Faecal shield of the tortoise beetle *Plagiometriona* aff. *flavescens* (Chrysomelidae: Cassidinae) as chemically mediated defence against predators

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Abstract: Larvae of *Plagiometriona* aff. *flavescens* carry a structure on their back made of faeces and exuviae, called faecal shield, which may protect larvae against natural enemies. Previous investigations suggested that the nature of such protection was chemical. To test if chemicals found in the faecal shield of *Plagiometriona* aff. *flavescens* provided defence for larvae, experiments in the field and in the laboratory (using the ant *Camponotus crassus*, and chicks *Gallus gallus* as model predators) were undertaken. Both field and laboratory experiments showed that live larvae with faecal shields, as well as baits treated with faecal shield extracts, were rejected by predators, confirming the chemical nature of this kind of defence.

Key Words: *Aureliana fasciculata*, chemical defence, larval defence, natural enemies, predation, Solanaceae

INTRODUCTION

Predators and parasitoids represent an important source of mortality for phytophagous insects (Cornell et al. 1998, Denno et al. 1990, Gratton & Denno 2003, Hawkins et al. 1997, Keese 1997, Oksanen & Ericson 1987). Natural enemies of Chrysomelidae (leaf beetles) belong to many different groups of organisms or taxa, varying from intracellular parasites such as fungi and bacteria to large predators like birds and lizards (see review in Nogueira-de-Sá & Vasconcellos-Neto 2003). Selective pressures imposed on these insects by natural enemies probably resulted in a diversity of defensive attributes including physical, behavioural and chemical adaptations (Olmstead 1996, Pasteels et al. 1988, Venclo et al. 1999). Chrysomelid beetles frequently use enteric products and exuviae as protection barriers, especially for their eggs and larvae (Olmstead 1994). Larvae of Chrysomelidae belonging to different subfamilies can carry masses of moulted skins and faeces, forming a shield-like structure (Eisner & Eisner 2000, Eisner et al. 1967). Various functions such as camouflage, protection against radiation and natural enemies, have been attributed to these structures (Müller & Hilker 2003 and references therein), although there are also cases in which faecal shields can serve as attractive cues for generalist predators and parasitoids (Müller & Hilker 1999, Schaffner & Müller 2001).

Some authors have suggested that shields provide physical protection to larvae (Eisner & Eisner 2000, Müller 2002, Müller & Hilker 2003, Root & Messina 1983). However, others have demonstrated that in different chrysomelids, including another species of the genus *Plagiometriona*, this kind of physical protection does not occur (Morton & Venc 1998, Nogueira-de-Sá & Trigo 2002, Venclo & Morton 1998, Venclo et al. 1999). In fact, shield effectiveness was demonstrated to be due to chemical components of host plant origin (Gómez et al. 1999, Morton & Venc 1998, Müller & Hilker 2003, Venclo et al. 1999). Therefore, the objective of this study was to verify if chemical compounds found in the shields of *Plagiometriona* aff. *flavescens* were able to deter predators from attacking larvae, under both field and laboratory conditions.

METHODS

Field bioassay – test for the chemical defence by faecal shield of *Plagiometriona* aff. *flavescens*

This experiment was conducted at Parque Estadual Intervales, in São Paulo State, south-eastern Brazil.
Experimental: baits were treated topically with shield extract of Plagiometriona aff. flavescens, and placed on unprotected plants.

Solvent control: baits were treated topically with MeOH (the solvent used to dilute the extract), and placed on protected plants as described in (1).

Untreated control: natural baits without the application of any substance, and placed on unprotected plants.

