Does the aggressiveness of the prey modify the attack behavior of the predator *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae)?

Rafael Braga da Silva[1,3], Alberto Soares Corrêa[2], Terezinha Maria Castro Della Lucia[2],
Alexandre Igor Azevedo Pereira[2], Ivan Cruz[3] & José Cola Zanuncio[2]

[1]Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, 13565–905 São Carlos–SP, Brasil.
rafaelentomologia@yahoo.com.br
[2]Departamento de Biologia Animal, Universidade Federal de Viçosa, 36571–000 Viçosa–MG, Brasil. correaalbertos@gmail.com;
aiapereira@yahoo.com.br; tdlucia@ufv.br; zanuncio@ufv.br
[3]Embrapa Milho e Sorgo, 35701–970 Sete Lagoas–MG, Brasil. ivancruz@cnpms.embrapa.br

ABSTRACT. Does the aggressiveness of the prey modify the attack behavior of the predator *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae)? The stink bug *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae) is a predator found in several Brazilian regions, which possesses desirable attributes as a natural control agent and in biological control programs. The aim of this study was to test if the attack behavior and predation success of *S. cincticeps* were affected by prey species. Larvae of *Tenebrio molitor* (L.) (Coleoptera, Tenebrionidae), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), and *Thyrinteina arnobia* (Stoll) (Lepidoptera, Geometridae) were offered to *S. cincticeps* in laboratory bioassays where predatory attack and prey defensive behaviors were observed for 2-hour periods. The attack behavior of *S. cincticeps* changed with the prey species offered. More than 25% of *T. molitor* and *S. frugiperda* larvae were immediately attacked, but *T. arnobia* was not immediately attacked by *S. cincticeps*. Successful attack (i.e., successful insertion of the predator stylets into the prey) depends on the region of the body attacked, with a greater proportion of successful attacks in the anterior than in the median or posterior regions. Larvae of *T. arnobia* and *S. frugiperda* displayed a sequence of abrupt head and body movements in response to *S. cincticeps* attack. Attempts of predation were more successful on *T. molitor* and *S. frugiperda* than on *T. arnobia*. Information about the differential attack behavior of *S. cincticeps* on different prey species is important for designing successful biological control programs using this hemipteran predator.

KEYWORDS. Biological control; ethogram of predation; fall armyworm; stink bug predator.

RESUMO. A agressividade da presa altera o comportamento de ataque do predador *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae)? O percevejo *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae) é um predador encontrado em várias regiões brasileiras, que possui atributos desejáveis como agente de controle natural ou em programas de controle biológico. O objetivo deste trabalho foi testar se diferentes espécies de presa podem afetar o comportamento de ataque e o sucesso de predação de *S. cincticeps*. Larvas de *Tenebrio molitor* (L.) (Coleoptera, Tenebrionidae), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae) e *Thyrinteina arnobia* (Stoll) (Lepidoptera, Geometridae) foram oferecidas a *S. cincticeps,* em bioensaios de laboratório, onde o comportamento de ataque do predador e o comportamento de defesa das presas foram observados por período de duas horas. O comportamento de ataque de *S. cincticeps* às presas apresentou variações nas frequências dos atos predatórios. O sucesso da inserção dos estiletes na presa depende da parte do corpo que é atacada (anterior, mediana ou posterior), apresentando maior sucesso quando o ataque ocorre na região anterior. As larvas de *T. arnobia* e *S. frugiperda* apresentaram maior frequência de movimentos bruscos quando atacadas por *S. cincticeps*, enquanto as de *T. molitor* não exibiram tais reações. Consequentemente, o sucesso da predação de *S. cincticeps* foi maior sobre *T. molitor* e *S. frugiperda* e consideravelmente menor em *T. arnobia*. Essas informações sobre as diferenças no comportamento de ataque de *S. cincticeps* a diferentes presas são úteis para os programas de controle biológico envolvendo hemípteros predadores.

PALAVRAS-CHAVE. Controle biológico; etograma de predação; lagarta–do–cartucho; percevejo predador.

