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EFFECTS OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN ON *NEZARA VIRIDULA* AND ITS PREDATOR *PODISUS MACULIVENTRIS* (HETEROPTERA: PENTATOMIDAE)

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ABSTRACT

Insecticidal effects of an encapsulated formulation of lambda-cyhalothrin on the southern green stinkbug *Nezara viridula* (L.) and one of its predators, *Podisus maculiventris* (Say), were investigated in the laboratory. Both pentatomids were exposed to the insecticide via contaminated drinking water and by residual contact. Nymphs and adults of *N. viridula* were more susceptible to the insecticide than nymphs of *P. maculiventris*, both by ingestion and contact exposure. For the respective ways of exposure, LC_{50} values calculated for *P. maculiventris* fourth instars were 30-190 times and 3-13 times higher than those of *N. viridula* fourth instars. Insecticidal activity of the pyrethroid by ingestion was 6-10 times greater against nymphs of *N. viridula* than against adults of the pest. In both the ingestion and residual contact experiments, nymphs of *P. maculiventris* recovered from initial knockdown. LC_{50} values for predator nymphs increased 1.7- to 2.7-fold between 24 and 48 h after the start of the experiment. Recovery from knockdown was not observed in *N. viridula*. The data from the current laboratory study suggest that encapsulated lambda-cyhalothrin may be effective for controlling the southern green stinkbug with little adverse effects on the predator *P. maculiventris*, but field experiments are needed to confirm this. Possible reasons for the differential toxicity of the insecticide to both pentatomids are discussed.

Key Words: lambda-cyhalothrin, pyrethroid, *Nezara viridula*, *Podisus maculiventris*, Pentatomidae, non-target effects

RESUMEN

Los efectos insecticidas de una mezcla encapsulada de lambda-cyhalothrin en la chinche hedionda verde de sur (southern green stink bug), *Nezara viridula* (L.) y uno de sus depredadores, *Podisus maculiventris* (Say), fueron investigados en el laboratorio. Ambos pentatómidos fueron expuestos al insecticida por medio de agua para beber contaminada y por el contacto del residuo del insecticida. Las ninfas y los adultos de *N. viridula* fueron más susceptibles al insecticida que las ninfas de *P. maculiventris*, por la ingestión y por la exposición por contacto. Para las respectivas formas de exposición, los valores LC_{50} calculados por las ninfas de *P. maculiventris* en el cuatro estadio fueron 30-190 veces y 3-13 veces más altos que los valores para las ninfas de *N. viridula* en el cuatro estadio. La actividad de la insecticida piretroide por ingestión fué 6-10 veces mayor contra las ninfas de *N. viridula* que contra los adultos de esta plaga. En ambos experimentos de ingestión y por contacto del residuo, las ninfas de *P. maculiventris* se recuperaron del derribo inicial. Los valores LC_{50} para las ninfas del depredador aumentaron 1.7 al 2.7 veces entre las 24 y 48 horas después de empezar el experimento. La recuperación del derribo inicial no fue observada en *N. viridula*. Los datos del estudio de laboratorio actual sugerieron que el lambda-cyhalothrin encapsulada puede ser efectivo para controlar la chinche hedionda verde de sur con pocos efectos adversos sobre el depredador *P. maculiventris*, pero se necesita llevar a cabo experimentos en el campo para confirmarlo. Se discuten las razones posibles para la toxicidad diferential del insecticida para ambos pentatómidos.

The southern green stinkbug, *Nezara viridula* (L.), is a highly polyphagous pest that is widely distributed in the tropical and subtropical regions of the world (Todd 1989; Panizzi et al. 2000). This pentatomid causes important economic damage to various field crops, including soybean, beans, rice, corn, cotton and tobacco. In Europe, it has been found increasingly in greenhouses, where it attacks vegetable crops like tomato, sweet pepper, and eggplant. Control of this pest is based largely on the intensive use of chemical pesticides, including carbamates, organophosphates and some pyrethroids (Jackai et al. 1990; Ballanger & Jouffret 1997; Panizzi et al. 2000). For instance, in Brazil it was estimated that in the mid 1990s over 4 million liters of insecticides were used annually to control stinkbugs in soybean (Corrêa-Ferreira & Moscardi 1996). Such massive use of insecticides not only increases production cost, it may also affect populations of beneficial insects and trigger pest resurgence problems.

