Adaptations of two specialist herbivores to movement on the hairy leaf surface of their host, *Solanum guaraniticum* Hassl (Solanaceae)

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ABSTRACT. Adaptations of two specialist herbivores to movement on the hairy leaf surface of their host, *Solanum guaraniticum* Hassl (Solanaceae). Plant trichomes can difficult the attachment and movement of small insects. Here, we examine the hypothesis that the success on the use of densely haired hosts by two cassidine species is determined by differential morphology and behavior. Larvae of *Gratiana graminea* (Klug, 1829) and *Gratiana conformis* (Boheman, 1854) move on the leaf surface of their host, *Solanum guaraniticum* Hassl by anchoring their tarsungulus on the trichome rays or by inserting the tarsungulus tip directly into epidermis. This kind of movement is only possible due to a similar tarsungulus shape among the species. Tarsungulus growth pattern is also similar between species, being relatively small on the posterior aperture, matching the diameter of the host plant trichome rays. The tarsungulus shape associated with differences on ontogenetic growth and attachment pattern allow these two Cassidine larvae to efficiently move on the pubescent leaf surface of their host.

KEYWORDS. Cassidinae; Gratiana; morphological adaptation; tarsungulus; trichomes.

RESUMO. Adaptações de dois herbívoros especialistas ao movimento, em folhas com tricomas do sua hospedeira, *Solanum guaraniticum* Hassl (Solanaceae). Os tricomas foliares podem dificultar a fixação e o movimento de pequenos insetos. Neste trabalho, testamos a hipótese de que o sucesso na exploração de plantas com folhas densamente cobertas por tricomas é determinado pela morfologia e comportamento diferenciados em duas espécies de cassídineos. As larvas de *Gratiana graminea* (Klug, 1829) e *Gratiana conformis* (Boheman, 1854) se movem sobre a superfície foliar de sua hospedeira, *Solanum guaraniticum* Hassl através do ancoramento de seus tarsungulos no raio dos tricomas estrelados da planta ou da inserção da ponta desta estrutura diretamente na epiderme. Tais tipos de movimento só são possíveis devido à forma dos tarsungulos, a qual é similar nas duas espécies. O padrão de crescimento dos tarsungulos das espécies estudadas é também similar e apresenta-se relativamente menor na abertura posterior, correspondente ao diâmetro dos raios dos tricomas estrelados. A forma dos tarsungulos, associada às diferenças no seu padrão de crescimento permite às larvas destas duas espécies de Cassidínios se moverem com eficiência sobre a superfície pubescente de sua planta hospedeira.

PALAVRAS-CHAVE. Adaptação morfológica; Cassidinae; Gratiana; tarsungulos; tricomas.

Trichomes represent a plant morphological trait that can impose resistance to herbivore insects (Levin 1973; Johnson 1975; Fernandes 1994; Wagner et al. 2004). Evidences from cultivated and wild plants support the idea that trichomes act as mechanical defense that impede movement on leaf surface, restrict access to food and/or diminish food digestibility and assimilation (Schillinger & Gallun 1968; Gilbert 1971; Rathcke & Pool 1974; Pillemer & Tinge 1976; Belcher & Thurston 1982; Brewer et al. 1983; Smith & Grodowitz 1983; Ramalho et al. 1984; Hoffman & McEvoy 1986; Fordyce & Agrawal 2001; Valverde et al. 2001; Kennedy 2003). In addition to mechanical defense, some plant glandular trichomes secret toxic chemicals and/or sticky exudates that may entrap and kill small insects (Thurston 1970; Smith et al. 1975; Tinge & Gibson 1978; Shade et al. 1979; Tinge & Laubengauer 1981; Duffey 1986; Neal et al. 1989; Yencho & Tinge 1994; van Dam & Hare 1998; Gurr & McGrath 2002).