The predacious stink bug *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae) is commonly found in natural and agricultural areas of the Neotropical region (Zanuncio *et al.* 2005). This predator feeds on insects in various orders, such as Coleoptera, Lepidoptera and Diptera. The distribution and feeding habits of *S. cincticeps* are desirable attributes for a potential agent for natural and applied biological control in integrated pest management (Zanuncio *et al.* 1998, 2005). While reproductive and morphological aspects of *S. cincticeps* have been reported in the literature (Azevedo & Ramalho 1999; Zanuncio *et al.* 2004, 2005), behavioral as-

pects of predator-prey interactions have been poorly studied.

Caterpillars exhibit a wide array of natural defenses against predators and parasitoids (Edmunds 1974). These defense mechanisms may be behavioral, morphological or physiological, moreover the defensive efficacy varies with the predator species (Gentry & Dyer 2002). Likewise, predators are exposed to risk during their attack on prey and have strategies to minimize the defensive actions of their prey (Edmunds 1974). Wignall & Taylor (2009) observed that the predator *Stenolemus bituberus* (Stål) (Hemiptera, Reduviidae) modifies its attack behavior according to the prey spe-

cies. The hypothesis is that the predator is able to assess the potential risk provided by its prey, and may in some situations give up their attack and abandon its prey.

Population density, the size and defensive tactics of prey and predator species, and attack techniques directly affect predation success (Azevedo & Ramalho 1999; Jeschke & Tollrian 2000; Al–Zyould & Sengonca 2004; Hajer & Hrubá 2007). The success of predation affects many aspects of the life history of predatory Hemiptera, including the reduction of feeding time and predation risk at early instars, as well as survival and fecundity (Grundy & Maelzer 2000; Matlock 2005; Zanuncio *et al.* 2005).

Therefore, predation success is affected by the extent of prey defense and predator attack strategies and tactics (Edmunds 1974; Gentry & Dyer 2002; Al–Zyould & Sengonca 2004; Wignall & Taylor 2009). The aim of this study was to describe the predatory attack behavior of adult *S. cincticeps* and the defensive behavior of larvae of three Lepidoptera species. We evaluated whether the predator modifies its attack behavior pattern according to the prey species.

MATERIAL AND METHODS

Insects used in the study were obtained from laboratory colonies and field collections. Adults of *S. cincticeps* used in the bioassays were obtained from a mass rearing unit at the Insect Biological Control Laboratory (BIOAGRO) of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil. The prey species used in the tests were *Tenebrio molitor* (L.) (Coleoptera, Tenebrionidae), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), and *Thyrinteina arnobia* (Stoll) (Lepidoptera, Geometridae). Larvae of *Spodoptera frugiperda* and *T. arnobia* are common preys of *S. cincticeps* in natural and agricultural environments (Zanuncio *et al.* 1998, 2004). Larvae of *T. molitor* were obtained from colonies at BIOAGRO. This prey species presents low defensive capability and was used as the standard control for the attack behavior of *S. cincticeps*. Larvae of *Spodoptera frugiperda* is the main pest in Brazilian maize crops (Cruz *et al.* 1999; Batista–Pereira *et al.* 2006); they were obtained from colonies maintained at the Insect Rearing Laboratory (LACRI) of the Brazilian Agricultural Research Corporation (EMBRAPA Maize and Sorghum) in Sete Lagoas, Minas Gerais State, Brazil. Larvae of *T. arnobia*, which is an important pest of eucalyptus in Brazil (Batista–Pereira *et al.* 2004), were collected from guava trees at the UFV campus.

Ninety virgin females of *S. cincticeps* (< 3 days-old) were placed individually in Petri dishes (14.5 cm X 1.5 cm) for 24 hours without food, but offered a cotton ball soaked in water for hydration. After this period, the cotton ball was removed and offered one specimen of fourth instar larva (2–3 cm length) of a prey species. The attack behavior of each predator and the defense behavior of the prey were visually observed and characterized over a two-hour period according to methods adapted from Lemos *et al.* (2005) (Fig. 1). Predator behavior in the bioassays was recorded as follows: (*a*)

