

# Lima bean–lady beetle interactions: hooked trichomes affect survival of *Stethorus punctillum* larvae

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**Abstract** We tested the hypothesis that Lima bean *Phaseolus lunatus* L. (Henderson cultivar) trichome density affects the survival of the acariphagous lady beetle *Stethorus punctillum* Weise. When isolated throughout larval development, 10% or less of *S. punctillum* larvae reared on two-spotted spider mite *Tetranychus urticae* Koch on small (rather than large) *P. lunatus* leaves survived until pupation. Although other factors might have contributed to larval mortality, we demonstrated that survival rate was related to trichome density (i.e., number of hooked trichomes per cm on the underside of leaves). Trichome density was greatest on the underside of small leaves and least on large leaves, indicating that survival rate increased as trichome density decreased. Hooked trichomes impaled *S. punctillum* larvae more often at the apex of the abdomen than at any other body part. Hooked trichomes also trapped *S. punctillum* adults, but they always managed to free themselves. This study highlights the negative effect of Lima bean hooked trichomes on a predacious lady beetle that attacks the two-spotted spider mite.

**Keywords** Biological control · Host-plant resistance · Rearing · Coccinellidae · Leguminosae · Tetranychidae

## Introduction

Compatibility of host-plant resistance and biological control in suppressing pest populations is an important goal of integrated pest management (Bottrell et al. 1998). Epidermal hairs or trichomes on leaves of plants can function as a resistance mechanism against attack by herbivores (Levin 1973). Hooked trichomes can have negative effects on herbivorous arthropods. For example, Johnson (1953) reported that the hooked trichomes on the growing shoots of French bean (*Phaseolus vulgaris* L.) impaled aphids (*Aphis craccivora* Koch), resulting in reduced longevity and reproduction and high larval mortality. Pillemer and Tingey (1976) found that hooked trichomes on the leaves of red kidney bean (*P. vulgaris*) and Lima bean (*Phaseolus lunatus* L.) trapped and caused mortality of nymphs and adults of *Empoasca* leafhoppers. Quiring et al. (1992) showed that hooked trichomes on *P. vulgaris* leaves impaled and subsequently reduced the longevity of adults of an agromyzid fly, *Liriomyza trifolii* Burgess. Kavousi et al. (2009) revealed that hooked trichomes on *P. vulgaris* leaves occasionally trapped and caused mortality of adults of the two-spotted spider mite, *Tetranychus urticae* Koch.

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Trichome-based plant resistance to attack from herbivores and the predation potential of natural enemies can be compatible (Barbour et al. 1997; Voigt et al. 2007; Loughner et al. 2008) or incompatible (Lambert 2007; Verheggen et al. 2009). Eisner et al. (1998) showed that hooked trichomes on leaves of an herb, *Mentzelia pumila* Nutt. ex Torr. & Gray, impaled and incapacitated larvae and adults of the convergent lady beetle, *Hippodamia convergens* Guérin-Ménéville, but not its prey, the aphid *Macrosiphum mentzeliae* Wilson. Shah (1982) showed that hooked trichomes on leaves of bush bean (*P. vulgaris*) impaled larvae of the two-spot ladybird beetle, *Adalia bipunctata* L., resulting in their death. Ricci and Cappelletti (1988) showed that hooked trichomes on scarlet runner bean, *Phaseolus coccineus* L., leaves inhibited the movement and impaled larvae of the lady beetle, *Clitostethus arcuatus* Rossi. Biddinger (1993) mentioned that hooked trichomes on Lima bean tore the integument of larvae and damaged the posterior integument of adults of *Stethorus punctum punctum* (LeConte) reared on caged Lima beans in greenhouse insectaries. Putman (1955) reported that hooked trichomes on scarlet runner bean, *P. coccineus*, tore the integument of developing larvae of the lady beetle *Stethorus punctillum* Weise.

*Stethorus punctillum* is an important, commercially available predator that suppresses tetranychid mite populations in greenhouses, interiorscapes, nurseries, and orchards (van Lenteren 2003; Biddinger et al. 2009). It is the only species of acariphagous coccinellid currently produced for augmentative biological control of pest mites, including *T. urticae*. *T. urticae* is a serious pest of more than 300 species of greenhouse plants (Zhang 2003). Experimental releases of *S. punctillum* to suppress *T. urticae* demonstrated predator establishment and pest suppression on pepper and cucumber plants but not tomato plants in greenhouses (Raworth 2001). The reason why *S. punctillum* did not establish in tomato was not determined, but could have been due to plant trichomes (Raworth 2001). Interestingly, Rott and Ponsonby (2000a, b) found that searching behavior and performance of *S. punctillum* was greatest on mite-infested pepper and tomato.

