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PREDATION BY *PODISUS MACULIVENTRIS* ON DIFFERENT LIFE STAGES OF *NEZARA VIRIDULA*

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Abstract

Predation capacity of fourth instars and male and female adults of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on the different life stages of the southern green stinkbug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) was measured in the laboratory. Both nymphal and adult predators displayed high predation rates on eggs, nymphs and adults of the southern green stinkbug. However, developmental times of fourth instar *P. maculiventris* on eggs or nymphal instars of *N. viridula* were longer than on fifth instars of the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), suggesting that *N. viridula* is suboptimal prey for the spined soldier bug. Preference experiments in which fourth instar *P. maculiventris* were given a choice between fourth instars of *N. viridula* and fifth instars of *S. littoralis* indicated that the stinkbugs were less vulnerable to predation than the caterpillars, mainly because of their greater agility. The potential role of *P. maculiventris* in augmentation or conservation biological control of the southern green stinkbug in greenhouse and field crops is discussed.

Key Words: *Nezara viridula, Podisus maculiventris*, Asopinae, predator, biological control

Resumen

La capacidad de depredación de cuartos instares y macho y hembra adultos de *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) sobre los diferentes estados del chinche verde hediondo sureño *Nezara viridula* (L.) (Heteroptera: Pentatomidae) fue medida en el laboratorio. Ambos depredadores de ninfas como de adultos demostraron alta capacidad de depredación sobre huevos, ninfas y adultos del chinche verde sureño. Sin embargo, los tiempos de desarrollos de los cuartos instares del *P. maculiventris* sobre los huevos o los instares ninfales de *N. viridula* fueron mayores que sobre los quintos instares de la rosquilla negra, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), sugiriendo que *N. viridula* es una presa poco optima para el chinche soldado. Experimentos de preferencia en donde a cuartos instares de *P. maculiventris* les fue dada la oportunidad de seleccionar entre cuartos instares de *N. viridula* y quintos instares de *S. littoralis* indicaron que los chinches fueron menos vulnerables a depredación que las larvas, básicamente debido a su gran agilidad. El papel potencial de *P. maculiventris* en el control biológico aumentativo o de conservación del chinche verde hediondo sureño en invernaderos y cultivos esta en discusión.

The southern green stinkbug, *Nezara viridula* (L.), has a worldwide distribution occurring throughout the tropical and subtropical regions of the Americas, Africa, Asia, Australasia and Europe (Todd 1989, Panizzi et al. 2000). In Europe, the growing number of reports in the North of France and Belgium during the 1990s indicates that this pentatomid is expanding its distribution northwards (Gallant 1996). The expansion of this pest in Europe is presumably related in part to its association with a number of greenhouse crops. In North America, the bug is distributed throughout the Southeast and in California it has been found increasingly since the mid 1980s (Hoffmann et al. 1991). The bug's expansion in South America is related to the increased acreage for soybean production (Panizzi et al. 2000).

Nezara viridula is a highly polyphagous pest attacking both monocots and dicots, but it appears to have a preference for leguminous plants (Todd 1989, Panizzi et al. 2000). Important economic damage is caused worldwide to field crops including soybean, rice, corn, cotton, and tobacco and to a number of vegetable crops like tomato, sweet pepper and eggplant, both in the field and in greenhouses. Control of this bug is largely based upon the use of conventional pesticides, including a number of carbamates, organophosphates and some pyrethroids (Jackai et al. 1990, Ballenger & Jouffret 1997, Panizzi et al. 2000). Nonetheless, in several regions efforts have been made to use biological control to suppress outbreaks of the southern green stinkbug. Many attempts have been made to establish parasitoids into newly invaded areas. The most important of these are the scelionid egg parasitoid *Trissolcus basalis* (Wollaston) and tachinid parasitoids belonging to the genus *Trichopoda*, that attack large nymphs and adults of *N. viridula* (Todd 1989, Corrêa-Ferreira & Moscardi 1996, Panizzi et al. 2000). Although predation is recognized to be an important mortality factor for *N. viridula* (see Todd 1989 for a review), much less attention has been given to the biocontrol potential of the pest's predator complex.

