *Pheidole*, 2 of *Crematogaster* and 2 of *Pseudomyrmex* (Dutra *et al.* 2006). The fruits are carbohydrate- and protein-rich (79.6% and 16.3% of the fruit dry mass, respectively). Although capuchin monkeys (*Cebus apella*) and seven birds species are the main seed dispersers of *U. baccifera* in the study area (Galetti & Pedroni 1994; Galetti & Pizo 1996), at least 3 large ant species may act as primary dispersers, including a large aggressive ponerine, *Pachycondyla villosa* (Plate 1B). Ants also visit the vegetative parts of the plants to collect pearl bodies, structures that are usually found on leaves, petioles and stalks of flowers and fruits. They exhibit protective behaviour against herbivores, as demonstrated by Machado & Freitas (2001) and Dutra *et al.* (2006).

Individuals from 1st to 3rd instar of *Smyrna blomfildia* (Fruhstorfer) (Nymphalidae: Coloburini) build frass chains, stick-like structures made of faecal pellets (frass) and silk, on the tip of which they rest (Machado & Freitas 2001).

Larvae of the moth *Pleuroptya silicalis* (Guenée) (Crambidae: Pyraustinae) build leaf rolls and fill them with silk and frass (Plate 2A). They feed inside the rolls and many individuals can be found inhabiting the same roll. When disturbed, larvae exhibit defensive behaviour by thrashing, dropping off the leaf and sometimes biting or, more rarely, regurgitating.

Urbanus esmeraldus (Butler) (Hesperiidae: Pyrginae) builds two types of leaf shelters during its ontogeny, but feeds outside the shelters. Larvae of 1st and 2nd instar build a peaked-roof shelter and rest on its "ceiling" (Plate 4C), whereas larvae from 3rd to 5th instar fold one side of the leaf (but unlike *P. silicalis* do not roll it completely), but only enough for them to hide underneath (Plate 4E). Larvae of 5th instar chew out the petiole of the leaf where they build the shelter and deposit large amounts of silk on the incision (Plate 4A, B and D). The leaves wither, and they feed on a different leaf. *Urbanus esmeraldus* also exhibit house cleaning behaviour by throwing its fecal pellets at long distances from the shelter (see Chapter 1).

We addressed the following questions concerning the natural history of *P. silicalis* and *U. esmeraldus* and their interaction with ants on shrubs of *Urera baccifera*: 1) Do larvae of these species have some preference for leaves of specific developmental stages? 2) If yes, is it related to ant foraging pattern on the plant? 3) Do ants have access to leaves with their petioles cut? 4) Do ants respond to frass of *P. silicalis* and of *U. esmeraldus* on the ground? 5) Is caterpillar survivorship greater in the absence of ants? 6) Are leaf rolls of *P. silicalis* effective against predation?

MATERIALS AND METHODS

Study area

Field work was carried out in the Santa Genebra Reserve, in the city of Campinas, São Paulo, southeast Brazil (22°49'S, 47°06'W). The reserve is predominantly covered by semi-deciduous mesophytic forest and the climate is defined as warm and wet, with dry winter and wet summer. The mean annual temperature is 21.6 °C and average rainfall is 1381 mm (Morellato & Leitão-FIlho

1995). Field experiments were performed with shrubs of *Urera baccifera* located along the main trail in the forest, about 1000 m long.

Field observations and experiments

Infestation of Urera baccifera *shrubs by lepidopteran larvae*

In order to discover if larvae of *Pleuroptya silicalis* and *Urbanus esmeraldus* use leaves of *Urera baccifera* according to some sort of preference, we recorded every shrub that had leaf shelters already built by the caterpillars. We classified the leaves into three age categories, according to characteristics such as brightness, coloration, leaf size and proximity to the apical gem. Young leaves were smaller than the others, dark green, brighter and located closer to the apical gem. Mature leaves were bigger and not as dark as young ones, less bright and not so close to the apical gem. Old leaves were normally the same size as mature leaves (although they were sometimes smaller), more withered and also yellowish green (based on Letourneau 1983).

Based on this classification, we recorded for each plant the number of leaves of each age category, as well as the total number of leaves of each plant. We recorded the number of shelters built in each leaf category as well. With respect to *U. esmeraldus* shelters, if there were more than one shelter of different sizes in the same leaf, we counted them as one. Early-instar larvae occasionally built consecutive shelters on the same leaf; in such cases one shelter was considered.

We analysed the data with G test, to check if larvae use young, mature and old leaves in the same proportion that they occur on plants. We recorded 50 plants with rolls of *P. silicalis* and 31 with shelters of *U. esmeraldus*.

Spatial pattern of ant foraging on shrubs of Urera baccifera

To check if ants forage differentially within the plant crown (top versus middle part of the crown), we conducted a field experiment examining removal by ants of live termites placed on leaves of *U. baccifera*. We tagged 28 pairs of plants of similar size and randomly assigned them as group 1 or 2. Each plant in group 1 had one live termite worker (*Neocapritermes opacus)* glued by its dorsum on the centre of one young leaf. We repeated the procedure with plants of group 2, which had termites glued on the centre of a mature leaf (adapted from Freitas & Oliveira 1996). After a two-hour interval, we checked if the termites had been removed or not. We performed a G test to compare termite removal between both groups. The plants had no reproductive organs (buds, flowers of fruits) at the time of the experiment, since these may affect ant visitation (Dutra *et al.* 2006), and had no leaf shelters or caterpillars.

Ant census and termite removal on leaves with previously cut petioles

In order to test if cutting the leaf petiole, as done by 5th instar larvae of *Urbanus esmeraldus* (Plate 4A), is an effective strategy against ant predation, we performed the following experiment.

In each of 44 plants, we chose a pair of similar-sized leaves and equally damaged by herbivory. One of the leaves had its petiole almost totally cut, simulating larval activity (Plate 1C), whereas the other remained with its petiole intact. This procedure was repeated for all plants. On day 1 we checked for ant presence on these leaves from 09:00 to 14:00 h. We continuously observed every plant for 10 minutes and recorded the maximum number of ants seen on each leaf. We compared the number of ants on each leaf treatment using a Wilcoxon test.

On the next day (day 2), we performed an experiment of termite removal with the same leaf pairs. We glued one live termite worker (*Neocapritermes opacus)* by its dorsum on all leaves in each experimental group (n = 40 pairs). Termites were glued in the morning (8:00 a.m.) and leaves were checked for termite removal 7 hours later. Termite removal on each leaf category was compared using a G test.

Effect of ants on larval survivorship in the field

We experimentally tested the suppressive effect of ants on the lepidopteran herbivores with an ant-exclusion experiment. We selected 34 individuals of *Urera baccifera* in the same phenological state (without flowers or fruits). We randomly assigned each plant as a treatment (ants excluded, n = 18 plants) or control (ants present, n = 16 plants). Each plant had one branch selected for records of herbivores. Branches of the treatment group had a barrier of Tree Tanglefoot™ (Tanglefoot Co. Grand Rapids, MI, USA) applied to the plant's trunk base, whereas control branches had Tanglefoot™ applied to only one side of the trunk. We recorded the initial number of larvae from all lepidopteran species present on the branches of both groups. We conducted weekly herbivore censuses during 8 weeks in March and April 2006. Grass bridges providing aerial access to the experimental branches were regularly pruned, and Tanglefoot™ was reapplied whenever necessary. Results were log (x+1) transformed and analysed using repeated-measures ANOVA (Zar 1999).