Efforts to improve rearing capacity, as well as identify plant substrates that do not limit the predation potential of *S. punctillum* would help the natural

enemy industry expand the production of this predator and other acariphagous coccinellids for augmentative releases against pest mites. In our efforts to initiate a colony of *S. punctillum*, we noticed that larvae were failing to complete development to the pupal stage in arenas containing spider mite-infested Lima bean leaves. This led to testing the hypothesis that trichome density affects the survival rate of *S. punctillum*. Research objectives of this study were (1) to determine the relationship of leaf size to density of hooked trichomes, (2) to determine the relationship between trichome density and survival rate of *S. punctillum*, and (3) to determine which body part of *S. punctillum* was most vulnerable to entrapment by the hooked trichomes.

## Materials and methods

### Plants and arthropods

Lima bean plants (cultivar Henderson) were grown from seed in plastic containers (57 cm long, 35.5 cm wide, and 16 cm deep) in an environmentally controlled (27°C, 30–40% RH, and 18 h photophase) greenhouse in Stoneville, Mississippi, USA. In each container, approximately 4 gm of fertilizer (Osmocote<sup>®</sup>, Smart-Release<sup>®</sup>, <http://www.scotts.com>) was mixed with 2.5 l of potting soil (Miracle-Gro<sup>®</sup>, Moisture-Control<sup>®</sup>, <http://www.scotts.com>), 2.5 l of coarse vermiculite, and water before adding seeds. A colony of prey mites (*T. urticae*) was established from a population from Syngenta Bioline (Oxnard, CA, USA) and was cultured year-round on *P. lunatus* plants in the above-mentioned greenhouse. Acariphagous *S. punctillum* were obtained from Applied Bionomics Ltd. (Sidney, BC, Canada). Several shipments of *S. punctillum* were required in our efforts to establish a laboratory colony. Predators were maintained in Petri dish arenas (25 mm deep, 90 mm wide) separated according to stage of development (egg, larval, pupal, and adult) in an environmental growth chamber (24°C, 60% RH, 16 h photophase).

### Estimating trichome density

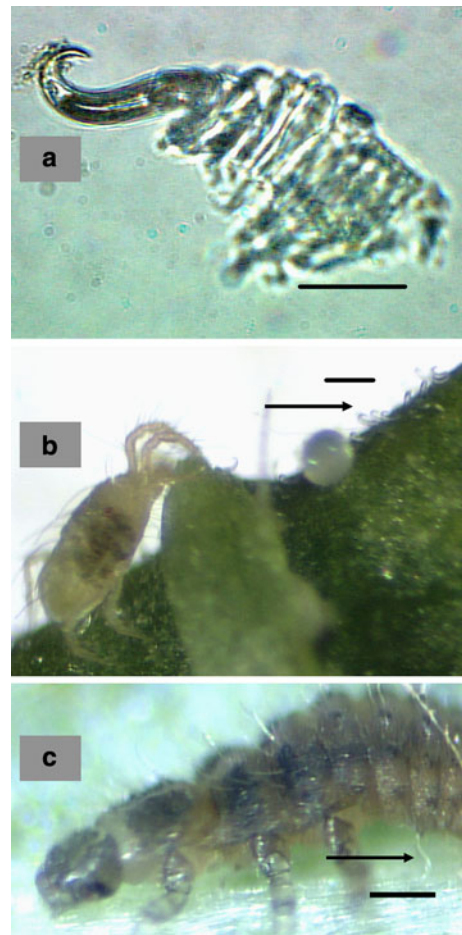
*Phaseolus lunatus* leaves ranging in age of 1–2 weeks were excised from upper and lower sections of randomly selected plants in the Stoneville greenhouse.

Relative leaf size categories (and age) were small (1 week old), medium (1.5 week old), and large (2 week old). Leaf size was determined by measuring the width (at the widest point) using a plastic ruler: 3–4 cm (small), 5–6 cm (medium), and 7–8 cm (large). Straight and hooked non-glandular trichomes were present on the *P. lunatus* leaves. Straight trichomes were along the main vein and lateral veins on the topside (adaxial) and, less often, the underside (abaxial) of the leaves. Hooked trichomes were along the main vein and lateral veins on the underside of the leaves. The tip of a hooked trichome was curved and pointed (Fig. 1a).