In several regions, efforts have been made to develop integrated pest management (IPM) programs against *N. viridula* (Panizzi et al. 2000). Biological control of the pest has focused mainly on the potential of parasitoids (Jones 1988). Releases of the scelionid egg parasitoid *Trissolcus basalis* (Wollaston) have successfully suppressed outbreaks of the southern green stinkbug in soybean (Corrêa-Ferreira & Moscardi 1996). Several arthropod predators also have an important impact on *N. viridula* populations. De Clercq et al. (2002) reported high predation rates by nymphs and adults of the predatory pentatomid *Podisus maculiventris* (Say) on the different life stages of the southern green stinkbug. This generalist predator is native to North America where it is commonly found in a variety of natural and agricultural ecosystems (De Clercq 2000). Although it appears to have a preference for larvae of lepidopterous and coleopterous insects, the predator frequently has been found in association with *N. viridula* in the southern United States (Drake 1920; Ragsdale et al. 1981; Stam et al. 1987). *Podisus maculiventris* has been used in European greenhouses since 1997 for augmentative biological control of caterpillar outbreaks, and predation on *N. viridula* may be an additional asset here for this beneficial insect (De Clercq et al. 2002).

In France, Ballanger & Jouffret (1997) reported effective control of *N. viridula* in soybean with foliar sprays of lambda-cyhalothrin. This contact and stomach insecticide belonging to the pyrethroid group has been used against a broad spectrum of pests in a variety of crops (Anonymous 2000). In 1999, a micro-encapsulated formulation (Zeon Technology™) of this pyrethroid was commercialised, offering reduced health hazards and an improved environmental profile (Ham 1999; Anonymous 2000). In the current study, insecticidal activity of an encapsulated formulation of lambda-cyhalothrin to *N. viridula* and its predator *P. maculiventris* was assessed in the laboratory. Both pentatomids were exposed to the insecticide via ingestion and residual contact. The implications of our findings for the control of the southern green stinkbug and the use of the predator *P. maculiventris* in IPM programs targeted against this and other agricultural pests are discussed.

Materials and Methods

Insect Cultures

A laboratory colony of *N. viridula* was established in 1999 with insects originating from field collections in France, Spain and Italy. Stinkbugs were fed on green bean pods (*Phaseolus vulgaris* L.) and sunflower seeds (*Helianthus annuus* L.). A culture of *P. maculiventris* was started in 1999 with specimens originating from a field collection in 1996 near Beltsville, Maryland, USA. The predators were fed mainly larvae of the greater wax moth, *Galleria mellonella* L. Colonies of all insects were maintained in growth chambers at 23 ± 1°C, 75 ± 5% RH and a 16:8 h light:dark photoperiod.

Chemicals

A commercial formulation of micro-encapsulated lambda-cyhalothrin (Karate Zeon®, 100 g/l) was obtained from Syngenta, Ruisbroek, Belgium.

Toxicity Bioassays

Exposure via ingestion. In these experiments, nymphs and adults of *N. viridula* and nymphs of *P. maculiventris* were exposed to insecticide through treated drinking water. Both pentatomids take up moisture in the absence of food and are regularly seen drinking even when food is available. Moisture can be supplied via plant materials, like green beans, but can also be provided as free water. Using gravimetric methods, we estimated that unfed newly molted fourth instars of *P. maculiventris* and *N. viridula* take up about 2 and 4.5 µl of free water, respectively, during a 24-h period.