identification of prey, the predator moves in a circle around the prey; (*b*) approximation of the prey, the predator moves towards the prey with the antennae forward V–shaped; (*c1*) the predator not execute the attack after initial contact with the prey, and it may touch the prey with its antennae and legs; (*c2*) immediate attack, the predator moves toward the prey with the stylet extended forward; (*d*) insertion of the mandibular stylet in the anterior, median or posterior region (third) of the prey; (*e1*) the predator continues the attack after the defense reaction of the prey (successful predation); (*e2*) release of the prey, the predator interrupts the attack after the defense reaction of the prey (unsuccessful predation). Behavior specific to the Lepidoptera larvae were recorded as follows: (*f1*) prey defense, abrupt and constant movement of the body and head; (*f2*) prey defense, slow and sparse movements of body and head; (*g*) the larva stays immobile resembles a stick (branch), provably decreasing the stimulation of predation of the predator (not shown in Fig. 1). The predation was recorded as successful when the prey was unable to move when repeatedly probed. The region of the prey body into which *S. cincticeps* inserted their mouthparts was recorded as anterior, median or posterior.
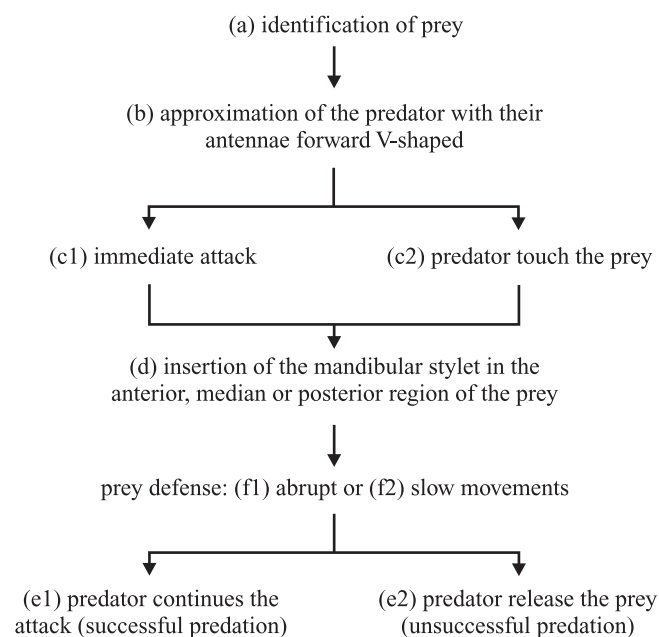

Fig. 1. Observed sequence of behavioral actions during two hours of predation of the stink bug *S. cincticeps* on *T. molitor*, *S. frugiperda*, and *T. arnobia*.

Behavioral bioassays were individually carried out between 10:00 and 17:00 h in a room with artificial, incandescent light and average temperature of 25 ± 3°C. The experimental design was completely randomized with 30 replicates for each predator-prey interaction (*i.e.*, total of 90 adults of *S. cincticeps*). The binomial data of successful predation and frequencies of predatory and defensive behavior were submitted to regression analysis with binomial distribution with logit link function (SAS Institute 2002).

RESULTS

The behavioral actions *c1* and *c2* of *S. cincticeps* (Fig. 1) differed significantly according to the prey species (likelihood ratio, $\chi^2 = 7.64$; d.f. = 2; p = 0.02). More than 25% of *T. molitor* and *S. frugiperda* larvae were immediately attacked, but none of the *T. arnobia* was immediately attacked by *S. cincticeps* (Fig. 2a). The success of insertion of the stylets (attack success) did not vary with the prey species (likelihood ratio, $\chi^2 = 0.24$; d.f = 2; p = 0.88) and there was no interaction among prey species and body part attacked (anterior, median and posterior region) (likelihood ratio, $\chi^2 = 1.37$; d.f. = 2; p = 0.85). However, there was significant difference in the attack success based on the prey body part attacked (likelihood ratio, $\chi^2 = 9.00$; d.f. = 2; p = 0.01). Attacks to the anterior region of the prey resulted in a greater rate of success, compared to those in the median and posterior regions (Fig. 2b).

hood ratio, $\chi^2 = 13.47$; d.f. = 2; p < 0.01). While > 90% of *T. arnobia* and *S. frugiperda* larvae exhibited abrupt movements of the body and head in response to attack by *S. cincticeps*, only 30% of *T. molitor* larvae exhibited these defensive movements (Fig. 3). Conversely, the specific behavior of mimicry (*g*) was observed in 10% of *T. arnobia* larvae, but none of the *T. arnobia* and *S. frugiperda* larvae exhibited such behavior. Mimicry was an effective defense, because *S. cincticeps* did not attack *T. arnobia* larvae that remained motionless during the 2-hour observation period.