The number of straight and hooked trichomes in variable cm lengths (that intersected the main vein) of randomly selected leaves (ten sample leaves per leaf size) was estimated using an Olympus® SZ11 zoom stereomicroscope (110× magnification) and computer-based imaging software (Image Pro® Plus, Media Cybernetics, Inc, MD, USA). Because of the transparency and small size of the trichomes, it was not possible to count trichomes in a circumscribed cm<sup>2</sup> area on the underside of leaves. Alternatively, we folded the leaves in half (along the middle) and pressed the folded edge firmly against a microscope slide. The trichomes that extended out from the folded edge of the leaf became visible under the stereomicroscope (110×). Thus, the number of trichomes per cm section of the leaf edge was used as an estimate of trichome density. Using the imaging software, the height (in μm) of hooked trichomes (on the same leaves used to determine trichome number) was determined by measuring the straight-line distance from the base of the trichome to the apex, excluding the curved tip. Note that the trichomes were angled from 20° to 90° from the leaf surface. The relationship of leaf size (small, medium, large) to straight and hooked trichome density (number per cm) and hooked trichome height were determined.

#### Estimating predator survival and frequency of entrapment

Three times per week, Lima bean leaves were placed in a plastic shoebox (with lid) and held in a table-top refrigerator until needed. In the first trial of this survival experiment, mite-infested small, medium, and large leaves were placed (with the underside facing upward) at the base of Petri dish arenas



**Fig. 1** Images of hooked trichome (at 600× magnification) dissected from Lima bean leaf tissue (a), underside of leaf (at 110×) displaying a cluster of hooked trichomes relative to the size of a *T. urticae* adult and egg (b), and lateral view of *S. punctillum* 2nd instar (at 110×) showing hooked trichome embedded in abdomen (c). Scale bar in Fig. 1a, b, and c represents 50, 100, and 100 μm, respectively

(25 mm deep, 90 mm wide). The quantity of mites (of all stages of development) on the leaves exceeded the amount that the predators could consume in a day. Two small leaves (to compensate for the small surface area), one medium leaf, and one large leaf were placed in separate arenas. Three replicate arenas for each of the three leaf sizes were used. Twenty-first instar larval *S. punctillum* from the laboratory colony, reared from adults purchased from commercial supplier, were placed in arenas on treatment leaves. Thus, there were 60 larvae per treatment group for a total of 180 larvae in this experiment. In

the second trial, mite-infested small and large leaves were placed in arenas (as before) and the quantity of mites on the leaves exceeded the amount that the predators could consume in a day. Two replicate arenas for each of the two leaf sizes were used. Twenty *S. punctillum* first instars from the same laboratory colony were placed in arenas on treatment leaves. Thus, there were 40 larvae per treatment group for a total of 80 larvae. Experimental arenas were maintained in an environmental growth chamber (24°C, 60% RH, 16 h photophase). In both trials, arenas were removed daily to record the number of larvae still alive, replace old leaves with fresh mite-infested ones, and observe the part of the body impaled on hooked trichomes, with the aid of the stereomicroscope. The relationship between hooked trichome density and *S. punctillum* survival during the larval stage and the frequency of body parts impaled on hooked trichomes were determined.

In a separate experiment, the frequency of *S. punctillum* adults that were trapped on hooked trichomes on mite-infested small or large *P. lunatus* leaves in Petri dish arenas (25 mm deep, 90 mm wide) was determined in 10-min observation periods over 15 consecutive days. Recently emerged adults from the laboratory colony were used in this experiment. Because of their small size and the difficulty of distinguishing the sexes without handling, the sex of the adults was not determined. Experimental arenas were maintained in an environmental growth chamber (24°C, 60% RH, 16 h photophase), but removed to record the behavior of adults, and replace old leaves with fresh ones, infested with mites, each day. The frequency of adults (20 adults in two replicate arenas per treatment group) that were trapped on hooked trichomes and the frequency at which they escaped were determined. The number of adults alive at the end of the experiment was determined.