Newly molted fourth instars and reproductively active adult female *N. viridula* were randomly collected from stock cultures and transferred to plastic petri dishes (9 cm diam) lined with absorbent paper. A replicate consisted of four insects in a dish for *N. viridula* nymphs, whereas adults were placed singly in dishes. Newly molted fourth instars of *P. maculiventris* were placed in petri dishes (9 cm diam) in groups of three. Each dish was supplied with a moisture source, consisting of a paper plug fitted into a plastic dish (2.5 cm diam). The paper plug was saturated with 2 ml of the insecticide in tap water. Control groups were supplied with tap water alone. At least 20 nymphs or 10 adults were tested with each of at least 10 concentrations. Choice of concentrations was based on preliminary range-finding tests. Test concentrations ranged from 0 to 200 mg a.i./l (11 concentrations) and from 0 to 100 mg a.i./l (10 concentrations) for *N. viridula* nymphs and adults, respectively, and from 0 to 800 mg a.i./l (13 concentrations) for *P. maculiventris* nymphs. Both pentatomids were exposed to the contaminated moisture source during a 1-wk period. To stimulate drinking behavior, the insects were not provided with food during the first 24 h. From the second day on, nymphs and adults of *N. viridula* were supplied with sunflower seeds as needed; predator nymphs were fed greater wax moth larvae *ad libitum*.

In range-finding tests, the ability of *P. maculiventris* in particular to recover from initial poisoning became apparent. Therefore, mortality counts were performed 1, 2, and 7 d after the initial treatment. Mortality percentages included dead and affected individuals. Insects were scored as affected when they were incapable of coordinated movement upon prodding with a fine brush.

Residual exposure. To evaluate residual contact activity of lambda-cyhalothrin, fourth instars of both pentatomids were exposed by tarsal contact to dry residues on filter paper. Whatman No. 41 filter papers were fitted into petri dishes (9 cm diam) and the dishes were sprayed in a Cornelis spray chamber (Van Laecke & Degheele 1993). Each dish was sprayed with 2 ml of insecticide suspension in water, yielding a homogeneous spray deposit on the filter paper of approximately 5 mg/cm^2. For the controls, dishes were sprayed with 2 ml of water. The plates were left to dry for about 1 h before introducing the insects. A replicate consisted of a petri dish containing three *P. maculiventris* or four *N. viridula* nymphs. Twenty to 40 insects were tested per concentration, with a minimum of 10 concentrations. Concentrations were chosen on the basis of range-finding tests and ranged from 0 to 200 mg a.i./l (12 concentrations) for *N. viridula* nymphs and from 0 to 400 mg a.i./l (10 concentrations) for *P. maculiventris* nymphs. To stimulate food searching by the nymphs and maximize contact with the treated surface, no food or moisture were provided during the first 24 h. From the second day on, the insects were supplied with water and food. Water was supplied via a soaked paper plug in a 2-cm-diam cup. Nymphs of *P. maculiventris* were given freshly killed wax moth larvae and those of *N. viridula* were offered sunflower seeds. Mortality counts were made after 1, 2, and 7 d, allowing for the assessment of recovery after initial knockdown.

Data Analysis

Mortality of the test insects after 1, 2, and 7 d was corrected for control mortality by Abbott's formula (Abbott 1925). Lethal concentration values and their 95% confidence limits were calculated from probit-regressions with POLO PC (LeOra Software 1987). All concentrations tested were used for LC-calculations; numbers (*n*) given in the footnotes of Tables 1 and 2 thus reflect actual numbers of insects tested and used in probit-regressions.

Results

In our laboratory setup, there was no indication of a repellent or antifeedant effect for encapsulated lambda-cyhalothrin. In ingestion assays, both *P. maculiventris* and *N. viridula* were regularly observed to suck on moisture sources contaminated with varying concentrations of the compound. In the residual contact experiment, nymphs of both pentatomids were usually on the treated surfaces (filter paper, petri dish walls) and only occasionally on the untreated lid.