Leaf rolls and herbivore survivorship in the field

We tested if hiding inside leaf rolls offers any protection against ant predation on *U. baccifera*. We chose 28 individuals of *Urera baccifera* and used live termite workers as herbivore models. We randomly selected a pair of similar-sized leaves on each plant, and glued underneath each one of them one termite (*Neocapritermes opacus*) by its dorsum, near the leaf edge. One of the leaves had one edge rolled and stapled, forming an artificial roll-like structure (adapted from Lill & Marquis 2003) (treatment), having one termite inside the roll. The other leaf had no roll built (control), but was stapled nonetheless. We checked for termite removal on control and treatment leaves 24 hours later. All plants had fruits, which increases ant visitation (Dutra *et al.* 2006). Termite removal in each category was compared with a G test.

Experiments in captivity

Ant response to frass of Pleuroptya silicalis *in the laboratory*

This experiment tested if frass of *P. silicalis* kept inside the shelter provides cues to potential predators such as ants. We used 5 colonies of the ant *Camponotus crassus* (Mayr), an abundant species that occurs naturally on shrubs of *U. baccifera* (Machado & Freitas 2001).

We obtained the frass needed for the experiments from larvae collected in the field and kept in the laboratory, as described in chapter 1.

For each colony tested, we presented pairs of artificial shelters constructed with filter paper and paperclip, forming a roll (adapted from Lill & Marquis 2003). One of the rolls contained fresh frass of *Pleuroptya silicalis* inside, whereas the other did not. The shelters were presented one at a time, in a tray of 40 x 20 cm connected to the ant nest by a paper bridge. Only one ant at a time was allowed in the tray, and during a ten-minute interval, we observed:

a- how many times the ant entered the shelter;
b- how long did it take to enter the shelter for the first time;
c- the total time the ant spent inside the shelter.

We performed 25 trials with the 5 colonies and each colony was tested twice a day (one time with a shelter containing frass and one time with an empty shelter). The ants tested were temporarily removed from the nest, and did not participate in subsequent trials. There was a fifty-minute interval between trials and the order of presentation of the two types of experimental shelters was decided by the flip of a coin. Before the trials the colonies were deprived of food for 48 hours. Artificial shelters were used only once and were discarded after the trials.

Overall ant visitation to both types of shelters, as well as the number of times they visited each type of shelter, was compared with a G test. Time elapsed until the ants entered the shelters was compared with a two-tailed t test, and total time spent inside the shelters was analysed using a Mann-Whitney U-test.

Ant response to frass of Urbanus esmeraldus *in the laboratory*

To test if frass of *Urbanus esmeraldus* induce ants to climb onto the plants (based on Del-Claro & Oliveira 1996; see chapter 1), we performed a laboratory experiment with artificial shrubs made of wooden sticks and foam (Figure 1). Each ant nest had access to one of these shrubs for about two weeks before the beginning of the experiments, so that the ants could get used to its presence near the nest and climb spontaneously onto it. For the experiments, we placed a clean filter paper (circle of 15 cm diameter) under the shrub. By the flip of a coin, we determined if the trial would contain frass (treatment) or small balls made of black paper used as control. At each trial we placed either frass or black paper balls on the filter paper. Observations started when the first ant stepped on the filter paper. During 10 minutes, we recorded:

a- how long ants took to climb onto the shrub for the first time;

b- the total number of times the ants climbed onto the shrubs.

We performed the tests with 6 colonies of *Camponotus crassus* and each colony was tested only twice a day, with an interval of 1 hour and 30 minutes between trials. The filter paper was discarded after every trial, and the shrubs cleaned with alcohol (to eliminate any chemical cues left by the ants). Ant colonies were deprived of food for 48 hours before the trials. We used a one-tailed t test to compare the total number of ants that climbed onto “shrubs”, and also to compare the time the ants took to climb onto “shrubs” for the first time.

Finally, the next step was to check if the frass deposition pattern on the ground influences ant visitation to the “shrubs”*.* Using the same type of artificial shrub previously described, we performed 20 trials with four *C. crassus* colonies. Each trial was randomly assigned as treatment or control. Treatment trials had 4 squares of filter paper (1.5 cm x 1.5 cm) containing frass scattered at a distance of 30 cm from the base of the artificial shrub (Figure 2). Control trials had the squares of filter paper with frass placed right next to the “shrub” base. Trials began when the first ant stepped on a piece of filter paper, and lasted 10 minutes. We registered the total number of ants that climbed onto the “shrubs” during each trial and compared these results using a Wilcoxon test. The number of trials at which ants climbed onto the shrubs was compared using a G test. Ant colonies were deprived of food for 48 hours before the trials.

RESULTS

Field observations and experiments

Infestation of Urera baccifera *shrubs by lepidopteran larvae*

Both species investigated, *Pleuroptya silicalis* and *Urbanus esmeraldus*, do not use leaves of *Urera baccifera* in the same proportion as these occur on shrubs. As shown in figure 3, the majority of leaf rolls built by larvae of *Pleuroptya silicalis* are in mature and old leaves (116 and 20 shelters, respectively), whereas only 3 rolls were found in young leaves. We thus demonstrated that there is some preference concerning leaf age (G = 33.488, d. f. = 2, p<0.0001).

Figure 4 presents the results for *Urbanus esmeraldus*. No shelters at all were found on young leaves of any of the shrubs recorded. As with *P. silicalis*, most shelters were recorded in mature leaves (31 out of 41 shelters). Although mature leaves are far more numerous, larvae still use the leaves of *U. baccifera* in a disproportionate manner (G =13.72, d. f. = 2, p = 0.001).

Spatial pattern of ant foraging on shrubs of Urera baccifera

Overall termite removal by ants was low; the number of termites removed did not differ significantly between young and mature leaves (G = 1.727, d. f. = 1, p = 0.188). Only 9 out of 28 termites were removed from young leaves, while 6 were removed from mature ones. Numbers are presented in table 1.

Ant census and termite removal on leaves with previously cut petioles

During the census we observed that small and medium-sized ants (*Crematogaster* spp. and *Camponotus crassus*, respectively) were perfectly able to climb onto the leaves which had their petioles previously cut. Unfortunately we did not observe any larger ant species.

Ants visited 8 leaves of the control group (with intact petioles) and 12 of the treatment group (with petioles cut), and the total number of ants observed in all trials was 10 and 14, respectively. The comparison of the number of ants between groups using a Wilcoxon test produced no significant difference ($Z = -1.3484$, $p = 0.1775$).

We did not detect any significant difference between control and treatment leaves for the termite removal experiment as well ($G = 0.1279$, d. f. = 1, $p = 0.7206$; see table 2A). Of the 40 plants used in this experiment, the number of termites removed was similar for control (30 removals) and treatment leaves (29 removals). This result supports the data obtained in the census.