### Statistical analysis

Experiments were set-up following a completely randomized design. A one-way analysis of variance (ANOVA) tested the significance of leaf size on the density of straight trichomes and the density and height of hooked trichomes. Repeated measures analysis of variance (RM-ANOVA) tested the significance of trichome density on percentage of

*S. punctillum* larvae alive at each observation date. A RM-ANOVA also tested the significance of body location on frequency of impalement on hooked trichomes. Absolute data were square root transformed and percentage data were arcsine transformed before the analysis. Means were considered significantly different if  $P \leq 0.05$ . The Holm–Sidak multiple comparison method was used to separate means after the one-way ANOVA and RM-ANOVA. A Kaplan–Meier survival analysis (KMSA), followed by a Log-Rank test of equality, was used to estimate the difference between the survival curves of larvae in the treatment groups. SigmaStat 3.0.1 (Systat Software Inc., Richmond CA, USA) and SAS 9.1.3 (SAS Institute Inc., Cary NC, USA) software assisted with analysis of data. All data presented herein represent non-transformed values.

### Results

Hooked trichomes were most dense on small leaves than on medium or large leaves ( $F = 128.8$ ,  $df = 2$ ,  $27$ ,  $P < 0.001$ ; Table 1). Hooked trichome density differed significantly among all treatment groups. Leaf size was also related to the height of hooked trichomes ( $F = 12.1$ ,  $df = 2$ ,  $27$ ,  $P < 0.001$ ); those on small and medium leaves were significantly shorter than those on large leaves (Table 1). However, the height of hooked trichomes was short in comparison to the size of the two-spotted spider mite *T. urticae* (see Fig. 1b). Leaf size was related to the density of straight trichomes ( $F = 79.0$ ,  $df = 2$ ,  $27$ ,  $P < 0.001$ ); straight trichome density differed significantly among all leaf sizes (Table 1). The density of straight trichomes was much lower than that of hooked trichomes. Although not reported, the height of straight trichomes was often much greater than that of hooked trichomes. No glandular substances exuded from the tip of hooked or straight trichomes.

Although the height of hooked trichomes was rather short in relation to the size of the acariphagous *S. punctillum*, this size disparity did not prevent entrapment of larvae on the curved tip (see Fig. 1c). The size of Lima bean leaves significantly affected the survival of *S. punctillum* larvae from the first through the fourth instar in the first trial ( $F = 28.8$ ,  $df = 2$ ,  $14$ ,  $P < 0.001$ ) and the second trial ( $F = 329.4$ ,  $df = 1$ ,  $7$ ,  $P < 0.001$ ). Survival was

**Table 1** Mean ± SE densities of straight and hooked trichomes on Lima bean leaves of three sizes

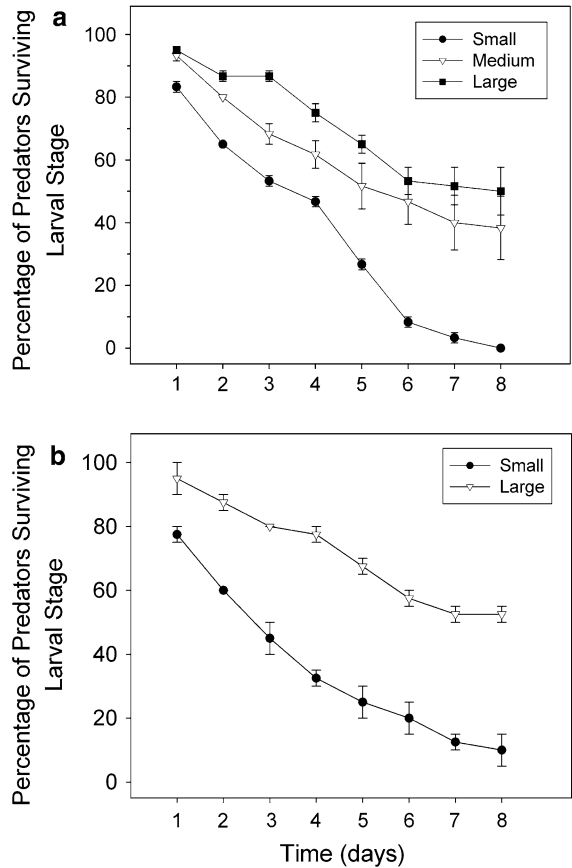
Leaf size	Leaf width (cm)	Straight trichome density (number per cm)	Hooked trichome density (number per cm)	Hooked trichome height (µm)
Small	3–4	1.11 ± 0.07 a	21.2 ± 0.8 a	70.4 ± 2.3 a
Medium	5–6	0.54 ± 0.04 b	10.5 ± 0.6 b	70.8 ± 3.5 ab
Large	7–8	0.31 ± 0.02 c	7.7 ± 0.3 c	87.4 ± 2.3 c

*n* Ten leaves per size category. Means followed by a different letter in a column are significantly different ( $P < 0.05$ )

significantly lower on small leaves than medium or large leaves, but not significantly different between medium and large leaves (Holm–Sidak method; Fig. 2a). In the second trial, survival was significantly less on small leaves than on large leaves (Fig. 2b).