Nymphs and adults of *N. viridula* were more susceptible to the insecticide than nymphs of their predator *P. maculiventris*, both by ingestion (Table 1) and contact exposure (Table 2). For the respective routes of exposure, LC_{50} values calculated for *P. maculiventris* fourth instars were 30-190 times and 3-13 times higher than those of *N. viridula* fourth instars. Further, insecticidal activity of the pyrethroid by ingestion was 6-10 times greater against nymphs of *N. viridula* than against adults of the pest. Lethal concentration values calculated for *N. viridula* nymphs were lower for ingestion exposure than for contact exposure, suggesting that lambda-cyhalothrin was more active by ingestion than by tarsal contact with dry residues. However, some of the nymphs in the ingestion bioassays were partially or fully in tarsal contact with the moist plug when drinking, so these insects may have been exposed to the insecticide both via ingestion and via contact with wet residues. Differences in biological activity due to exposure routes were less apparent in the predatory pentatomid *P. maculiventris*.

In both the ingestion and residual contact experiments, nymphs of *P. maculiventris* recovered from initial knockdown. LC_{50} values for predator nymphs increased 1.7- to 2.7-fold between 24 and 48 h after the start of the experiment. Recovered predators were able to attack prey and feed normally. Comparisons of the LC_{50} values for *P. maculiventris* fourth instars after 2 and 7 d, however, show that some of the individuals that appeared to have recovered after 2 d died before reaching the fifth stadium when they were continuously exposed to contaminated drinking water or filter paper during a 7 d period. At 23°C, optimally fed fourth instars of both pentatomids usually reach the next stadium in 4-5 d (De Clercq & Degheele 1992). There was little or no recovery from knockdown in *N. viridula*. Here, LC_{50} values after 1 and 2 d were generally similar and further decreased with exposure time. Likewise, slopes were equal after 1 and 2 d ($\chi^2, P > 0.05$), but were significantly increased after 7 d of exposure. Only in the ingestion study with adults, LC_{90} values suggest there may have been some recovery at the upper end.

Discussion

In our experiment, both nymphs and adults of *N. viridula* were susceptible to encapsulated lambda-cyhalothrin. However, nymphs suffered about 5 times greater mortality when exposed by ingestion than by residual contact. For the control of *N. viridula*, the practical significance of ingestion exposure may be limited given that pyrethroids have no systemic properties. It is unlikely that the insect would ingest the compound in the field, except when it would drink from fresh spray deposits. Although lambda-cyhalothrin is widely recommended for the control of a broad range of insect pests, including stinkbugs, there are few studies reporting on the insecticidal effects of this pyrethroid on the southern green stinkbug. Ac-

TABLE 1. TOXICITY OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN TO *N. VIRIDULA* AND *P. MACULIVENTRIS* BY INGESTION AFTER 1, 2, AND 7 DAYS.

	LC value[a]				
Insect	LC_{10}	LC_{50}	LC_{90}	χ^2 (*df*)	Slope ± SE
			1 day		
Nezara fourth instar	0.32 (0.12-0.61)	4.61 (3.12-6.50)	66.98 (39.35-147.46)	8.46 (8)	1.10 ± 0.11
Nezara female adult	2.79 (0.42-6.06)	28.70 (15.29-82.31)	295.72 (96.63-646.70)	8.03 (7)	1.26 ± 0.25
Podisus fourth instar	20.85 (12.67-29.68)	144.53 (116.50-181.80)	1001.83 (671.00-1776.99)	7.56 (10)	1.52 ± 0.15
			2 days		
Nezara fourth instar	0.30 (0.10-0.60)	3.83 (2.47-5.7)	49.43 (28.48-116.13)	10.72 (8)	1.15 ± 0.11
Nezara female adult	2.41 (0.13-6.19)*	40.04 (18.14-253.32)*	663.79 (141.99-1,798,890)*	13.84 (7)	1.05 ± 0.23
Podisus fourth instar	28.00 (16.29-40.8)	248.97 (193.15-340.43)	2214.20 (1279.44-5112.59)	7.77 (10)	1.35 ± 0.15
			7 days		
Nezara fourth instar	0.12 (0.05-0.22)	0.97 (0.64-1.32)	7.64 (5.57-11.63)	2.14 (8)	1.41 ± 0.16
Nezara female adult	1.36 (0.05-3.66)*	10.26 (3.92-26.16)*	77.23 (29.10-2164.97)*	24.40 (7)	1.46 ± 0.25
Podisus fourth instar	33.25 (20.83-46.08)	183.28 (146.88-234.81)	1010.36 (673.73-1838.99)	12.09 (10)	1.73 ± 0.17