The experiment was carried out with 30 host plant individuals submitted to the treatments described above during six experimental periods of 2 d each. Preliminary experiments showed that the period of 2 d was enough to expose larvae to predators. The treatment to be applied to each of the 30 experimental plants was randomly selected in the beginning of each experimental period. The same host plant individual was submitted to the same treatment only once. Therefore, at the end of the six experimental periods, each host plant had been submitted to all treatments once, resulting in 30 replicates for each treatment. The experiments were carried out during the summer, when the populations of the cassidines and predators of herbivorous insects were large (Nogueira-de-Sá & Vasconcellos-Neto 2003). At the end of each experimental period, we recorded if baits had disappeared or if they were intact. For analysis, baits that had disappeared were scored as one and intact baits were scored as zero. Baits that had been partially attacked (recorded as bite marks) but not completely consumed were discarded from analysis. Cochran’s Q test was used to compare the proportions of disappeared and intact baits among treatments with a post hoc test for multiple comparisons (Zar 1999, p. 268).

Laboratory bioassays

Carpenter ants, Camponotus crassus (Formicidae: Myrmicinae) were used as an invertebrate predator model, and chicks, Gallus gallus (Galliformes: Phasianidae), as a vertebrate model. Two kinds of bioassays were carried out, with the capturing frequencies by these predators as variable. In one bioassay, we used live 3rd or 4th instar larvae with and without shields, removed experimentally with fine forceps. In a second bioassay we used individual baits (frozen larvae...
of *Spodoptera frugiperda* submitted to one of the three chemical treatments described below.

1. Experimental: baits were treated topically with faecal shield extract of *Plagiometiona* aff. *flavescens* (see above).
2. Solvent control: baits were treated topically with MeOH.

**Camponotus crassus** bioassay

We collected *Camponotus crassus* colonies at Estação Experimental de Mogi Guacu (Fazenda Campininha), in São Paulo State, south-eastern Brazil (22°18’S, 47°10’W). In the laboratory, the colonies were kept in a plastic container (20 × 20 × 5 cm) which walls were treated with Fluon™ to prevent the ants from escaping. We placed a test tube (20 cm length, 2 cm diameter, 2 cm height) with damp cotton in the bottom covered with red plastic film, in the container where the ants nested. The nest container was linked to a foraging arena (another plastic container of 16 cm diameter, 5 cm height) with food was supplied and where the experiments were carried out. The colonies were fed daily with a honey-water solution; and a frozen 5th or 6th instar larvae of *Spodoptera frugiperda* was supplied weekly. We conducted the bioassays in the foraging arena to avoid defensive behaviours of the ants that might occur due to proximity to the nesting chamber. Colonies of *Camponotus crassus* were kept under 14/10 h, LD photoperiod, at 24 °C.

A double quantification bioassay was carried out as follows. Two days before the beginning of every bioassay a larva of *Spodoptera frugiperda* was offered to the colony as described above, as the first control. This indicated if the colony was hungry and the ability to feed on the tested prey. When the larva was not consumed, the colony was considered unable to attack prey and was not used in the experiments. When the first control was consumed, we started the bioassay 48 h after that was offered, by offering an individual larva or bait, depending on the bioassay, as mentioned above. The treatment was defined randomly. Larvae or baits were placed on a fresh leaf of *Aureliana fasciculata* with the petiole dipped into a vial with water to prevent wilting. This system was kept in the centre of the foraging arena. One day later, the behaviour of the ants towards the test organisms was recorded. The recorded behavioural categories were; (1) captured, when the experimental larva or bait disappeared from host plant leaf after 24 h, or (2) not captured, when ants left the experimental larva or bait intact after 24 h. In this case, we offered a second *Spodoptera frugiperda* control larva, which was placed on an *Aureliana fasciculata* leaf as well, after removing the experimental larva or bait of the colony. This procedure was applied to check if the experimental larva or bait was not consumed because the ants rejected it or if the colony was no longer hungry. When the ants consumed the second control within 24 h, the behavioural category 'not captured' was confirmed. When the second control was not consumed, that trial was rejected from further analysis.

In both larva and bait bioassays frequencies of captured and not captured organisms were compared using a G test for contingency table, followed by subdivided contingency table as a *post hoc* test when needed (Zar 1999, p. 504).