Survival curves differed significantly between treatments in the first trial (KMSA; Log-Rank test,  $\chi^2 = 15.06$ ,  $df = 7$ ,  $P = 0.03$ ) and second trial (KMSA; Log-Rank test,  $\chi^2 = 15.06$ ,  $df = 7$ ,  $P = 0.035$ ). On small leaves, survival rate of *S. punctillum* larvae decreased to 0% by the eighth day, corresponding to the late fourth instar (Fig. 2a). On medium and large leaves, survival rate of larvae was 40% and 60%, respectively. In the second trial, on small leaves, survival rate decreased to 10% by the eighth day, corresponding to the late fourth instar (Fig. 2b). On large leaves, survival rate was 52.5% by the eighth day.

Body part (location) had a significant effect on the frequency of impalement of *S. punctillum* larvae on small leaves ( $F = 41.5$ ,  $df = 4, 28$ ,  $P < 0.001$ ), medium leaves ( $F = 25.1$ ,  $df = 4, 28$ ,  $P < 0.001$ ) and large leaves ( $F = 26.8$ ,  $df = 4, 28$ ,  $P < 0.001$ ) in trial 1 (Table 2). Impalement was significantly more frequent at the tip of the abdomen (pygopodium) than at other regions of the abdomen, mouthparts, legs, or combination of two body parts (on the same individual) on small, medium, and large leaves in trial 1 (Holm–Sidak method). Similar results were observed in trial 2. Body part had a significant effect on the frequency of impalement of *S. punctillum* larvae on small leaves ( $F = 14.3$ ,  $df = 4, 28$ ,  $P < 0.001$ ) and large leaves ( $F = 5.5$ ,  $df = 4, 28$ ,  $P = 0.002$ , Table 2). Impalement was significantly more frequent at the tip of the abdomen (pygopodium) than at other regions of the abdomen, mouthparts, legs, or combination of two body parts in trial 2 on small leaves. On large leaves in trial 2, impalement at the abdomen tip was significantly greater than at the



**Fig. 2** Mean ± SE percentage of *S. punctillum* surviving the larval stage across eight days on small, medium, and large Lima bean leaves in trial 1 (a) and small and large leaves in trial 2 (b). Sample size was 24 and 16 observations for trials 1 and 2, respectively

mouthparts, legs, or combination of two body parts (Table 2). No significant differences in rate of impalement at the mouthparts, legs, abdomen (other than the tip), or a combination of two body parts on the same individual on large leaves was observed. On occasion, hemolymph was observed to have leaked from the point where the tip of the trichome entered



**Table 2** Mean  $\pm$  SE percentage of predator (*S. punctillum* larvae) body part impaled on hooked trichomes on small, medium, and large Lima bean leaves in trials 1 and 2

*n* 40 observations for each of the leaf size categories in both trials. Means followed by a different letter in a column are significantly different ( $P < 0.05$ )

Trial	Body part	Small	Medium	Large
1	Abdomen (tip)	87.9 $\pm$ 4.8 a	82.4 $\pm$ 7.1 a	81.5 $\pm$ 12.4 a
	Abdomen (other)	3.1 $\pm$ 3.1 b	11.2 $\pm$ 6.4 b	4.2 $\pm$ 4.2 b
	Mouth	8.9 $\pm$ 4.6 b	3.3 $\pm$ 2.2 b	1.8 $\pm$ 1.8 b
	Leg	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b
	Combination	0.0 $\pm$ 0.0 b	3.1 $\pm$ 3.1 b	0.0 $\pm$ 0.0 b
2	Abdomen (tip)	70.9 $\pm$ 10.0 a		58.3 $\pm$ 14.8 a
	Abdomen (other)	9.2 $\pm$ 6.1 b		18.7 $\pm$ 13.1 ab
	Mouth	4.3 $\pm$ 3.0 b		10.4 $\pm$ 7.0 b
	Leg	0.0 $\pm$ 0.0 b		0.0 $\pm$ 0.0 b
	Combination	15.5 $\pm$ 8.8 b		0.0 $\pm$ 0.0 b

the integument. All impaled larvae, incapable of escaping, died within 18–24 h, probably from desiccation or starvation.