The present study investigates the predation capacity of the spined soldier bug, *Podisus maculiventris* (Say), on *N. viridula*. This generalist predator, belonging to the pentatomid subfamily Asopinae, has been used since 1997 in European greenhouses for the augmentative biological control of caterpillar outbreaks (De Clercq et al. 1998, De Clercq 2000) and predation on *N. viridula* may be an additional asset for this beneficial. In the Southern United States, *P. maculiventris* is a common predator of lepidopterous and coleopterous insects in various crops (Richman & Mead 1980) but it has also been observed to prey on *N. viridula* (Jones 1918, Drake 1920, Underhill 1934, Ragsdale et al. 1981, Stam et al. 1987). However, the ability of *P. maculiventris* to prey upon the southern green stinkbug is largely unknown. The objective of this study was to quantify consumption of the different life stages of *N. viridula* by nymphs and adults of *P. maculiventris* under laboratory conditions. In addition, prey preference of nymphal predators was tested by giving them a choice between nymphs of *N. viridula* and larvae of the cotton leafworm, *Spodoptera littoralis* (Boisduval).

Materials and Methods

Insects

A laboratory colony of *N. viridula* was established in 1999, using insects originating from different field collections in France, Spain and Italy. Stinkbugs were fed on pods of green bean (*Phaseolus vulgaris* L.) and on seed kernels of sunflower (*Helianthus annuus* L.). A culture of *P. maculiventris* was started in 1999, using specimens originating from a field collection in 1996 near Beltsville, MD. The predators were fed mainly larvae of the greater wax moth, *Galleria mellonella* L., and of the cotton leafworm, *S. littoralis*, but were occasionally also provided with larvae of the yellow mealworm, *Tenebrio molitor* L. Cotton leafworms were reared on an artificial diet modified from Poitout & Bues (1970). Colonies of all insects were maintained in growth chambers at 23 ± 1°C, 75 ± 5% relative humidity and a 16:8 (L:D) photoperiod.

Consumption Experiments

Predation by fourth instars and female and male adults of *P. maculiventris* on the different life stages of the southern green stinkbug was measured in petri dishes. Predator nymphs were newly (<12 h) molted, adults were 3-5 days old and were starved for 24 h before testing. Predators were placed in individual disk-vented petri dishes (9 cm diameter, 2 cm high) lined with paper towelling. For nymphs, each predator-prey combination was replicated 20 times; for adults, 10 replicates were done for each sex and prey stage. Predator individuals were offered either 1 batch of eggs (with an average of 80 eggs per batch), 15 first instars, 8 second instars, 6 third instars, 6 fourth instars, 4 fifth instars or 2 adults (one male and one female) of *N. viridula* per day. A slice of green bean and 5 sunflower seed kernels were supplied as food to nymphal or adult prey. Predators were supplied with moisture via a soaked paper plug fitted into an Eppendorf centrifugation tube. Predation by *P. maculiventris* adults was recorded after 24 h. Predation by males and females was compared using the non-parametric Mann-Whitney test. For nymphs, predation was measured throughout the stadium. Dead prey were replaced every day to keep prey density constant throughout the experiment. Two-day-old nymphal prey were replaced to minimize variability in prey size and behavior. In addition to predation rates, developmental durations of predator nymphs were recorded in each experiment.

A control group of 20 *P. maculiventris* fourth instars was presented with late instars of the noctuid *S. littoralis*, which can be considered optimal prey for this pentatomid (De Clercq 2000). Individual predators were offered 5 fifth instar cotton leafworms per day. A slice of green bean and a source of free water were also supplied. Dead prey and prey over two days old were replaced as above. Predation rates and developmental durations of predator nymphs were recorded. Developmental times of *P. maculiventris* fourth instars were compared among diets using a non-parametric Kruskal-Wallis analysis of variance and multiple comparison tests. The experiment-wise probability of type I error was controlled by the Bonferroni method.