On the other hand, certain insects developed behavioral traits to surpass the barriers imposed by plant trichomes and guarantee their success in using pubescent hosts. Larvae of some Lepidoptera and Coleoptera use their mandibles to cut and remove leaf trichomes in order to clear a patch of hairs before establish a feeding site (Hsiao 1986; Hulley 1988; Medeiros & Vasconcellos-Neto 1994; Zalucki 2001; Medeiros & Moreira 2005). The gregarious larvae of *Mechanitis isthmia* (Lepidoptera: Ithomiinae) avoid the leaf trichomes of their host by spinning a network of silk over the spines tops, above which they crawl safely (Rathcke & Pool 1974). Also, there are evidences for the existence of insect morphological modifications to overcome the barriers imposed by trichomes and their exudates (Kennedy 1986 a, b; Moran 1986; Southwood 1986). For example, *Dicyphus errans* (Hemiptera, Miridae) presents very long tibia and modified tarsi structure that avoid the leg contact to the the tip of the host glandular trichomes, which secret a gummy exudeate when touched (Southwood 1986). Recent studies showed for larvae of six cassidine species a convergence on tarsungulus shape associated with differences on ontogenetic growth, which contribute to an efficient movement on both glabrous and pubescent surfaces (Medeiros et al. 2004).
Seven species have been ascribed for the neotropical genus Gratiana Spath, 1913. Five of them are known to feed on Solanum L. (Solanaceae) and tend to be host specific (Siebert 1975; Buzzi 1988, 1994; Borowiec 1996, 1999; Olckers et al. 2002). The effects of Solanum trichomes as a defense mechanism against herbivores are widely studied, mainly regarding the role of their exudates (Gibson 1978; Tingey & Gibson 1978; Obrycki & Tauber 1984; Gregory et al. 1986; Neal et al. 1989; Yencho & Tingey 1994; Hill et al. 1997). However, the corresponding mechanical effects are relatively poorly explored. Medeiros & Moreira (2002) showed that the stellate trichomes of Solanum sisymbriifolium Lam. (Solanaceae) act as a mechanical barrier to Gratiana spadicea (Klug, 1829) (Cassidinae) larval movement, mainly for the first three instars. To move across the pubescent host leaf surface, G. spadicea larvae present a tarsungulus morphology and growth pattern adapted to anchor their legs on the trichomes, and thus moving above them (Medeiros & Moreira 2002).

At the Northwest of Rio Grande do Sul State, Gratiana conformis and G. graminea are commonly found sharing the same individual plants of Solanum guaraniticum (Medeiros et al. 1996; Nogueira-de-Sá et al. 2004), whose leaf surface is densely pubescent. The aim of this study is to characterize the larval leg morphologies and morphometries of these two Cassidinae, and to describe the way by which they attach and move on the hairy leaf surface of Solanum guaraniticum.

MATERIAL AND METHODS

Insects and plants. Adults and immatures of G. conformis and G. graminea were field collected from nearby wild S. guaraniticum plants, and kept in a laboratory chamber (25 ± 1°C; L14:D10; ca. 70% RH), existing at the Zoology Laboratory of University of Jui (UNIJUI), Jui, RS, Brazil (28°23′18″ S). The plants used for insect feeding, to study behavioral trials and trichome characterization, were field collected also from the vicinity of UNIJUI Campus. Leaves of the same age were used, and there was no indication they presented different size or trichome density.

Leaf characterization. Leaf squares of S. guaraniticum (ca. 1 cm²) were cut with a pair of scissors and immediately fixed in FAA 50% solution. They were immersed overnight in acetone, and then critical point dried. The dried leaf squares were mounted on aluminum stubs and coated with gold-palladium, using a Balzers Union® sputter coater. They were observed and photographed in a Jeol 5800® scanning electron microscope. For morphometric characterization, the stellate trichomes were removed at random with fine pointed forceps from the abaxial surface (ca. 0.5 mm² per surface) of S. guaraniticum leaves (n = 20 leaves; three trichomes per leaf). The corresponding groups of detached trichomes were slide mounted in water and the images obtained from a microscope connected to a digitizing plate and a video camera. The length, diameter at the tip, middle and base of the trichome rays and peduncle were taken by using the Image tool® software.

Stellate trichome density was recorded for the abaxial leaf surface (ca. 1 mm²) of fully expanded leaves (n=30).