Occasionally, larvae escaped from hooked trichomes by molting to the next instar. For example, eight, four, and seven exuviae were found on hooked trichomes on small, medium, and large leaves, respectively, during days 1–4 in trial 1. No exuviae were found on hooked trichomes on any treatment leaves on days 5–8. Considering that each larva casts three exuviae during its development, and the total sample size was 60 larvae (20 larvae in each of three arenas) per treatment group, 4.4, 2.2, and 3.9% of *S. punctillum* instars escaped, via molting, from hooked trichomes on small, medium, and large leaves, respectively. In trial 2, six and four exuviae were found on the tip of hooked trichomes on small and large leaves, respectively, during days 1–4. None were found on trichomes during days 5–8. With a total sample size of 40 larvae (20 larvae in each of two arenas) per treatment group, 5.0% and 3.3% of *S. punctillum* instars escaped, via molting, from hooked trichomes on small and large leaves, respectively. Straight trichomes (lacking hooks) never impaled *S. punctillum* larvae.

Occasionally, hooked trichomes trapped *S. punctillum* adults. During 10-min observation periods over 15 consecutive days, hooked trichomes on small and large leaves trapped a combined 11 and 6 adults, respectively. It is unclear if hooked trichomes trapped the same adults more than once over this time. Trichomes impaled adults at the mouth, tip of the abdomen, and leg at a frequency of 45, 27, and 27%, respectively, on small leaves, and at a frequency of 50, 33, and 17% on large leaves, respectively. All

adults freed themselves from hooked trichomes by violent body twisting and circling motions around the insertion point of the trichome into the body, within approximately two min after initial entrapment. The insertion point may have been superficial because no apparent mortality of adults resulted from previous impalement. Temporary entrapment of ovipositing *S. punctillum* females or defecating males or females on hooked trichomes was not observed in this study. All adults were alive at the end of this experiment. Straight trichomes did not impale *S. punctillum* adults. Neither hooked nor straight trichomes impaled spider mite nymphs and adults.

## Discussion

The observation that the density of hooked trichomes was greatest on the underside of small *P. lunatus* leaves (rather than medium or large leaves) was not reported before. In this study, trichome density decreased with leaf size (which was related to leaf age) suggesting that small leaves less than one week in age possess their total complement of hooked trichomes for the remainder of their development. Related research found that trichome density decreased with leaf age and development in *P. vulgaris* (Pillemer and Tingey 1976) and trichome density was very high in young leaves but decreased as leaves matured and expanded in size in three birch species (Valkama et al. 2004). Trichome density was higher on young leaves on young plants than on young leaves on mature plants (Pérez-Estrada et al. 2000).

This study shows that the relative frequency of *S. punctillum* larvae escaping from *P. lunatus* hooked trichomes is low at high trichome density; impalement often results in death, probably from starvation or desiccation. The observation that 0–10% of *S. punctillum* larvae completed development to the pupal stage when confined with prey on small *P. lunatus* leaves in trials 1 and 2, respectively (see Fig. 2a, b), was not expected. The fact that 60% of *S. punctillum* larvae survive to pupation in arenas with large *P. lunatus* leaves (in this study), and the observation that only 60% of larvae survive on spider mites on filter paper in the absence of any leaves (EWR and ZW, unpublished data), suggests that unidentified sources of mortality still impede larval development. In other words, up to 40% mortality may not be a result of larval entrapment on leaf trichomes. Ongoing research is determining the prey-mediated influence of plant defensive chemicals (putative allelochemicals) on *S. punctillum* development.

Putman (1955) reported that hooked trichomes on scarlet runner bean *P. coccineus* tore through the integument of developing larvae of *S. punctillum*, but did not provide survival rates or indicate a relationship between trichome density and predator survival. Similarly, Biddinger (1993) found that hooked trichomes on Lima bean tore through the integument of *S. punctum* larvae and damaged the posterior integument of adults (during defecation or oviposition) but did not provide any survival rates.

The way that *S. punctillum* larvae crawl over the leaf surface likely affects their susceptibility to impalement on hooked trichomes. Larvae usually maintain contact with the leaf surface using the apex of the abdomen (i.e., pygopodium). This structure serves to support the body of the larva as it crawls backward or forward and reduces the likelihood of falling from the leaf. In laboratory arenas, extensive searching behavior of larvae results in greater contact between trichomes and the pygopodium than the other body parts. The infrequent occurrence of mouthpart impalement of predator larvae implies that contact with hooked trichomes can occur during feeding. Spider mite females can oviposit on top of hooked trichomes. Predator feeding involves piercing an egg and sucking-out its contents. This could result in the inadvertent contact between mouthparts and the tip of a hooked trichome just beneath the base of the now deflated egg.