Effect of ants on larval survivorship in the field

The presence of ants did not influence lepidopteran infestation levels on *Urera baccifera* shrubs. Considering all species together, we observed no difference between treatment (ants excluded) and control (ants present) plants (see table 3). When we analysed the data for each species separately (*U. esmeraldus* and *P. silicalis*), we also found no significant difference between treatment and control plants (see table 3). We also observed no interaction between time and ant treatment in any of the analyses performed. Time was the only factor affecting herbivore survival in both species. One plant of each experimental group was lost during the eight-week period. Mean number of larvae in the beginning and end of the experiment are presented in table 4.

Leaf rolls and herbivore survivorship in the field

From a total of 27 leaf pairs, termites were removed from 18 leaf rolls and from 20 leaves without rolls, as shown in table 2B. Difference of termite removal between control and treatment leaves is not significant ($G = 0.7307$, d. f. = 1, $p = 0.1887$). We had to discard one plant because one of the leaves fell before the end of the experiment.

Experiments in captivity

Ant response to frass of Pleuroptya silicalis *in the laboratory*

Overall ant visitation to artificial rolls was low. From a total of 25 rolls of each category, ants visited 10 rolls with frass, and 13 without frass ($G = 1.447$, d. f.

= 1, p = 0.229). Taking into account all times ants entered each type of roll (treatment or control), including if they entered more than one time in the same trial, the categories also do not differ, with ants entering 17 times in rolls with frass and 16 in rolls without frass (G = 0.1767, d. f. = 1, p = 0.6742).

Finally, time elapsed until the ants entered the shelters for the first time also did not differ between control and treatment (two tailed t test, t = 0.7189, d. f. = 20, p = 0.4805). Total time spent by ants inside shelter category was analysed with a Mann-Whitney U-test, and no significant difference was detected (U = 0.679, p = 0.497).

Ant response to frass of Urbanus esmeraldus *in the laboratory*

Ants took the same time to climb onto artificial shrubs that had either frass or black paper balls around their base (t = 0.2112, d. f. = 17, p = 0.4176). However, ants climbed significantly more onto artificial shrubs which had filter paper with frass placed under their base (figure 5). In 18 trials, the total number of ants that climbed onto "shrubs" with frass was 61, whereas on trials with black paper balls the number of ants that climbed onto "shrubs" was 33 (one-tailed t test, t = -3.3896, d. f. =17, p = 0.0017).

Ants climbed more often (G = 14.1733, d. f. = 1, p < 0.001) and in greater numbers (Wilcoxon test, Z = -2.4853, p = 0.0129) onto "shrubs" of the control groups (with frass placed right next to the "shrub" base) than onto treatment "shrubs" (with frass placed away from the "shrub" base) – see figures 6 and 7.

DISCUSSION

The results demonstrate a preference of the larvae of *Pleuroptya silicalis* and *Urbanus esmeraldus* for leaves of specific developmental stage (i. e., mature leaves). We tried to correlate this preference with ant distribution pattern on the shrubs of *Urera baccifera*. Our results, however, show that ants forage equally within the crown of *U. baccifera*, since there is no significant difference in termite removal from young and mature leaves. Thus there is no apparent relationship between larval preference and ant foraging on *U. baccifera.* One can argue that the observed infestation pattern is related to the nutritional quality of young leaves. Young leaves may be nutritionally richer than older ones and provide the larva a faster development (Damman 1987). On the other hand, the infestation pattern observed could also be due to the presence of toxic compounds in the young leaves. Host plant chemistry mediates food choice by many species of herbivores, and is also well known to affect plant quality and to cause negative impacts on herbivores (see Ode 2006 and references therein). Toxic plant substances can deter feeding by herbivores and confer a great selective advantage to the plant if they are not easily metabolized by herbivores into nontoxic derivatives (McKey 1979). In some plant species, young leaves can contain greater concentrations of secondary compounds (e.g., alkaloids, tannins, cyanogenic glycosides) than mature ones (McKey 1979). However, we are not aware if this would be the case of *U. baccifera*, as we have no record of the presence of secondary compounds in this plant.

Small and medium-sized ants were perfectly able to climb on leaves with petioles previously cut (treatment). During the ant census they were seen on both control (with intact petioles) and treatment leaves and no significant difference was detected, which suggests that the cut petiole is no barrier and do not prevent them from reaching the leaf surface. This result is reinforced by the fact that termite removal from control and treatment leaves did not differ significantly. Predation pressure from crawling invertebrates such as ants is, according to these results, not affected by the integrity of the leaf petiole. We should bear in mind that only 5th instar larvae chew out the petiole, and birds are more important predators of older larvae and pupae, whereas arthropods are probably more important predators of eggs and early larvae (Scoble 1995). Thus this larval behaviour could more likely be a strategy to escape from avian predators. Because leaves with the petiole cut soon wither, the visual effect can be deceptive for insectivorous birds (Heinrich & Collins 1983).

Another hypothesis is that chewing out the leaf petiole can reduce parasitoid attack. Parasitoids are well known to use a variety of cues, especially chemical and visual, to make decisions about where and for how long to search for hosts at different locations (Vinson, 1976; Godfray 1994; Quicke 1997). The use of vibrations is less known, but has been reported to be important in determining the foraging success of at least 13 species of parasitoids (Meyhöfer & Casas 1999). Vibrations may be the main stimuli used by parasitoids when their hosts are concealed feeders, as is the case for the leaf miner of the genus *Phyllonorycter* (Lepidoptera: Gracillariidae) and its parasitoid *Sympiesis sericeicornis* (Hymenoptera: Eulophidae) (Djemai *et al.* 2004). An incision on the leaf petiole could probably reduce the transmission of host vibrations to other parts of the plant, making it more difficult for some vibration-guided parasitoid to detect the signals and thus the location of the caterpillar.

We noticed that the plants began to produce fruits between weeks number 3 and 4 of the ant-exclusion experiment, and we are aware that ant visitation to *U. baccifera* is higher during the fruiting season (Dutra *et al.* 2006). However, ants had no influence on lepidopteran infestation levels on *U. baccifera* shrubs, because there was no difference in larval survivorship between treatment (ants excluded) and control (ants present) plants. Dutra *et al.* (2006) performed this same experiment during the years of 2003 and 2004, and their results showed that ants were effective in reducing herbivore infestation in both years, with significantly more herbivores infesting ant-excluded than ant-allowed plants. The fact that ants did not influence herbivore survival in 2006 may be explained by a much higher infestation level by herbivores in this year than in the years of the experiments by Dutra *et al.* (2006). Herbivore infestation levels in 2006 are about twice as high as those recorded in 2003 and 2004. At higher herbivore infestation levels ant predation may not be sufficient to produce a significant suppressing effect – fluctuations in herbivore abundance can lead to temporal variability in the outcomes of ant-herbivore-plant interactions, as demonstrated by other authors (Bentley 1977; Barton 1986; de la Fuente & Marquis 1999). Because predation pressure depends on ant visitation rates to the host plant (Freitas 1991; Freitas &

Oliveira 1996), another possible and non-exclusive explanation is that ant visitation to *U. baccifera* shrubs in 2006 might have been low.

In a way or another, our results reinforce the hypothesis that herbivore abundance is a key factor in determining the effectiveness of ant protection to *U. baccifera*, as suggested by Dutra (2004). When herbivore infestation levels increase, ant visitation may not be sufficient to reduce the negative effects of herbivores on plants.