Leg morphometrics. Leg length of both Gratiana species (n=10 per instar per species) was measured as the distance from the proximal margin of the coxa to tibial tip (Fig. 1a). To measure the tarsungulus, individuals (n= 10 per instar per species) were clarified in a hot, 10% KOH solution, dehydrated in an ethanol series (50, 70, 90 and 100% ethanol), immersed in xylol, and slide mounted in Canada balsam. The width of the tarsungulus basis and its aperture at anterior, median and posterior positions (Fig. 1b) were also measured. The tarsungulus consists of the distal portion of larval legs, and results from the fusion of the tarsus and pre-tarsus (Crowson 1981; Lawrence 1991). To compare the growth pattern of these leg structures with other Gratiana body parts, we also measured the larvae head capsule width (n=10 per instar per species), which corresponded to the distance between the posterior stemmata. Data were submitted to a regression analysis (α = 0.05). The best-fit equations were determined by comparing the sum-of-squares using the F-test (Graph pad Prism 3.0).

Fig. 1. A - Schematic representation of a cassidine (Gratiana spadicea) larval leg (cx = coxa, fm = femur, tb = tibia, tr = trochanter, ts = tarsungulus), and corresponding length measure (LL arrow); B – schematic representation of its tarsungulus, and corresponding measures (a = anterior aperture, m = median aperture, p = posterior aperture, and tw = tarsungulus width). (from Medeiros & Moreira, 2002).
Larval movement. To describe which leaf structures are used as attachment for larval movement on *S. guaraniticum* abaxial leaf surface, focal observations (n=20 individuals per instar per species) were made under a stereomicroscope during three minutes. As a given individual moved during observations, the corresponding leg position in relation to either the ray of different types of trichomes or epidermis was identified, and recorded. The data were transformed to frequencies and compared using Chi-square tests (α = 0.05).

RESULTS

Leaf characterization. Leaves of *S. guaraniticum* are densely covered by stellate and simple glandular trichomes. The stellate type presents a puricellular peduncle and, in general, seven to eight lateral rays arising from a common point (Fig. 2). These correspond to the multiangulated type described by Mentz *et al.* (2000). The number of stellate trichomes in the abaxial leaf surface was 12.78 ± 0.45/mm² (X ± SE; n=30). The corresponding trichome rays were longer (0.47 ± 0.011 mm; n = 144) and thinner (25.6 ± 4.5 µm) than the peduncles (0.29 ± 0.012 mm length, and 52.3 ± 7.3 µm thickness; n = 42).

Leg morphometrics. The tarsungulus of both *Gratiana* larvae are heavily sclerotized, corresponding to a terminal hooked structure with a sharp tip in continuation to the distal edge of the tibia (Fig. 3). Although *G. conformis* larvae and adult body is slightly larger than that of *G. graminea*, their larvae share the same pattern of ontogenetic growth of the body parts considered here. Tarsungulus aperture increases in size from its posterior to anterior end. For both *Gratiana*, exponential growth was recorded for the head capsule, leg length, and tarsungulus width (Fig. 4). Also, the anterior portion of the aperture grows exponentially throughout the larval instars (Figs. 5a and d). In contrast, the posterior and median portions of the tarsungulus aperture increase relatively

| Table I. Ratios between the mean larval head capsule width, and that of leg length (LL), tarsungulus width (TW), tarsungulus aperture on the anterior (AA), median (MA), and posterior (PA) portions of *Gratiana conformis* and *G. graminea*. |
|-----------------|-------------|-------------|---|---|---|
| Species         | Larval instar | LL  | TW  | AA | MA | PA |
| Gratiana conformis | I            | 1.16 | 5.49 | 7.80 | 18.31 | 36.92 |
|                 | II           | 1.11 | 5.61 | 9.36 | 24.90 | 44.33 |
|                 | III          | 1.06 | 5.75 | 10.48 | 35.03 | 60.92 |
|                 | IV           | 1.02 | 5.94 | 10.23 | 38.34 | 71.22 |
|                 | V            | 0.97 | 6.11 | 9.62 | 39.41 | 85.60 |
| Gratiana graminea | I            | 1.09 | 7.06 | 9.07 | 22.41 | 41.17 |
|                 | II           | 1.08 | 6.55 | 9.72 | 25.35 | 48.28 |
|                 | III          | 1.03 | 6.87 | 11.00 | 31.20 | 57.27 |
|                 | IV           | 0.98 | 7.33 | 12.30 | 31.61 | 64.48 |
|                 | V            | 1.03 | 7.04 | 9.24 | 30.59 | 80.03 |

Fig. 2. *Solanum guaraniticum*. Overview of the abaxial leaf surface showing the stellate (st) and the simple glandular trichomes (gt).