**Gallus gallus** bioassay

One-day-old chicks were obtained from a commercial hatchery and taken to the laboratory, where they were kept together for 1 wk. The animals were maintained under 25 °C, natural photophase and fed with commercial corn-based food and water *ad libitum*. After 7 d, the birds were placed singly in experimental cages (30 × 30 × 40 cm) and were visually isolated from each other. On their 9th, 10th and 11th day of life, chicks were deprived of food for 2 h, and then trained to become familiar with the palatable larvae of mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae), offered in Petri dishes. They were given 2 min to accept or reject the mealworm. There were one or two training sessions every day. An individual bird that never managed to find/eat the larvae was not used in the experiment.

On the day following the third training day we conducted the double quantification bioassay. Chicks were deprived of food for 2 h, and then offered the first control larva: a mealworm to check if the chick was hungry. When the first control was eaten, one experimental larva or bait was offered, depending on the bioassay (see above), and the bird response in relation to the offered organism was recorded as: (1) captured, when the larva or bait was consumed, and (2) not captured, when the larva or bait was pecked and released. When the chick did not try to prey upon or attack the larva or bait, the result for that individual was not considered (as in Chai 1990). In the response category (2), after offering the experimental larvae or bait, we offered to the chick another mealworm (second control). When the bird ignored or rejected the second control, that trial was rejected for further analysis. Each individual chick was never used in more than one trial. The frequency of captured and non-captured larvae or baits in each bioassay were compared as in the ant experiments.

**RESULTS**

In the field bioassay, the disappearance of *Spodoptera frugiperda* was significantly different among the
treatments (Q = 50.6, df = 5, P < 0.01; Figure 1). On protected plants, disappearance was always lower compared with unprotected ones, but there was no significant difference among treatments. On unprotected plants the disappearance of baits treated with shield extract was significantly lower than solvent controls and untreated controls.

In the laboratory bioassays using ants, capturing frequencies of live larvae with shields (6.67%, n = 30) or without shields (13.8%, n = 29) did not differ significantly (G_{Yates corr} = 0.23, df = 1, P = 0.63). However, the frequency of predation upon baits treated with shield extract was significantly lower (40%, n = 20) than upon solvent controls (90%, n = 20) or untreated controls (86.7%, n = 15) (G = 14.6, df = 2, P < 0.001). Observing ant behaviour in relation to the offered prey, it was verified that ants tried to drag the prey away at first contact with the larvae or the baits. However, when larvae with shields or baits treated with shield extract were offered, some ants groomed their antennae and cleaned contaminated mouthparts by rubbing them against the substrate. This behaviour is described in the literature as a typical reaction to chemical repellents (Eisner et al. 1967, Morton & Vencl 1998).

In the laboratory bioassays using chicks, the frequency of predation upon live larvae with shields was significantly lower than larvae without them (57.1% n = 21 and 89.5% n = 19, respectively) (G_{Yates corr} = 3.92, df = 1, P = 0.048). Nevertheless, pecked and released larvae always died. The frequency of predation upon baits treated with shield extract was significantly lower (50%, n = 28) than upon solvent controls (91.7%, n = 24) or untreated controls (88.2%, n = 17) (G = 14.3, df = 2, P < 0.001). Some chicks that pecked and released live larvae with shields and baits treated with shield extract tried to clean their beaks by pressing them onto the cage floor.

**DISCUSSION**

Early in our studies, we demonstrated that the faecal shield of *Plagiometriona aff. flavescens* larvae has a role in defence in the field (Nogueira-de-Sá & Trigo 2002). According to the results presented in the previous paper, shields are important in protecting larvae, and comparisons of larvae mortality without shields or with natural shields substituted by an artificial non-noxious shield showed that such protection was not physical, suggesting that the protection must be due to chemicals in this structure. In this work we have tested and confirmed this suggestion for *Plagiometriona aff. flavescens* larvae in field and laboratory conditions.