Although overall ant visitation to artificial rolls was low, there was no detectable difference between visitation to rolls containing frass (control) or to empty ones (treatment). Workers of *Camponotus crassus* did not exhibit any change in behaviour (attraction/aversion) due to the presence of frass inside the rolls, so we can infer that there is no chemical signaling related to it. Chemical cues from frass and its effects are better studied for predatory wasps (Weiss 2003) and parasitoids (Vinson 1976). Nevertheless, frass may play an important defensive role for *P. silicalis*. We demonstrated that leaf rolls *per se* are not an effective barrier against predation, because termite removal from artificial rolls and from plain leaves did not differ statistically. Artificial rolls were very similar to natural ones in size and shape, but contained no frass nor silk inside. Frass could represent a mechanical barrier blocking the way into the rolls or, at least, making it much more difficult for ants to enter. This could work both against predators (including ants) and small parasitoids that, lacking a long ovipositor, need to go inside the shelter in order to sting the larva (D. Janzen, pers. comm.). Some insects use their own faeces as a physical or chemical defence against natural enemies. Vasconcelos (1991) describes ant-proof shelters built by two species of lepidopteran herbivores that feed on a myrmecophytic melastome. One of them, *Stenoma charitarca* (Meyrick) (Oeciphoridae), builds ant-proof tunnels filled with silk and frass inside which the larva moves, along the plant stem. Other known examples of faeces as defensive devices include faecal coverings, faecal structures that offer refuge, or direct defence through discharge of fluids toward approaching animals (see Weiss 2006 for more details).

The presence of *U. esmeraldus* frass beneath the artificial shrubs induces increased numbers of ants to climb on plants, similar to hemipteran exudates that function as chemical cues and attract ants onto plants (Del-Claro & Oliveira 1996). In the case of *U. esmeraldus*, however, ant attraction represents a risk rather than an advantage to the caterpillars, since the ants are potential predators. As described in chapter 1, the larvae throw their faecal pellets at long distances, so that they fall away from the tree base and are scattered on the ground. This fact could make it difficult for foraging ants to find the host plant and the caterpillar. Indeed, we demonstrated that ants climbed significantly more onto shrubs which had frass placed right next to its base, compared to shrubs which had frass placed at a distance of 30 cm away from its base. Although this is a conservative experiment (because faecal pellets can reach much greater distances – see chapter 1), it demonstrates clearly that frass ejection behaviour (and the scattered deposition of frass on the ground) is a selective advantage because it directly affects ant visitation to the shrubs. Weiss (2003) demonstrated the importance of frass ejection behaviour for the hesperiid *Epargyreus clarus* (Cramer) that, in the presence of its own frass, suffers more attacks from the predatory wasp *Polistes fuscatus*. Few studies have examined ant attraction to potential host waste products, and to our knowledge this is the first study to demonstrate that ants use lepidopteran frass as cues to climb onto plants, increasing herbivore vulnerability to ant predation.

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Table 1. Number of termites (*Neocapritermes opacus*) removed or not removed on *Urera baccifera* according to different leaf age categories. Groups did not differ significantly (n = 28 pairs) (G = 1.727, d.f. = 1, p = 0.188).

	Leaf age	
	Group 1(young)	**Group 2 (mature)**
Removed	9	6
Not removed	19	22

Table 2. Number of live termite workers (*Neocapritermes opacus*) removed or not removed on *Urera baccifera* from **A-** leaves with intact petioles (control), or with previously cut petioles (treatment), n = 40 leaf pairs (G = 0.1279, d.f. = 1, p = 0.7206) and **B-** artificial leaf rolls (treatment), and from plain leaves without rolls (control), n = 27 leaf pairs (G = 0.7307, d.f. = 1, p = 0.1887).

	A		**B**	
	Control	**Treatment**	**Control**	**Treatment**
Removed	30	29	20	18
Not removed	10	11	7	9

Table 3. Repeated-measures ANOVA on the infestation levels of *Pleuroptya silicalis*, *Urbanus esmeraldus* and all species of lepidopteran larvae on *Urera baccifera,* for the ant exclusion experiment. Analyses were performed using log (x+1) transformed data.

Source of variation	SS	DF	MS	F	p
Pleuroptya silicalis					
Ant treatment	0.23465	1	0.23465	0.21193	0.6486
Error	33.21618	30	1.10721		
Time	4.55840	6	0.75973	4.01063	0.0009
Time x Treatment	1.47168	6	0.24528	1.29483	0.2617
Error	34.09746	180	0.18943		
Urbanus esmeraldus					
Ant treatment	0.06303	1	0.06303	0.12034	0.7311
Error	15.71186	30	0.52373		
Time	5.50533	6	0.91755	8.13098	0.000000
Time x Treatment	0.40953	6	0.06825	0.60484	0.7262
Error	20.31241	180	0.11285		
All species					
Ant treatment	0.00294	1	0.00294	0.00316	0.9556
Error	27.94223	30	0.93141		
Time	15.83769	6	2.63962	10.79137	0.000000
Time x Treatment	0.63882	6	0.10647	0.43527	0.8546
Error	44.02877	180	0.24460		

Table 4. Mean number of larvae (± standard error) of all lepidopteran species, *Pleuroptya silicalis* and *Urbanus esmeraldus* in the first and last week of the ant-exclusion experiment. Ants were excluded from treatment plants (n = 17) and present in control plants (n = 15 plants).

	Week 0		**Week 7**	
	Treatment	**Control**	**Treatment**	**Control**
all species	1.882 (± 0.342)	2.533 (± 0.533)	0.941 (± 0.764)	0.667 (± 0.41)
P. silicalis	0.941 (± 0.348)	1.733 (± 0.53)	0.765 (± 0.765)	0.533 (± 0.401)
U. esmeraldus	0.941 (± 0.218)	0.800 (± 0.355)	0.059 (± 0.059)	0.067 (± 0.067)

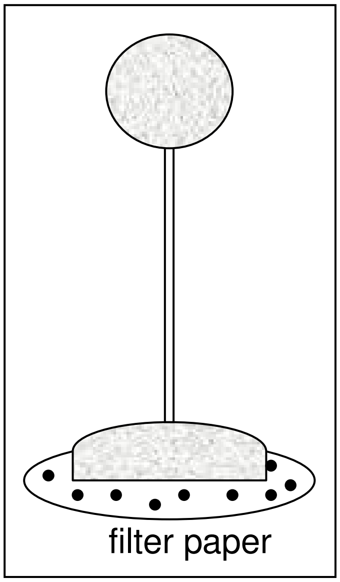

Figure 1. Artificial shrub used in the experiments of ant response to frass of *Urbanus esmeraldus*. The top and base were made of foam, connected by a wooden stick (“stem”). On the filter paper we placed either frass pellets (treatment) or black paper balls (control).