[a]LC values and slopes in mg a.i./l; LC-values are followed by 95% fiducial limits except when marked with an asterisk (*) where 90% fiducial limits are given.
n = 456, 137, and 411 for *N. viridula* fourth instars, *N. viridula* females and *P. maculiventris* fourth instars, respectively.

TABLE 2. TOXICITY OF AN ENCAPSULATED FORMULATION OF LAMBDA-CYHALOTHRIN TO *N. VIRIDULA* AND *P. MACULIVENTRIS* BY RESIDUAL CONTACT AFTER 1, 2, AND 7 DAYS.

Insect	LC value[a]				
	LC_{10}	LC_{50}	LC_{90}	χ^2 (*df*)	Slope ± SE
1 day					
Nezara fourth instar	4.96 (2.02-8.08)	19.92 (13.79-27.18)	80.02 (53.53-160.92)	22.23 (9)	2.12 ± 0.20
Podisus fourth instar	15.92 (3.39-28.64)	66.63 (42.88-93.82)	278.78 (169.62-981.19)	13.48 (7)	2.06 ± 0.30
2 days					
Nezara fourth instar	4.75 (3.13-6.41)	19.06 (15.80-22.62)	76.50 (60.05-105.97)	8.21 (9)	2.12 ± 0.20
Podisus fourth instar	26.45 (1.27-51.00)	178.84 (106.72-1038.60)	1209.01 (392.16-482,011)	17.54 (7)	1.54 ± 0.29
7 days					
Nezara fourth instar	4.80 (2.98-6.38)	11.29 (9.21-13.15)	26.55 (22.20-34.79)	3.38 (9)	3.45 ± 0.49
Podisus fourth instar	43.80 (10.62-66.79)*	147.81 (111.93-252.08)*	498.82 (278.24-4031.84)*	13.75 (7)	2.43 ± 0.53

[a]LC values and slopes in mg a.i./l; LC-values are followed by 95% fiducial limits except when marked with an asterisk (*) where 90% fiducial limits are given. *n* = 416 and 255 for *N. viridula* fourth instars and *P. maculiventris* fourth instars, respectively.

cording to Ballanger & Jouffret (1997) and Gouge et al. (1999), the pest can be adequately controlled in soybean with non-encapsulated lambda-cyhalothrin at 20-30 g a.i./ha. In topical application experiments on third instars of *N. viridula*, Baptista et al. (1995) reported LD_{50} values of 0.82-0.25 µg/g for a technical grade formulation of lambda-cyhalothrin 3-24 h after treatment; the pyrethroid was 20-40 times more active against the pest than monocrotophos.