Fig. 3. Proportion of *T. molitor*, *S. frugiperda* and *T. arnobia* larvae (n = 30 per species) that displayed abrupt and constant movements of the body and head in response to attack by *S. cincticeps*. Proportions grouped by the same letter are not significantly different (chi-square test, p < 0.05).
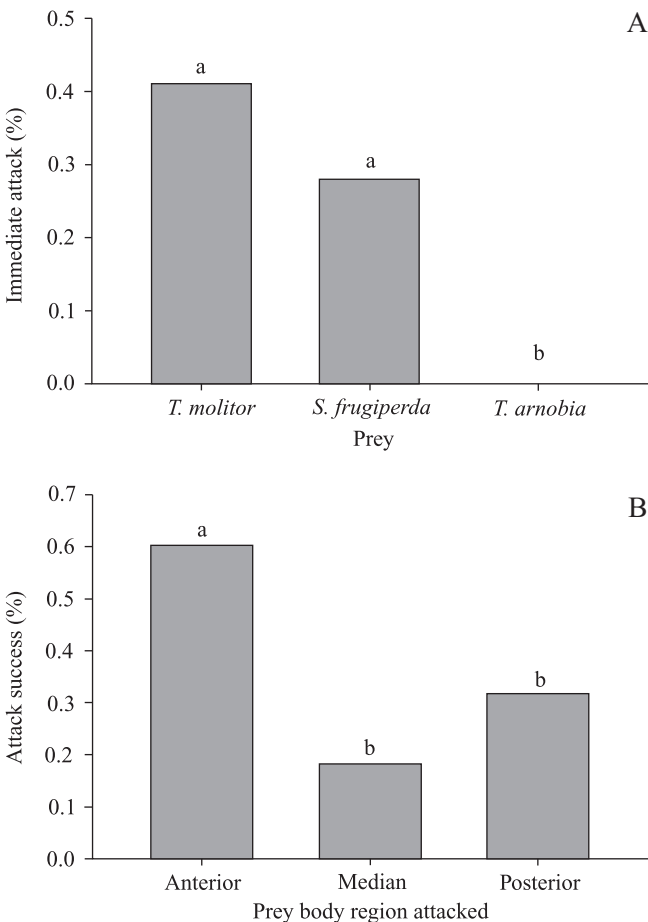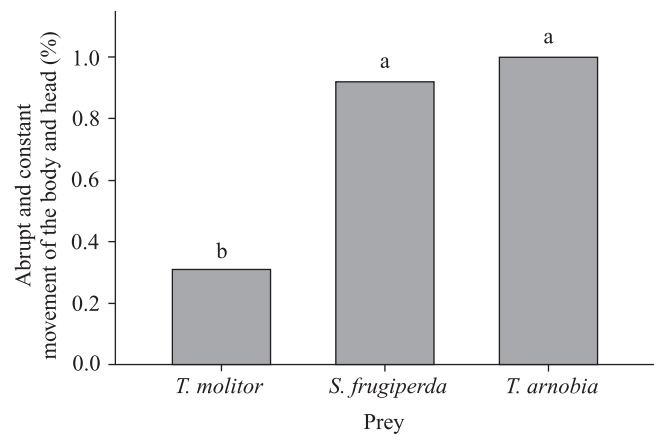

Fig. 2. (A) Proportion of larvae that were immediately attacked by *S. cincticeps* and (B) attack success of *S. cincticeps* on *T. molitor*, *S. frugiperda* and *T. arnobia* larvae (n = 30 per species). Proportions grouped by the same letter are not significantly different (chi-square test, p < 0.05).