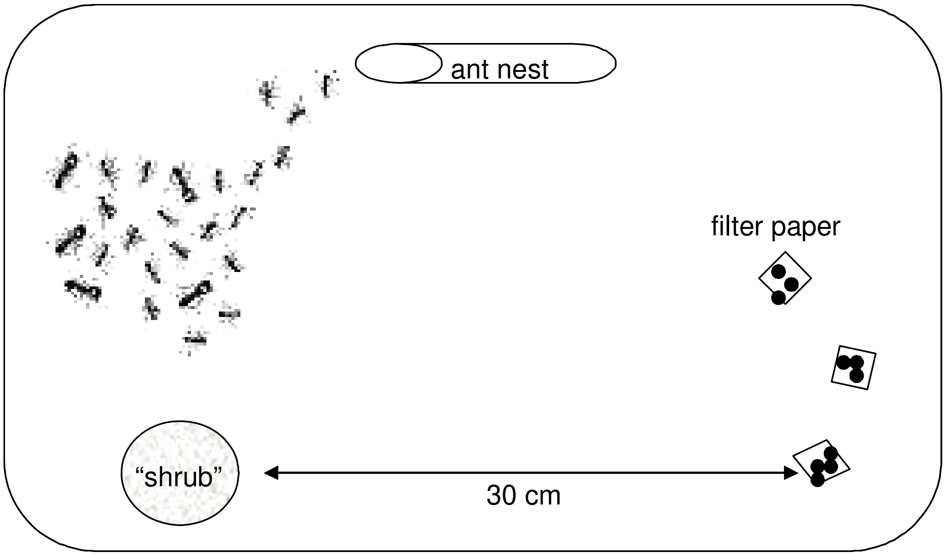

Figure 2. Representation of the artificial shrub placed in the tray (40 x 20 cm) containing the ant nest, in order to check if the frass deposition pattern on the ground influences ant visitation to the "shrub". Control trials had frass placed right next to the "shrub" base, and treatment trials had frass placed far from the base (as represented above).

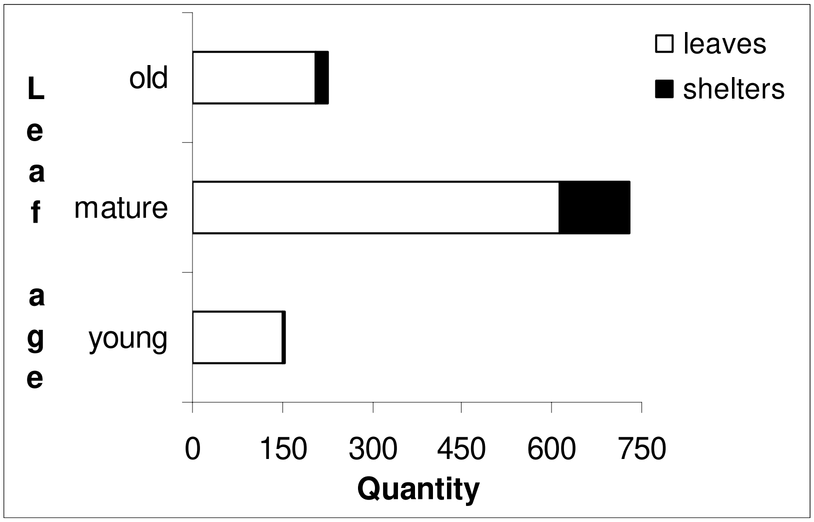

Figure 3. Total number of shelters of *Pleuroptya silicalis* in relation to leaves of *Urera baccifera* of different age categories (G = 33.488, d.f. = 2, p<0.0001). N = 50 plants.

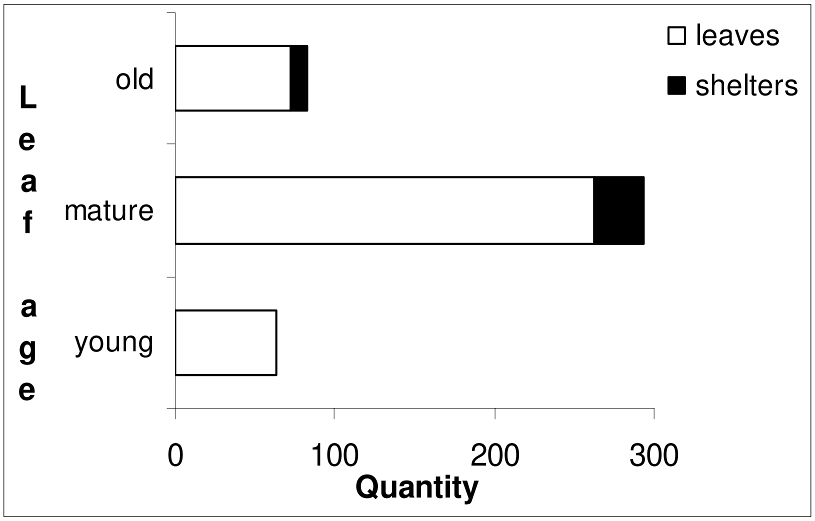

Figure 4. Total number of shelters of *Urbanus esmeraldus* in relation to leaves of *Urera baccifera* of different age categories (G =13.72, d.f. = 2, p = 0.001). N = 31 plants.

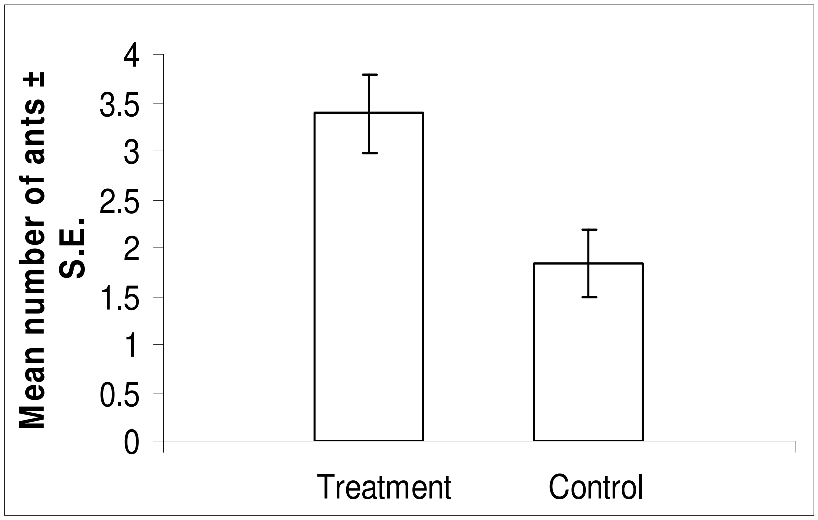

Figure 5. Number of ants (*Camponotus crassus*) climbing onto artificial shrubs with frass of *Urbanus esmeraldus* around its base (treatment) or black paper balls (control). Total number of ants differ significantly between treatments (one-tailed t test, t = -3.3896, p = 0.0017, d.f. =17).