Preference Experiment

To investigate the prey preference of *P. maculiventris*, fourth instars of the predator were given a choice between caterpillars of *S. littoralis* and nymphs of *N. viridula*. Twenty *P. maculiventris* fourth instars were placed singly in 14 cm diameter petri dishes, lined with absorbent paper. They were presented with 2 fifth instars of *S. littoralis* and 2 fourth instars of *N. viridula*. Predator nymphs were newly (12-24 h) molted and had not fed before the experiment; prey insects were about one day old. Slices of green bean were added to provide food for the prey and moisture for the predators. Interactions between predator and prey were monitored 5, 15, 30, 45 and 60 min after the start of the experiment. In addition, survival of the prey was recorded after 24 h.

Results

Consumption Experiments

All life stages of *N. viridula* were readily attacked by nymphal *P. maculiventris* (Table 1). Fourth instars of the predator consumed on average 9 eggs, 20 first instars, 9 second instars, 3 third instars, 2.5 fourth instars, 2 fifth instars or 1 adult of the southern green stink bug during the total stadium. Male and female adults of *N. viridula* were similarly attacked: 58% of the adults killed were males. Survival of the predator nymphs was 90-100% in all treatments. Developmental times indicate, however, that eggs and nymphs of *N. viridula* were suboptimal prey for *P. maculiventris* (Table 1). Fourth instars took 1.5-3 days longer ($H = 66.19$, df = 7, $P < 0.001$; Kruskal-Wallis ANOVA) to reach the next stadium on these prey stages than on fifth-instar caterpillars of *S. littoralis*. Fourth-instar *P. maculiventris* killed on average 6 fifth-instar cotton leafworms during the total stadium.

Predation rates of adult *P. maculiventris* on different life stages of the southern green stinkbug are reported in Table 2. Whereas females of the predator did not accept eggs of *N. viridula* as food, males consumed about 4 eggs over a 24-h period. Predation rates on nymphal instars and adults did not differ ($P > 0.05$; Mann-Whitney tests) between male and female *P. maculiventris*. Adult predators killed on average 3.5-7.5 first instars, 2.5 second instars, 3.5 third instars, 1.5 fourth instars, 0.7 fifth instars or 0.5 adults of the pest per day.

Preference Experiment

Three out of 20 predator nymphs did not attack prey within the first 5 min of the experiment and had not done so by the end of the 60-minute observation period (Fig. 1). Seven out of the remaining 17 individuals (41%) directed their first attack towards *N. viridula*. However, after 30 min none of these predators had succeeded in capturing a *N. viridula* nymph. After 1 h, four out of these seven individuals had successfully attacked a larva of *S.littoralis*, whereas the remaining three had not resumed attacking prey. All tested predators had killed at least one prey item after 24 h. Overall, 72.5% of the *S. littoralis* larvae offered had been killed after 24 h versus only 5% of the *N. viridula* nymphs.

Discussion

Although the different life stages of *N. viridula* were attacked by fourth instars and adults of *P. maculiventris*, our results indicate that the southern green stinkbug is not optimal prey for the spined soldier bug. Developmental rate of fourth-instar *P. maculiventris* on adult *N. viridula* was similar to that on late-instar cotton leafworms, which can be considered optimal food for development of the predator (De Clercq 2000), but when eggs or nymphs of different instars were provided, developmental rates were lower than on caterpillar prey. Slower development on eggs and nymphs of *N. viridula* than on fifth-instar *S. littoralis* is believed to be related mainly to size and mobility of the prey. Predators had difficulty detecting and feeding on eggs and early instars of *N. viridula*. Feeding on lepidopteran prey of small size has also been found to prolong development in *P. maculiventris* (De Clercq & Degheele 1994, De Clercq et al. 1998). Further, the predators had to spend more time capturing the more agile late instars of *N. viridula* than they did to kill a larva of *S. littoralis*. When *P. maculiventris* was given a choice between *N. viridula* nymphs and *S. littoralis* larvae, nearly half of the first attacks were directed against *N. viridula*. However, nymphs of the southern green stinkbug reacted with agility when the predator positioned its rostrum onto the prey body and tried to insert its stylets, and they