Susceptibility of the spined soldier bug, *P. maculiventris*, to classical and novel insecticides has been studied to some extent (see De Clercq 2000 for a review). Besides direct and residual contact, predatory pentatomids can be poisoned by drinking contaminated free water or plant sap (in case of systemic compounds) or by feeding on contaminated prey (De Clercq et al. 1995). In the current study, *P. maculiventris* was less vulnerable to lambda-cyhalothrin than *N. viridula*, both in the ingestion and contact exposure bioassays. Based on lethal concentration values, nymphs of the predator were somewhat more susceptible to the compound by residual contact than by ingestion. The LC_{50} value for fourth instars of *P. maculiventris* exposed to lambda-cyhalothrin via ingestion was similar to that found in an earlier study for nymphs treated similarly with deltamethrin (158 mg a.i./l, Mohaghegh et al. 2000). In a number of studies, *P. maculiventris* and related asopines have demonstrated a better tolerance to pyrethroids than their lepidopterous prey (Yu 1988; Zanuncio et al. 1993; Picanço et al. 1996). Yu (1988) hypothesized that thickness and lipid content of the cuticle may affect the penetration rate of the lipophilic pyrethroids and may thus be responsible for differences in toxicity to the heavily sclerotized pentatomid predators and their soft-bodied caterpillar prey. The finding of Baptista et al. (1995) that lambda-cyhalothrin was 5-9 times more toxic to the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) than to *N. viridula* supports this hypothesis. It may, however, not explain the differences found in our study because *P. maculiventris* and *N. viridula* are both pentatomids with a similarly sclerotized cuticle. Different drinking rates explain only in part the observed differences in toxicity of lambda-cyhalothrin to the studied pentatomids by ingestion. Preliminary gravimetric tests indicated that unfed fourth instars of *N. viridula* ingested twice as much free water during a 24-h period than did those of *P. maculiventris*, despite similar body weights (approximately 12.5 mg). Alternatively, higher detoxification or excretion rates may be responsible for the lower susceptibility of *P. maculiventris* to lambda-cyhalothrin as compared to *N. viridula*. Pyrethroids are known to be metabolised in insects mainly by esterases and microsomal oxidases (Shono et al. 1979). Yu (1987, 1988) found, however, that these enzyme activities were generally lower in the spined soldier bug than in its caterpillar prey. Likewise, first tests have shown about 8-fold higher esterase activities in the phytophagous pentatomid *N. viridula* than in the predatory pentatomid *P. maculiventris* (unpublished data). Recently, it has been shown that glutathione S-tranferases also may play a significant role in detoxifying pyrethroids (e.g., Kostaropoulos et al. 2001). In this context, it is worth noting that Yu (1987) reported about 2-fold higher glutathione transferase activity toward CDNB in the spined soldier bug than in its lepidopterous prey. Further studies on the pharmacokinetics of lambda-cyhalothrin are warranted to explain the difference in toxicity of the compound to the studied pentatomids.

A number of field studies have demonstrated adverse effects of lambda-cyhalothrin on various beneficial arthropods, including predatory heteropterans, although in some cases negative effects on field populations of natural enemies were transient (Pilling & Kedwards 1996; Cole et al. 1997; van den Berg et al. 1998; Al-Deeb et al. 2001; Stewart et al. 2001). Encapsulation of broad-spectrum insecticides, including pyrethroids, is aimed at improving their selectivity and suitability for IPM programs (Scher et al. 1998; Ham 1999). However, several studies comparing encapsulated and non-encapsulated formulations of various insecticides have shown highly variable results with a range of beneficial arthropods (see Pogoda et al. 2001 for references). Pogoda et al. (2001) reported that a micro-encapsulated formulation of lambda-cyhalothrin was as toxic as an emulsifiable-concentrate formulation of the compound to the oriental fruit moth, *Grapholita molesta* (Busck). These workers also found that the encapsulated formulation of lambda-cyhalothrin was less toxic than the emulsifiable concentrate to a pyrethroid-resistant population of the phytoseiid mite *Typhlodromus pyri* Scheuten but more toxic to a pyrethroid-susceptible population of the predator.

The current laboratory trials indicate that encapsulated lambda-cyhalothrin has a good insecticidal activity against the southern green stinkbug and is relatively safe to the predatory stinkbug *P. maculiventris*. However, field studies are in place to test further the selectivity of this insecticide toward *P. maculiventris*. Further research also is needed to determine if the studied encapsulated formulation of lambda-cyhalothrin is more selective toward *P. maculiventris* and other natural enemies compared with other formulations of the compound.

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