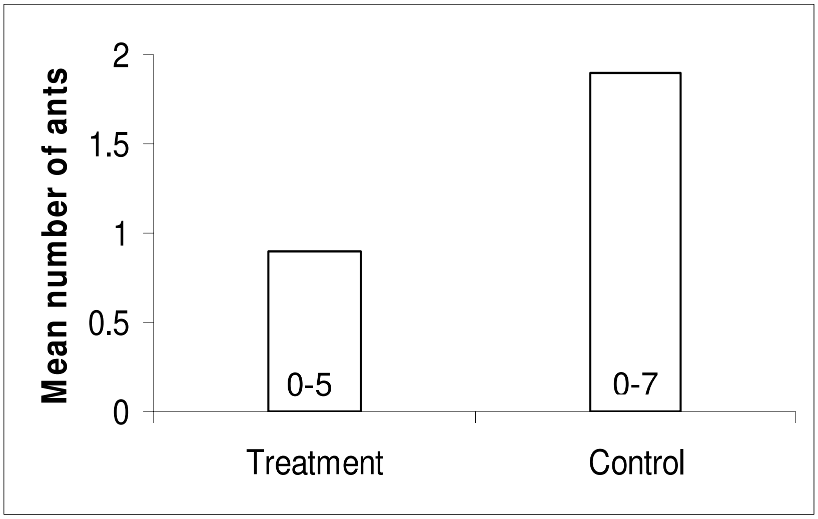

Figure 6. Mean number of ants (*Camponotus crassus*) that climbed onto treatment (frass placed on the ground, far from the "shrub" base), and onto control artificial shrubs (frass placed next to the "shrub" base) during trials in captivity. N = 20 trials, Wilcoxon test Z = -2.4853, p = 0.0129. Numbers inside bars represent ranges.

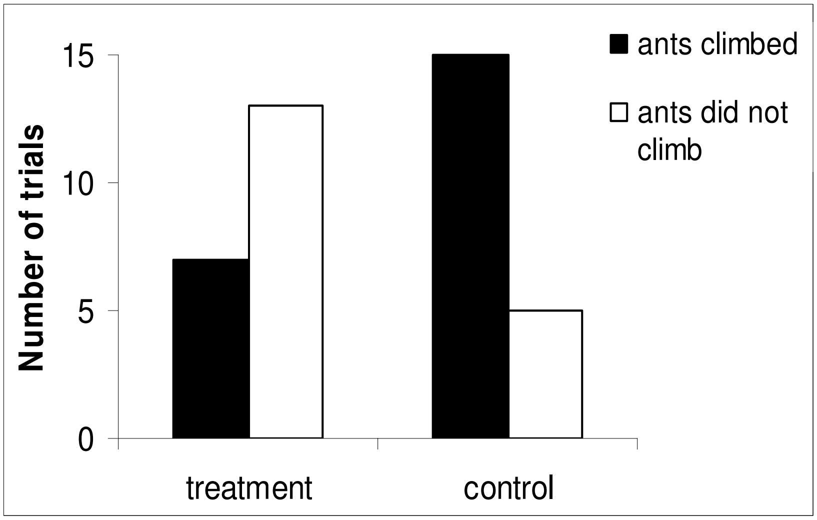

Figure 7. Number of trials at which ants (*Camponotus crassus*) climbed/did not climb onto artificial shrubs, according to the experimental group. Treatment trials had frass placed on the ground far from the shrub base, whereas control trials had frass placed next to the shrub base. N = 20 trials (G = 14.1733, d.f. = 1, p< 0.001).

Conclusão Geral

Lagartas de ambas as espécies estudadas passam por 5 estádios de desenvolvimento e apresentam diferentes mecanismos de defesa contra predação. *Pleuroptya silicalis* constrói abrigos em forma de tubo, enrolando as folhas de *Urera baccifera*, e deposita grandes quantidades de seda e fezes em seu interior. Formigas não exibem mudanças de comportamento devido à presença de fezes no interior dos abrigos; portanto, as fezes nos abrigos não atraem ou repelem estes predadores. Por outro lado, elas podem exercer um papel crucial no sucesso do abrigo como proteção contra predação por formigas, pois demonstramos experimentalmente que apenas o abrigo, sem fezes ou seda dentro, não é capaz de fornecer proteção a um herbívoro simulado (cupins) contra predação. As fezes podem ainda funcionar como uma barreira mecânica, impossibilitando (ou ao menos dificultando) a entrada de predadores ou até mesmo parasitóides com ovipositor curto que necessitem entrar para alcançar o hospedeiro. Por outro lado, as fezes podem emitir sinais químicos a parasitóides, desta forma prejudicando a larva. Porém, este papel ainda precisa ser investigado experimentalmente.

Larvas de *Urbanus esmeraldus* constróem dois tipos diferentes de abrigos foliares ao longo de seu desenvolvimento e larvas de 5º estádio cortam o pecíolo das folhas das quais se alimentam e onde descansam. Este comportamento, porém, não está relacionado à atividade de formigas na plantas, pois diferentes espécies de formigas conseguem transpor o pecíolo cortado e atingir o limbo foliar. O pecíolo cortado pode atrapalhar a transmissão de vibrações provenientes da lagarta pela planta, prejudicando eventuais parasitóides que dependam deste tipo de sinal para localização do hospedeiro. Uma outra hipótese é de que o corte no pecíolo, ao fazer a folha murchar precocemente, funcione como defesa contra predação por aves, já que estas podem utilizar sinais visuais indicativos de presença e/ou atividade de lagartas para localização de presas. Por fim, tal comportamento pode eliminar compostos tóxicos da planta; porém estudos adicionais são necessários para verificar a presença de tais substâncias em *Urera baccifera.*

Outra característica comportamental de *Urbanus esmeraldus* é arremessar suas fezes a grandes distâncias, espalhando-as pelo chão ao redor da planta hospedeira. Em laboratório, a presença de fezes no chão é, de fato, um estímulo para formigas subirem na planta – fezes arremessadas longe da base da planta induzem menos formigas a subirem nas plantas do que fezes localizadas ao redor de sua base.

Ao contrário do observado em anos anteriores, a presença de formigas não foi suficiente para diminuir a infestação por todas as 3 espécies de lepidópteros que ocorrem em *Urera baccifera*. Os níveis de infestação de 2006 são aproximadamente o dobro dos observados em 2003 e 2004. Além disso, a visitação às plantas pelas formigas pode ter sido menor em 2006. Portanto, o caráter mutualístico da interação entre formigas e *Urera baccifera* varia temporalmente de acordo com os níveis de infestação dos herbívoros e também visitação pelas formigas.