Table 1. Predation rates and developmental times of fourth instar *P. maculiventris* offered different life stages of *N. viridula* or fifth instars of *S. littoralis* (Means ± SE, n = 20)

Prey	Predation rate[a]	Developmental time (days)[b]
S. littoralis fifth instar	5.8 ± 0.4	4.55 ± 0.13 a
N. viridula egg	8.9 ± 1.1	6.30 ± 0.27 c
N. viridula first instar	20.5 ± 1.5	5.95 ± 0.20 bc
N. viridula second instar	9.3 ± 0.5	7.75 ± 0.27 d
N. viridula third instar	3.3 ± 0.3	6.00 ± 0.29 bc
N. viridula fourth instar	2.4 ± 0.3	6.50 ± 0.30 cd
N. viridula fifth instar	2.0 ± 0.2	7.37 ± 0.39 d
N. viridula adult	1.0 ± 0.1	5.22 ± 0.21 ab

[a]Mean number of prey killed during the total stadium.
[b]Means within a column followed by the same letter are not significantly different ($P > 0.05$, Kruskal-Wallis test with Bonferroni correction).

TABLE 2. PREDATION RATES BY MALE AND FEMALE ADULTS OF *P. MACULIVENTRIS* ON DIFFERENT LIFE STAGES OF *N. VIRIDULA* (MEANS ± SE, N = 10)

N. viridula stage	Predation rate[a]	
	Male	Female
Egg	4.2 ± 1.2 a	0.0 ± 0.0 b
First instar	3.4 ± 0.7 a	7.6 ± 1.6 a
Second instar	2.4 ± 0.6 a	2.4 ± 0.9 a
Third instar	3.3 ± 0.4 a	3.6 ± 0.5 a
Fourth instar	1.6 ± 0.4 a	1.6 ± 0.2 a
Fifth instar	0.7 ± 0.2 a	0.6 ± 0.2 a
Adult	0.6 ± 0.2 a	0.3 ± 0.1 a

[a]Mean number of prey killed per day; means within a row followed by the same letter are not significantly different $P > 0.05$, Mann-Whitney test)

often managed to escape. Predators were sometimes seen pursuing an escaping nymph of *N. viridula* with extended rostrum but usually they quickly gave up the chase. When encountering a larva of *S. littoralis* in the process, the attention was easily diverted to the caterpillar. Although fifth-instar cotton leafworms reacted by vigorous thrashing movements when the predator inserted its stylets into the prey body, most of them did not succeed to escape from the hold of the predator once the stylets were firmly fixed. These observations indicate that more caterpillars than stinkbug nymphs were killed in choice tests basically because of their greater vulnerability.

Prey preferences and predation rates observed in this study may have been biased by the fact that stock colonies of *P. maculiventris* were maintained mainly on lepidopteran larvae. Selective adaptation to the food received during culturing cannot be ruled out and may have influenced the outcome of the experiments. Nonetheless, although the majority of Asopinae are highly polyphagous, reviews of the literature suggest that they have a preference for slow-moving, soft-bodied insects, primarily larval forms of Lepidoptera, Coleoptera, and Hymenoptera (McPherson 1980, Schaefer 1996, De Clercq 2000). Most predatory pentatomids appear to have more difficulty capturing heavily sclerotized prey. The only known exception is the oriental asopine *Amyotea malabarica* (F.), that has demonstrated a strong preference for *N. viridula* over lepidopterous prey (Singh 1973, Singh et al. 1973).