The intensity of defensive behavior (slow or abrupt movements) significantly differed among the prey species (likeli-

Successful predation by *S. cincticeps* also varied according to the prey species (likelihood ratio, $\chi^2 = 7.78$, d.f. = 2, p = 0.02). More than 60% of larvae of *T. molitor* and *S. frugiperda* were successfully attacked, and only about 40% of *T. arnobia* (Fig. 4).
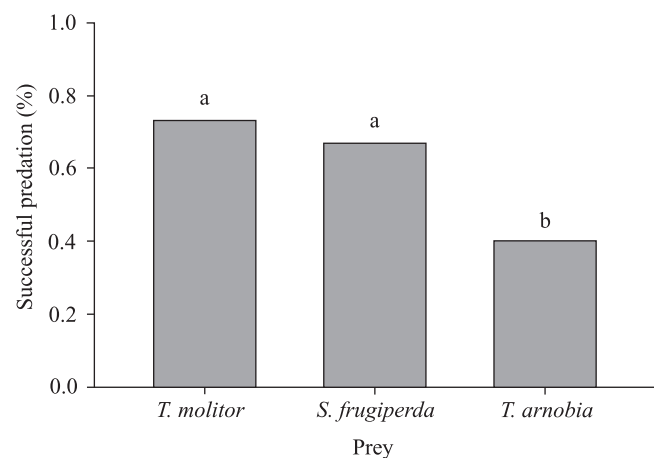

Fig. 4. Proportion of successful predation on *T. molitor*, *S. frugiperda* and *T. arnobia* larvae (n = 30 per species) by *S. cincticeps*. Proportions grouped by the same letter are not significantly different (chi-square test, p < 0.05).

DISCUSSION

The preys exhibit different responses to the attack by predators and parasitoids. They may hide, flee, bite, regurgitate compounds causing injury and even kill the predator (Edmunds 1974; Gentry & Dyer 2002; Lemos *et al.* 2005). Each species of prey can modify the attack behavior affecting the success of predation by hemipteran predators (De Clercq *et al.* 1998; Lemos *et al.* 2003; Wignall & Taylor 2009), as demonstrated in this study with *S. cincticeps* and the preys *T. molitor*, *S. frugiperda* and *T. arnobia*.

The approaching behavior of *S. cincticeps,* with the antennae forward, suggests a visual and chemical recognition of the prey as observed for *Podisus rostralis* (Stål) (Hemiptera, Pentatomidae) during the attack on *Bombix mori* L. (Lepidoptera, Bombycidae) (Lemos *et al.* 2005). Additionally, *S. cincticeps* touch their prey with their antennae and legs, supporting the idea that predatory stink bugs also use tactile clues in prey selection. Similar observations have been made by others to support the conclusion that predators may use tactile (Lemos *et al.* 2005), visual (Haynes *et al.* 2002; Nakamatsu & Tanaka 2005) and chemical clues (Haynes *et al.* 2002; Mendel *et al.* 2004; Mochizuki & Yano 2007) to locate and recognize their prey. We noticed that initial recognition of the prey may directly influence the attack behavior of *S. cincticeps*, suggesting that it is able to adapt its behavior according to the prey being offered (Wignall & Taylor 2009). None of the larvae *T. arnobia* larvae was immediately attacked compared to 26% of *S. frugiperda* and 40% of *T. molitor*.

Mimicry of various colorations, behaviors, and morphologies is a primary defensive tactic that can prevent natural enemies from detecting their prey (Lev–Yadun & Inbar 2002; Gentry & Dyer 2002). Despite the simplified experimental environment provided in our bioassay, which makes this strategy less effective, the mimetic behavior of *T. arnobia* to resemble a stick was very effective against the predator *S. cincticeps*. The caterpillars that maintained the defensive behavior were not attacked.

This trial further demonstrated that successful predation can be affected by the region of the body attacked. The success of the attack by *S. cincticeps* depends on the body region where the stylets are inserted into the prey. The most efficient attacks occurred when the predator inserted its stylets in the anterior region of the body of the preys, avoiding the bites of the preyed larvae, as mentioned by Lemos *et al.* (2005). However, when the predator attacks the preys in the median and posterior regions, it became vulnerable to the bites and aggressiveness of the prey. The parasitoid *Euplectrus separatae* Kamijo (Hymenoptera, Eulophidae) oviposits in areas of the body of the prey *Pseudaletia separata* (Walk.) (Lepidoptera, Noctuidae), which are less susceptible to defense reaction, thereby avoiding the removal of eggs and young stages of the parasitoid (Nakamatsu & Tanaka 2005).