Apêndice

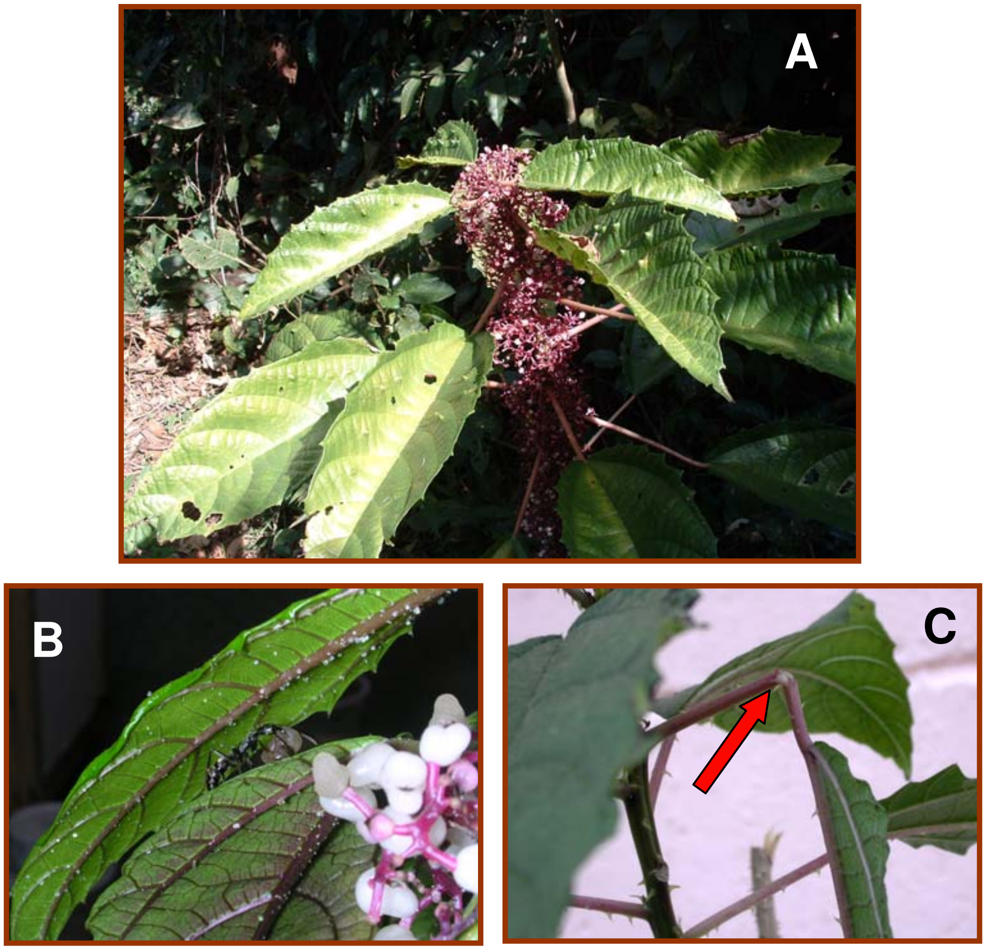

Plate 1. A) *Urera baccifera* (Urticaceae) in fruiting season. B) The ant *Pachycondyla villosa* carrying a fruit. C) Leaf petiole artificially cut, simulating the activity of 5th instar *Urbanus esmeraldus* larva.

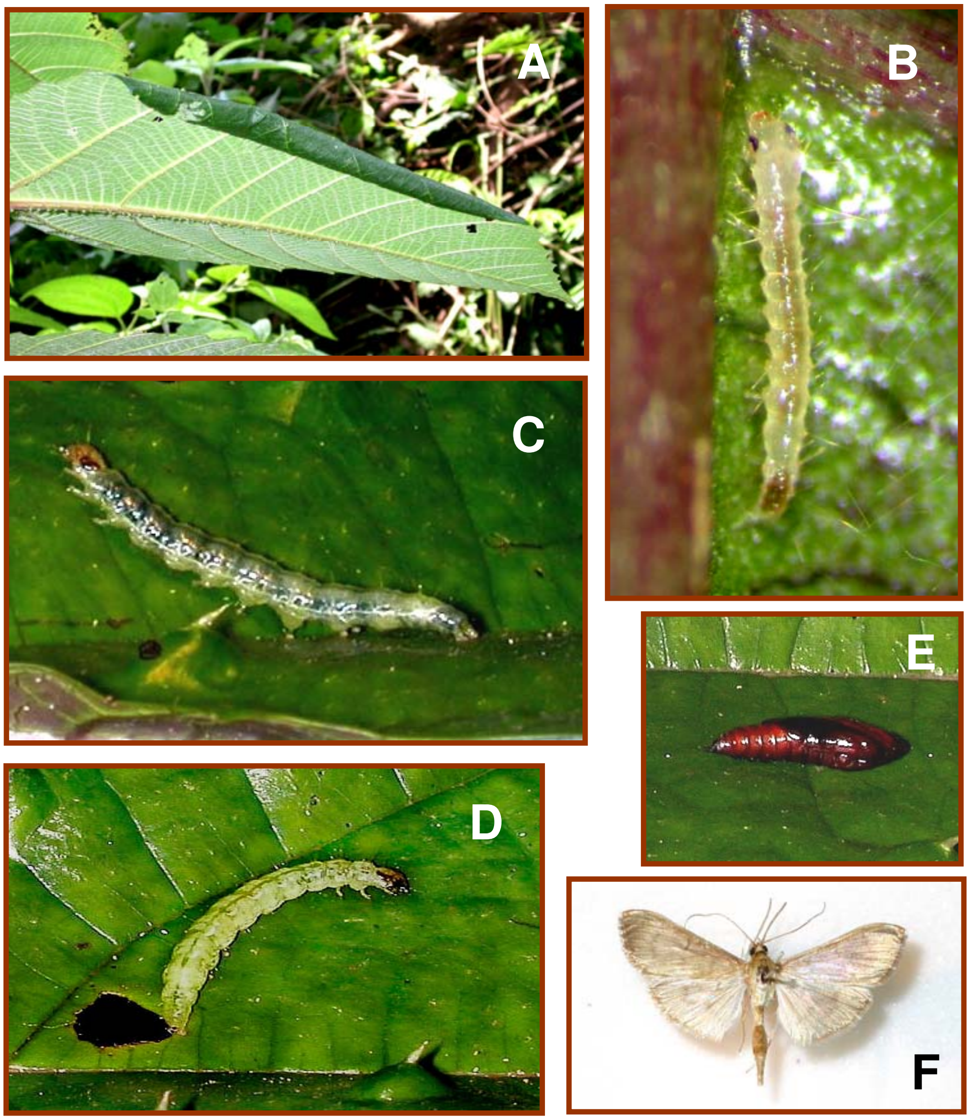

Plate 2. A) Leaf roll made by *Pleuroptya silicalis* in *Urera baccifera* (Urticaceae). B) First instar larva of *Pleuroptya silicalis* (Lepidoptera: Crambidae); larval length is ca. 6 mm. C) Fifth instar larva (length is ca. 30 mm). D) Fifth instar larva, two days before pupation (length is ca. 21 mm). E) Pupa (length is ca. 15 mm) . F) Adult – dorsal view.