The observation that hooked adults tend to free themselves apparently without any negative effects is in conflict with those previously reported for other *Stethorus* species. Walters (1974) commented that hooked trichomes on *P. vulgaris* leaves lacerated the membranes of the terminal abdominal segments of adult *Stethorus loxtoni* Britton and Lee, *Stethorus nigripes* Kapur, and *Stethorus vagans* (Blackburn) resulting in reduced longevity. However, Walters (1974) did not provide survival rates or indicate a relationship between trichome density and predator survival. Trichomes are much more dense and larger on leaves of *P. vulgaris* than *P. lunatus* (Pillemer and Tingey 1976; Oku et al. 2006). This could explain why *S. punctillum* adults had reduced longevity on *P. vulgaris* leaves in the Walters (1974) study and not on *P. lunatus* leaves in this study.

The mobility of the two-spotted spider mite was apparently unaltered on Lima bean leaves of any size (e.g., trichome density). This mite tends to build-up large populations on small leaves. This observation is probably a response to the greater nutrient quality (i.e., higher nitrogen content) of young rather than old leaves (Wilson 1994). The way that nymphs and adults crawl over the leaf surface may reduce contact with hooked trichomes and consequently entrapment by these trichomes. *T. urticae* have long-legs (see Fig. 1b), relative to the rest of their body, which could allow them to move over the top of the hooked tip of the trichomes in *P. lunatus*, thereby avoiding impalement. The lower height and lower density of hooked trichomes on *P. lunatus* in comparison to *P. vulgaris* (Pillemer and Tingey 1976; Oku et al. 2006) may explain why *T. urticae* is less frequently entrapped on *P. lunatus* leaves. Kavousi et al. (2009) showed that hooked trichomes on *P. vulgaris* occasionally trapped and caused the mortality of *T. urticae*. Neither *T. urticae* nor *S. punctillum* was entrapped by or impaled on straight trichomes on Lima bean. The lack of a curved tip as well as the low density of straight trichomes on the leaf underside might explain the absence of an effect on both arthropods.

With regard to mass rearing, the unsuitability of spider mite-infested small *P. lunatus* leaves for rearing *S. punctillum* larvae will stimulate research to find an alternative substrate. Putman (1955) and Biddinger (1993) recommended using smooth-leaved fava bean *Vicia faba* L. as an alternate substrate for prey and predator in a rearing system. Scriven and

Fleschner (1960) used oranges as substrates for rearing a *Stethorus* sp. and its prey *Tetranychus pacificus* McGregor. According to Raworth (2001), just 64% of *S. punctillum* larvae survived to pupation, for reasons unknown, in arenas containing spider mites (*T. urticae*) on strawberry leaf discs.

Although *S. punctillum* is commercially available (van Lenteren 2003), the cost of producing it is quite high, i.e. ranging from 30 to 50 USD per 100 adults (Applied Bionomics Ltd., <http://www.appliedbionomics.com>). Thus, there is a need for a more cost-effective rearing system.

In conclusion, this study highlights an inverse relationship between Lima bean hooked trichome density and survival of *S. punctillum* larvae. The lack of a curved, pointed tip and low density could explain, in part, the absence of negative effects of straight trichomes on *S. punctillum*. This study shows that hooked trichome-based resistance in Lima bean has a negative effect on a predacious coccinellid that attacks the two-spotted spider mite.