Results of field bioassays testing predation upon baits treated with shield extracts agree with the chemical protection suggested by Nogueira-de-Sá & Trigo (2002). Other authors also suggested that chemicals from the faecal shield, which may be derived from the host plant, were responsible for the protection of cassidine larvae against predators (Evans et al. 2000, Gómez et al. 1999, Morton & Vencl 1998, Müller & Hilker 2003, Vencl et al. 1999). However, in those studies no field bioassays testing the chemical nature of protection in leaf beetles against natural predators were conducted on their host plants. When undertaking this experiment, arthropods were collected on the vegetation along the studied trail using a sweep net; most potential predators were spiders, and there were a few ants and heteropterans. On three occasions we observed the spiders *Misumenops* sp. (Thomisidae), *Achaearanea tessellata* and *Theridion calcynatum* (Theridiidae) preying upon cassidine larva (Nogueira-de-Sá 2004).

Ant bioassays showed that the capturing percentage of live larvae was lower than that of baits treated with shield extracts. We verified that *Plagiometriona aff. flavescens* larvae have another defensive mechanism against *Camponotus crassus* ants, namely chemical camouflage, in which larvae matched the pattern of cuticular hydrocarbons of its host plant (Nogueira-de-Sá 2004). This kind of defence strategy was first verified for larvae of the ithomiine butterfly *Mechanitis polymnia* that feed on *Solanum* species (Portugal 2001). Because chemical cues are so important for ants, such alternative defensive mechanisms could be the main factor responsible for the higher survivorship of live larvae– even without shields – when compared with baits treated with chemicals from shields in the ant bioassays. Another explanation is
the lack of defensive behaviours in baits. Chrysomelidae larvae rely on some behavioural characteristics described as responses to natural enemies (see Jolivet & Verma 2002 and references therein). Specifically, moving the body, fortifying defensive compounds and waving the shield after a disturbance to cover stimulated areas of the body were observed for larvae of different Chrysomelidae species (Blum 1994, Olmstead 1996).

Although chicks preyed upon larvae with shields less frequently than larvae without it, those prey always died after being pecked, independent of the presence of the shield. Therefore, we suggest that protection provided by chemicals present in the shield can be considered efficient only if chicks become able to associate the image of the shield to unpalatability and learn to avoid larvae that carry such structures. As larvae die, this kind of aversive learning may have been established via kin selection as proposed by Guilford (1990). Although we did not conduct experiments with aversive learning based on unpalatability, it was demonstrated for birds, like Parus major and Parus ater (Hilker & Köpf 1994), and chicks (Begossi & Benson 1988, Hough-Goldstein et al. 1993).

Although we did not conduct any bioassays testing host plant extracts, chemical defence in shields derived from host plants was claimed by several authors (Evans et al. 2000, Gómez et al. 1999, Jolivet & Verma 2002, Morton & Vencl 1998, Müller & Hilker 1999, 2003; Vencl & Morton 1998, Vencl et al. 1999). We still do not know what substances are important in defending the larvae of Plagiometriona aff. flavescens. Vencl et al. (1999) detected steroidal alkaloids, saponins, fatty acids and phytol derivatives in the faecal shield of Plagiometriona cladava, which are derived from its host plant Solanum dulcamara (Solanaceae). These compounds together probably provided protection against larvae predation. The characterization of chemicals from shields of Plagiometriona aff. flavescens and leaves of its host plant, Aureliana fasciculata, is under investigation in our laboratory.

In conclusion, this work supports the efficacy of shields of Plagiometriona aff. flavescens as a defensive strategy in larvae, enhancing its survivorship and, as suggested in other studies, proves the chemical nature of this defence.

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LITERATURE CITED


KEESE, M. C. 1997. Does escape to enemy-free space explain host specialization in two closely related leaf-feeding beetles (Coleoptera: Chrysomelidae)? *Oecologia* 112:81–86.