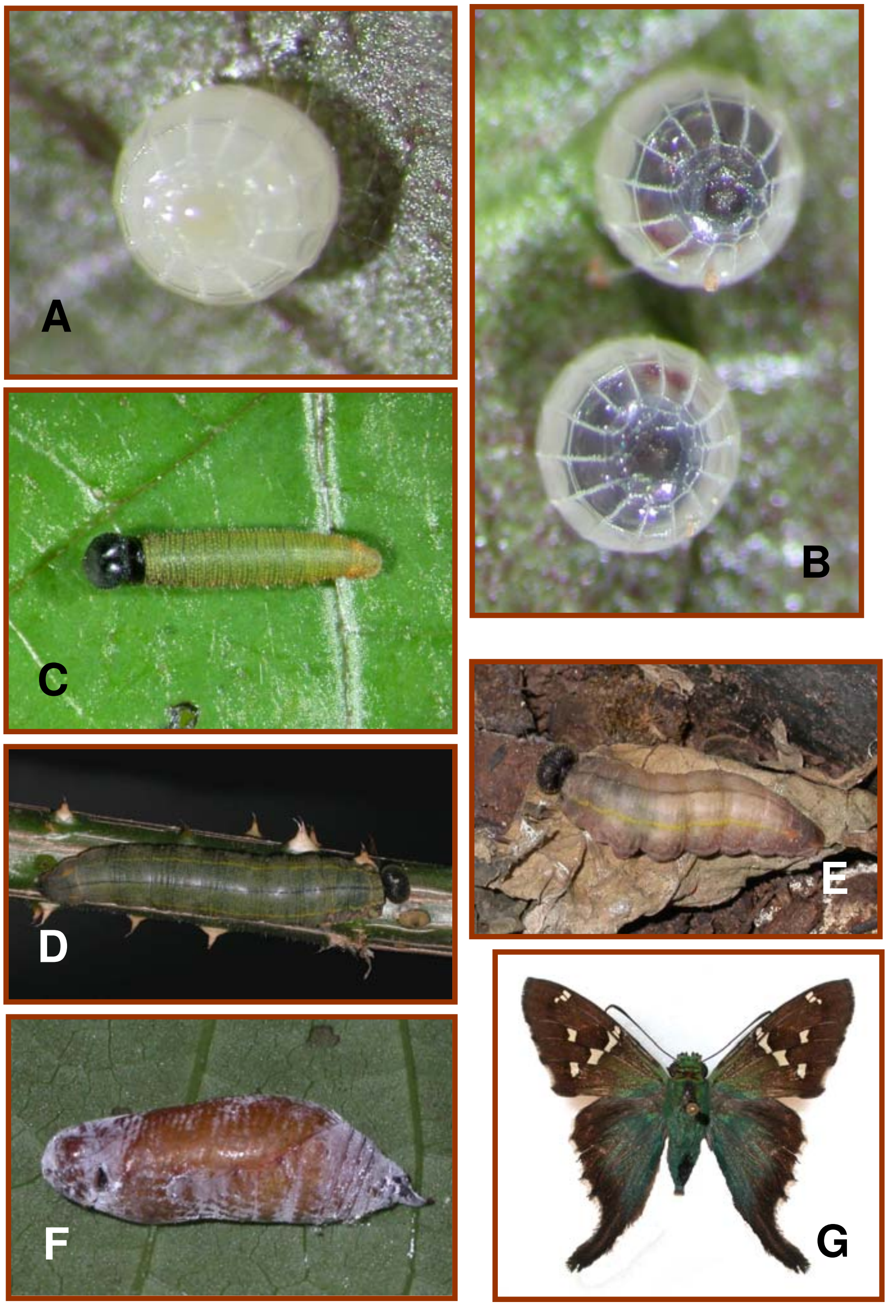

Plate 3. A) Egg of *Urbanus esmeraldus* (Lepidoptera: Hesperiidae) - top view (height and diameter equal 1.1 mm). B) Eggs (top view) one day before eclosion. C) Second instar larva (length is ca. 8 mm). D) Fifth instar larva (length is ca. 45 mm) on the stem of *Urera baccifera* (Urticaceae). E) Prepupa seeking refuge to pupate in the soil (length is ca. 27 mm). F) Pupa (length is ca. 24 mm). G) Adult – dorsal view.

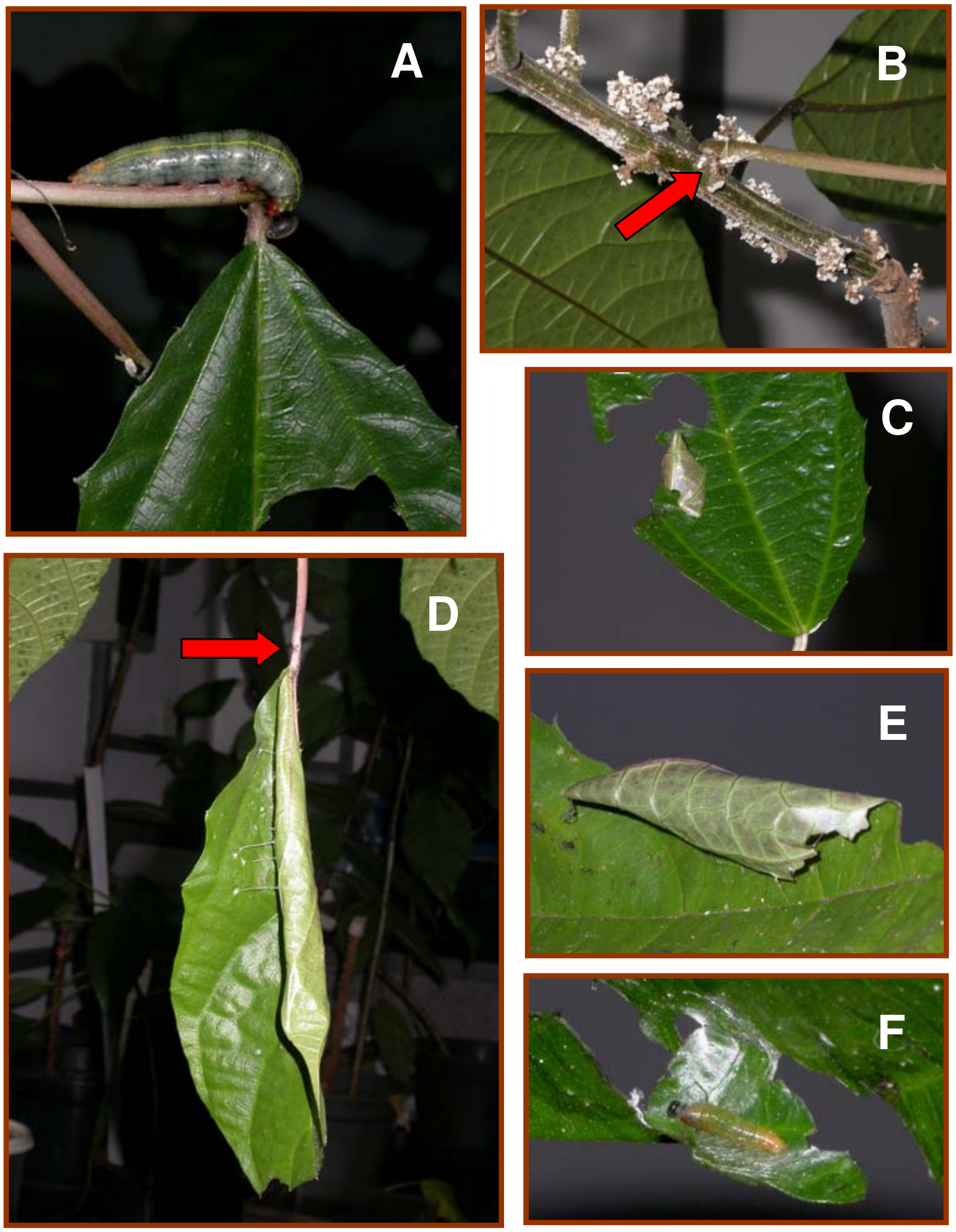

Plate 4. A) Fifth instar larva of *Urbanus esmeraldus* depositing silk after chewing out the petiole of the leaf. B) Detail of the incision with silk deposited on it. C) Peaked-roof shelter, built by larvae from 1st - 3rd instar. D) and E) Roll-like shelter, made by 4th – 5th instar larvae (arrow in E shows the spot firstly chewed out by the larva). F) Second instar larva resting on the "ceiling" of the peaked-roof shelter.