Defensive responses by the prey were triggered when *S. cincticeps* partially inserted their stylets into the prey. Larvae of *Trichoplusia ni* (Hübner) (Lepidoptera, Noctuidae) exhibit low aggressiveness; therefore, they are often successfully attacked by predators (Marston *et al.* 1978). Larvae of *B. mori* are not commonly predated because they frenetically move their head and body in response to attack (Lemos *et al.* 2005). We observed a similar defense behavior for *T. arnobia* and *S. frugiperda*, what reduced their predation compared to *T. molitor*, which was a calmer larvae. Abrupt movements of the body and head by the prey often resulted in the interruption of the attack by *S. cincticeps*, demonstrating the importance of defensive behavior of the prey to the success of predation (Azevedo & Ramalho 1999; Lemos *et al.* 2005; Matlock 2005). This behavior was observed for the larva of *T. arnobia*, which increases the body movements and enhances the attack with the mandibles, because it has a more flexible body and, in turn, suffers less attacks by the predator. After inserting the mandibular stylets, *S. cincticeps* injects enzymes and substances to paralyze its prey and start extra-oral digestion of its hemocel (Cohen 1995; Oliveira *et al.* 2006).

Our study demonstrates that *S. cincticeps* attack behavior can be modified according to the species predated and the success depends on its strategy to avoid aggressive reactions of the prey (Gentry & Dyer 2002). Differences in the frequency and the absence of some behavioral actions by *S. cincticeps* demonstrate that this predator can adapt its attack behavior according to the prey species. Understanding the attack behavior of *S. cincticeps* and the response of different prey species is fundamental to guarantee the success of biological control programs using this predator species.

REFERENCES

Al–Zyould, F. & C. Sengonca. 2004. Prey consumption preferences of *Serangium parcesetosum* Sicard (Col., Coccinellidae) for different prey stages, species and parasitized prey. **Journal of Pest Science 77**: 197–204.

Azevedo, F. R. de & F. de S. Ramalho. 1999. Efeitos da temperatura e da defesa da presa no consumo pelo predador *Supputius cincticeps* (Stål) (Heteroptera: Pentatomidae). **Pesquisa Agropecuária Brasileira 34**: 165–171.

Batista–Pereira, L. G.; C. F. Wilcken; S. D. Pereira Neto & E. N. Marques. 2004. Comportamento de chamamento de *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae) em *Psidium guajava*, *Eucalyptus grandis* e em Dieta Artificial. **Neotropical Entomology 33**: 21–28.

Batista–Pereira, L. G.; K. Stein; A. F. Paula; J. A. Moreira; I. Cruz; M. L. C. Figueiredo; J. Perri & A. G. Corrêa. 2006. Isolation, identification, synthesis, and field evaluation of the sex pheromone of the Brazilian population of *Spodoptera frugiperda*. **Journal of Chemical Ecology 32**: 1085–1099.

Cruz, I.; M. L. C. Figueiredo; A. C. Oliveira & C. A. Vasconcelos. 1999. Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. **International Journal of Pest Management 45**: 293–296.

Cohen, A. C. 1995. Extra-oral digestion in predaceous terrestrial Arthropoda. **Annual Review of Entomology 40**: 85–103

De Clercq, P.; F. Merlevede & L. Tirry. 1998. Unnatural prey and artificial diets for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). **Biological Control 12**: 137–142.

Edmunds, M. 1974. **Defence in Animals: a survey of anti-predator defences.** Harlow, Longman, 357 p.

Gentry, G. L. & L. A. Dyer. 2002. On the conditional nature of neotropical caterpillar defenses against their natural enemies. **Ecology 83**: 3108–3119.

Grundy, P. & D. Maelzer. 2000. Predation by the assassin bug *Pristhesancus plagipennis* (Walker) (Hemiptera: Reduviidae) of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in the laboratory. **Australian Journal of Entomology 39**: 280–282.

Hajer, J. & L. Hrubá. 2007. Wrap attack of the spider *Achaearanea tepidariorum* (Araneae: Theridiidae) by preying on mealybugs *Planococcus citri* (Homoptera: Pseudococcidae). **Journal of Ethology 25**: 9–20.

Haynes, K. F.; C. Gemeno; K. V. Yeargan; J. G. Millar & K. M. Johnson. 2002. Aggressive chemical mimicry of moth pheromones by a bolas spider: how does this specialist predator attract more than one species of prey? **Chemoecology 12**: 99–105.