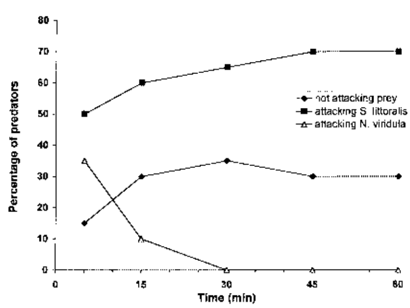

Fig. 1. Percentage of fourth-instar *P. maculiventris* not attacking prey, attacking a fifth-instar *S. littoralis*, or attacking a fourth-instar *N. viridula* 5, 15, 30, 45, and 60 min after the start of the preference experiment (n = 20).

In North America, predation by *P. maculiventris* on late instars of *N. viridula* has been observed in the field on a number of occasions. In most of these cases, the predator was seen preying on late instars of *N. viridula* (Jones 1918, Drake 1920, Stam et al. 1987) but Ragsdale et al. (1981), using serological assays, also noted significant predation on eggs of the pest. Based on his field records, however, Drake (1920) believed that another asopine, *Euthyrhynchus floridanus* (L.), was a more important natural enemy of the southern green stinkbug in Florida. Mead (1976) also listed *N. viridula* as a major prey of *E. floridanus* in Florida. In South America, *Podisus nigrispinus* (Dallas) is the most abundant predatory pentatomid in soybean and alfalfa fields, where it has been observed to attack a variety of pentatomid pests including *N. viridula* (Saini 1994). In Japan, Kiritani (1964) listed the holarctic asopine *Zicrona caerulea* (L.) as a predator of the southern green stinkbug in rice. A number of other heteropterans, mainly belonging to Reduviidae, have been noted to be predators of *N. viridula* with biocontrol potential (Drake 1920, Ambrose 1999, Grundy & Maelzer 2000).

Although the southern green stinkbug appears to be suboptimal prey for *P. maculiventris* compared to caterpillars, nymphs and adults of the predator were able to kill high numbers of eggs, nymphs and adults of the pest in the laboratory, suggesting that it may contribute to suppressing *N. viridula* outbreaks. In field crops, natural populations of *P. maculiventris* or *P. nigrispinus* could be augmented with laboratory-reared individuals. However, given the relatively high price for these soldier bugs (e.g., the market price for a fourth instar of *P. maculiventris* currently averages $0.25), augmentative releases may only be feasible for high-value crops, such as greenhouse crops. In European greenhouses, where damage by *N. viridula* has been increasingly reported in recent years, releases of *P. maculiventris* nymphs could help keep the pest in check especially when infestations are localized (i.e., in "hot spots"). On the other hand, measures may be warranted to conserve natural or augmented populations of predatory pentatomids, for instance when using chemical pesticides or biological control agents for the control of *N. viridula* and other pests in the crop. When targeting chemical treatments against *N. viridula*, it is worth considering that *Podisus* spp. are generally more tolerant to pyrethroids than to organophosphates or carbamates (Yu 1988, Picanço et al. 1996, Mohaghegh et al. 2000). Field-collected eggs of *Podisus* spp. and other asopines have been found to be heavily parasitized by the scelionid egg parasitoid *T. basalis* (Buschman & Whitcomb 1980, Orr et al. 1986, Corrêa-Ferreira & Moscardi 1995). As a consequence, augmentative releases of this parasitoid against phytophagous pentatomids like *N. viridula* could adversely affect populations of co-occurring predaceous pentatomids.

Because laboratory experiments using small arenas without refugia tend to overestimate the killing capacity of a predator (Wiedenmann & O'Neil 1992), further studies will assess predation by *P. maculiventris* on *N. viridula* under greenhouse conditions. Also, more field studies are needed to evaluate the impact of pentatomid predators on populations of the southern green stinkbug in major field crops.

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