Fig. 3. Tarsungulus of *Gratiana conformis* (A), and *G. graminea* (B) larvae.
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slight with larval instars for both species (Figs. 5b, c, e and f). As a consequence, the growth ratio between the head capsule width and for both middle and posterior aperture size progressively increases among instars, while the corresponding ratio remains similar for the anterior aperture, leg length and tarsungulus width (Table I).

**Larval movement.** The larvae of both *Gratiana* species move mainly by anchoring the tarsungulus hook on the trichome rays, but occasionally they also move by inserting the tarsungulus tip directly into the epidermis. This second type of movement is more frequent for latter instar larvae (Fig. 6).

**DISCUSSION**

Plant trichomes can confer resistance to movement and feeding to herbivorous insects, and also to their natural enemies (Levin 1973; Shah 1982; Belcher & Thurston 1982; Brewer et al. 1983; Gamarra et al. 1998; Fernandes 1994). Also, their negative effects are in many cases inversely related to the size of the organisms, and so the younger are generally more vulnerable to their harmfulness (Schillinger & Gallun 1968; Tingey & Launbengayer 1981; Hoffman & McEvoy 1986a; Welles & Hoxie 1988; Van Dam & Hare 1998; Fordyce & Agrawal 2001; Zalucki et al. 2002). Considering that many Cassidinae are very closely associated to their hosts (Buzzi 1988, 1994; Jolivet & Hawkeswood 1995), and that their larvae are relatively small, it is reasonable to expect that they must present adaptations to surpass barriers imposed by high density of trichomes. Here, we found that both *Gratiana* species present a similar tarsungulus shape that allow larvae to move on the very pubescent leaf surface of their host, *S. guaraniticum*. Such a pattern has already been described for other Cassidinae larvae (Medeiros & Moreira 2002; Medeiros et al. 2004). The tarsungulus seems to represent a well adapted attachment structure to exploit leaves varying from glabrous to densely pubescent.

The tarsungulus hook allows the larval anchorage on the trichomes of hairy leaf surfaces. In fact, larvae that exploit densely pubescent leaves as those of *S. guaraniticum*, must move above and/or between the trichomes by anchoring the tarsungulus hook on the trichome rays, such as observed herein. Medeiros et al. (2004) showed that the higher frequencies of this type of movement are registered for larvae that feed on more densely pubescent plants, in particular those with higher density of stellate trichomes. Larval anchorage in this way is only possible due to the tarsungulus morphology, and probably to variation on their morphometry, as the tarsungulus aperture at the median and posterior portions grows isometrically along the larval stage, while the other body parts grow exponentially. Also, these aperture dimensions present a correspondence with the trichome peduncle and ray diameter, where larvae anchor the tarsungulus. The same pattern was observed for other cassidine species that exploit pubescent hosts, while on the contrary those associated to glabrous plants present exponential growth on those body parts (Medeiros & Moreira 2002; Medeiros et al. 2004).
The sharp end of the tarsungulus allows the larvae to attach and move by inserting the tip directly into the leaf epidermis. This type of movement was described for oak aphids, and called “tiptoeing” or walking on the claws by Kennedy (1986a, b). It occurs preferentially on glabrous or low trichome density surfaces that permit the larvae to move among their rays, and seems to be a common strategy of many insects, since leaf surfaces are generally smooth and relatively easy to perforate with a sharp claw. The Gratiana larvae here studied also walk on the claws, but in a relatively low frequency, except during the fifth instar. This movement is difficult for small larvae because their legs are shorter than the trichome peduncle and central ray, so they cannot reach the epidermis under high density of stellate trichomes, as occurs in S. guaraniticum leaves. Hence, their movement must occur above the trichomes, which is possible given the morphological modifications of the tarsungulus.

Thus, from a morphological perspective, it seems clear that the tarsungulus shape and ontogenetic growth pattern favors the larvae of Gratiana conformis and Gratiana graminea to successfully move on the hairy surface of their host.

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