Jeschke, J. M. & R. Tollrian. 2000. Density–dependent effects of prey defences. **Oecologia 123**: 391–396.

Lemos, W. P.; F. S. Ramalho; J. E. Serrão & J. C. Zanuncio. 2003. Effects of diet on development of *Podisus nigrispinus* (Dallas) (Het., Pentatomidae), a predator of the cotton leafworm. **Journal of Applied Entomology 127**: 389–395.

Lemos, W. P.; J. C. Zanuncio & J. E. Serrão. 2005. Attack behavior of *Podisus rostralis* (Heteroptera: Pentatomidae) adults on caterpillars of *Bombyx mori* (Lepidoptera: Bombycidae). **Brazilian Archives of Biology and Technology 48**: 975–981.

Lev–Yadun, S. & M. Inbar. 2002. Defensive ant, aphid and caterpillar mimicry in plants? **Biological Journal of the Linnean Society 77**: 393–398.

Marston, N. L.; G. T. Schmidt; K. D. Biever & W. A. Dickerson. 1978. Reaction of five species of soybean caterpillars to attack, by the predator, *Podisus maculiventris*. **Environmental Entomology 7**: 53–56.

Matlock, R. B. 2005. Impact of prey size on prey capture success, development rate, and survivorship in *Perillus bioculatus* (Heteroptera: Pentatomidae), a predator of the Colorado potato beetle. **Environmental Entomology 34**: 1048–1056.

Mendel, Z.; F. Assael & E. Dunkelblum. 2004. Kairomonal attraction of predatory bugs (Heteroptera: Anthocoridae) and brown lacewings (Neuroptera: Hemerobiidae) to sex pheromones of *Matsucoccus* species (Hemiptera: Matsucoccidae). **Biological Control 30**: 134–140.

Mochizuki, M. & E. Yano. 2007. Olfactory response of the anthocorid predatory bug *Orius sauteri* to thrips–infested eggplants. **Entomologia Experimentalis et Applicata 123**: 57–62.

Nakamatsu, Y. & T. Tanaka. 2005. How does the ectoparasitoid wasp *Euplectrus separatae* (Hymenoptera: Eulophidae) recognize a suitable oviposition site on the host larva *Pseudaletia separata* (Lepidoptera: Noctuidae)? **Applied Entomology and Zoology 40**: 185–191.

Oliveira, J. A.; M. G. A. Oliveira; R. N. C. Guedes & M. J. Soares. 2006. Morphology and preliminary enzyme characterization of the salivary glands from the predatory bug *Podisus nigrispinus* (Heteroptera: Pentatomidae). **Bulletin of Entomological Research 96**: 251–258.

SAS Institute, 2002. SAS/STAT User's Guide, Version 9.1. SAS Institute.

Wignall, A. E. & P. W. Taylor. 2009. Alternative predatory tactics of an araneophagic assassin bug (*Stenolemus bituberus*). **Acta Ethologica 12**: 23–27.

Zanuncio, J. C.; V. C. Batalha; R. N. C. Guedes & M. C. Picanço. 1998. Insecticide selectivity to *Supputius cincticeps* (Stal) (Het., Pentatomidae) and its prey *Spodoptera frugiperda* (J. E. Smith) (Lep., Noctuidae). **Journal of Applied Entomology 122**: 457–460.

Zanuncio, J. C.; M. C. Lacerda; J. S. Zanuncio Junior; T. V. Zanuncio; A. M. C. da Silva & M. C. Espindula. 2004. Fertility table and rate of population growth of the predator *Supputius cincticeps* (Heteroptera: Pentatomidae) on one plant of *Eucalyptus cloeziana* in the field. **Annals of Applied Biology 144**: 357–361.

Zanuncio, J. C.; E. B. Beserra; A. J. Molina–Rugama; T. V. Zanuncio; T. B. M. Pinon & V. P. Maffia. 2005. Reproduction and longevity of *Supputius cincticeps* (Het.: Pentatomidae) fed with larvae of *Zophobas confusa*, *Tenebrio molitor* (Col.: Tenebrionidae) or *Musca domestica* (Dip.: Muscidae). **Brazilian Archives of Biology and Technology 48**: 771–777.