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## References

- Barbour JD, Farrar RR Jr, Kennedy GG (1997) Populations of predaceous natural enemies developing on insect-resistant and susceptible tomato in North Carolina. *Biol Control* 9:173–184
- Biddinger DJ (1993) Toxicity, stage specificity, and sublethal effects of abamectin and several classes of insect growth regulators to *Platynota idaeusalis* (Lepidoptera: Tortricidae) and *Stethorus punctum* (Coleoptera: Coccinellidae). PhD. Dissertation, The Pennsylvania State University
- Biddinger DJ, Weber DC, Hull LA (2009) Coccinellidae as predators of mites: Stethorini in biological control. *Biol Control* 51:268–283
- Bottrell DG, Barbosa P, Gould F (1998) Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annu Rev Entomol* 43:347–367
- Eisner T, Eisner M, Hoebeke ER (1998) When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proc Natl Acad Sci USA* 95:4410–4414
- Johnson B (1953) The injurious effects of the hooked epidermal hairs of French beans (*Phaseolus vulgaris* L.) on *Aphis craccivora* Koch. *Bull Entomol Res* 44:779–788
- Kavousi A, Chi H, Talebi K, Bandani A, Ashouri A, Naveh VH (2009) Demographic traits of *Tetranychus urticae* (Acari: Tetranychidae) on leaf discs and whole leaves. *J Econ Entomol* 102:595–601
- Lambert AM (2007) Effect of prey availability, facultative plant feeding, and plant defenses on a generalist insect predator. *Arthropod-Plant Interact* 1:167–173
- Levin DA (1973) The role of trichomes in plant defence. *Q Rev Biol* 48:3–15
- Loughner R, Goldman K, Loeb G, Nyrop J (2008) Influence of leaf trichomes on predatory mite (*Typhlodromus pyri*) abundance in grape varieties. *Exp Appl Acarol* 45:111–122
- Oku K, Yano S, Takafuji A (2006) Host plant acceptance by the phytophagous mite *Tetranychus kanzawai* Kishida is affected by the availability of a refuge on the leaf surface. *Ecol Res* 21:446–452
- Pérez-Estrada LB, Canto-Santan Z, Oyama K (2000) Variation in leaf trichomes of *Wigandia urens*: environmental factors and physiological consequence. *Tree Physiol* 20:629–632
- Pillemer EA, Tingey WM (1976) Hooked trichomes: a physical barrier to a major agricultural pest. *Science* 193:482–484
- Putman WL (1955) Bionomics of *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) in Ontario. *Can Entomol* 87:9–33
- Quiring DT, Timmins PR, Park SJ (1992) Effect of variations in hooked trichome densities of *Phaseolus vulgaris* on longevity of *Liriomyza trifolii* (Diptera: Agromyzidae) adults. *Environ Entomol* 21:1357–1361
- Raworth DA (2001) Development, larval voracity, and greenhouse releases of *Stethorus punctillum* (Coleoptera: Coccinellidae). *Can Entomol* 133:721–724
- Ricci C, Cappelletti G (1988) Relationship between some morphological structures and locomotion of *Clitostethus arcuata* Rossi (Coleoptera: Coccinellidae), a whitefly predator. *Frustr Entomol* 11:195–202
- Rott AS, Ponsonby DJ (2000a) The effects of temperature, relative humidity and host plant on the behaviour of *Stethorus punctillum* as a predator of the two-spotted spider mite, *Tetranychus urticae*. *BioControl* 45:155–164
- Rott AS, Ponsonby DJ (2000b) Improving the control of *Tetranychus urticae* on edible glasshouse crops using a specialist coccinellid (*Stethorus punctillum* Weise) and a generalist mite (*Amblyseius californicus* McGregor) as biocontrol agents. *Biocontrol Sci Technol* 10:487–498
- Scriven GT, Fleschner CA (1960) Insectary production of *Stethorus* species. *J Econ Entomol* 53:982–985
- Shah MA (1982) The influence of plant surfaces on the searching behaviour of coccinellid larvae. *Entomol Exp Appl* 31:377–380
- Valkama E, Salminen J-P, Koricheva J, Pihlaja K (2004) Changes in leaf trichomes and epicuticular flavonoids during leaf development in three birch taxa. *Ann Bot* 94:233–242



- van Lenteren JC (2003) Commercial availability of biological control agents. In: van Lenteren JC (ed) Quality control and production of biological control agents, theory and testing procedures. CABI Publishing, Oxon, pp 167–179
- Verheggen FJ, Capella Q, Schwartzberg EG, Voigt D, Hauruge E (2009) Tomato-aphid-hoverfly: a tritrophic interaction incompatible for pest management. *Arthropod-Plant Interact* 3:141–149
- Voigt D, Gorb E, Gorb S (2007) Plant surface–bug interactions: *Dicyphus errans* stalking along trichomes. *Arthropod-Plant Interact* 1:221–243
- Walters PJ (1974) A method for culturing *Stethorus* spp. (Coleoptera: Coccinellidae) on *Tetranychus urticae* (Koch) (Acarina: Tetranychidae). *J Austral Entomol Soc* 13:245–246
- Wilson LJ (1994) Plant-quality effect on life-history parameters of the two spotted spider mite (Acari: Tetranychidae) on cotton. *J Econ Entomol* 87:1665–1673
- Zhang Z-Q (2003) Mites of greenhouses: identification, biology and control. CABI Publication, Oxon