VIE ET MILIEU - LIFE AND ENVIRONMENT, 2007, 57 (1/2) : 39-43

EFFECTS OF TWO ECDYSTEROID ANALOGS (RH-0345 AND RH-2485) ON THE TOTAL GROWTH OF *EUPOLYBOTHRUS NUDICORNIS* (MYRIAPODA, CHILOPODA)

O. DAAS [1], P. SCAPS [2], N. BOUZERNA [1], M. DESCAMPS [2]*

[1] Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, 23000 Annaba, Algeria
[2] Laboratoire d'Ecologie Numérique et d'Ecotoxicologie, EA 3570, FR 1818, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France
** Corresponding author: patrick.scaps@univ-lille1.fr*

PESTICIDES
ECDYSTEROID ANALOGS
GROWTH
HEMOLYMPH PROTEINS
OVARIAN PROTEINS
EUPOLYBOTHRUS NUDICORNIS

ABSTRACT. – We studied the effects of two ecdysteroid analogs (RH-0345 and RH-2485) on the growth and on the hemolymph and ovarian protein concentrations of the centipede *Eupolybothrus nudicornis* used as a model of predators of soil fauna. Sublethal doses of RH-0345 and RH-2485 significantly reduce total body and ovarian weights compared with those of controls. The decrease in ovarian weight is proportional to the decrease in total body weight because the gonadal somatic index remains constant and is the same among controls and intoxicated animals. RH-0345 and RH-2485 also reduce significantly the total protein concentrations in both the hemolymph fluid and ovarian tissue of females. Taken together, the observed decrease in total body and ovarian weight and total protein production is indicative of a decrease in total growth.

INTRODUCTION

Today, use of pesticides in order to control pests causes much concern in the general population. Since it is impossible to control all pest species without resorting to some use of pesticides, these are required to be specific and environmentally safe. In this context, natural and synthetic compounds capable of interfering with the processes of growth, development, reproduction and metamorphosis of the target pests have been developed in the search for safer insecticide technologies. These chemicals have been called insect growth regulators (for review see Hoffmann & Lorenz 1998). As they work at extremely low levels, they are very specific and are not toxic to mammals; several compounds are already used. Among these compounds, novel insecticides that mimic the action of the molting hormone, the steroidal 20-hydroxyecdysone, are currently developed by the industry. Bisacylhydrazines are non-steroidal ecdysteroid agonists of 20-hydroxyecdysone and exhibit their insecticidal activity via interaction with ecdysteroid receptor proteins (Dhadialla *et al.* 1998).

Even though the effects of these compounds are known in insects (reviewed in Dhadialla *et al.* 1998), no work exists on effects on other terrestrial arthropods such as centipedes. So, the aim of this study was to test the influence of two bisacylhydrazine ecdysteroid analogs (RH-0345 and RH-2485), used as insecticides, on the growth and on the hemolymph and ovarian protein concentrations of one of the predators of the soil fauna, the centipede *Eupolybothrus nudicornis* (Gervais, 1837) (= *Eupolybothrus elongatus* = *Bothropolys elongatus*) which could be directly affected by exposure to these compounds or affected following ingestion of preys containing these molecules.

MATERIALS AND METHODS

Centipedes: experiments were conducted on mature female *Eupolybothrus nudicornis* collected during the spring or the autumn of 1999 from the area of Annaba (Eastern Algeria) where they are relatively common (Daas *et al.* 1996). Animals were maintained individually in the laboratory on regularly moistened filter paper. They were fed three times a week with insects (cockroach larvae, flies and mosquitoes) and spiders.

Chemicals and toxicity tests: RH-0345 (halofenozide) and RH-2485 (methoxyfenozide) were developped by Rohm & Haas Company (Pennsylvania, USA). They were a gift of G Smagghe (Laboratory of Agrozoology, University of Gent, Belgium). RH-0345 and RH-2485 were dissolved in acetone to prepare a concentration of 10 µg/µl for experimental use. 3 µl were injected by means of a microsyringe between the third and fourth dorsal segment. Toxicity tests were performed during a 45-day period. Dose levels injected were based on previous finder range test (Daas *et al.* 2005) and were chosen in order not to be lethal during the experiment. Control animals were maintained in the same conditions and injected with 3 µl of acetone.

Sample preparation: ovaries were dissected and homogenized in 0.05 M Tris-HCl, 0.15 M NaCl (pH 7.4) buffer. After centrifugation (5 000 X g for 10 min at 4°C), the supernatant, defined as soluble oocyte proteins (Fabre *et al.* 1990) was anal-

ysed. Hemolymph samples (3 µl) were taken from the hemolymphatic sinus of the same individuals between the third and the fourth dorsal segment. They were completed to 300 µl with the same buffer and were stored in the refrigerator at 4°C until analysis for total hemolymph protein concentration.

Protein quantification in hemolymph and ovaries: the amounts of proteins in hemolymph and ovaries were measured 7, 15, 21 and 45 days after injection according to Bradford (1976). 100 µl of hemolymph extract or supernatant were adjusted to 4 ml with a solution of brillant blue Coomassie (100 mg dissolved in 50 ml ethanol, 100 ml phosphoric acid and 850 ml water). The absorbance was read at 595 nm and the protein content of the sample determined with respect to a bovine serum albumine calibration curve. Data were expressed as µg of proteins per µl of hemolymph liquid and mg of proteins per µg of ovary, respectively.

Data analysis: the results were expressed as mean ± standard deviation (S.D.). A one-way ANOVA was used to compare the amounts of proteins in hemolymph and ovaries from acetone injected animals (controls) and animals exposed to RH-0345 and RH-2485 as a function of time. This analysis was followed by application of the Student-Newmans-Keuls multiple comparison method. The level of significance was set at $p < 0.05$.

RESULTS

Mean total body weight

The same results were obtained with individuals collected in spring or autumn (Fig. 1A, B). The mean total body weight of control females remained stable. A significant decrease of the mean total body weight of individuals intoxicated with RH-2845 and RH-0345 compared to controls was noticed 45 days after the start of the experiment. The effect was more pronounced with RH-2485 but the difference between the treatments with RH-0345 and RH-2845 was not significant.

Mean ovarian weight

The same results were obtained with individuals collected in spring or autumn (Fig. 2A, B). The mean ovarian weight of control females remained stable. No significant differences were detected between the controls and the different treatments 7 days after intoxication. The mean ovarian weight of females collected in spring and intoxicated with RH-0345 or RH-2485 was significantly reduced from 15 to 45 days compared to controls. A sig-

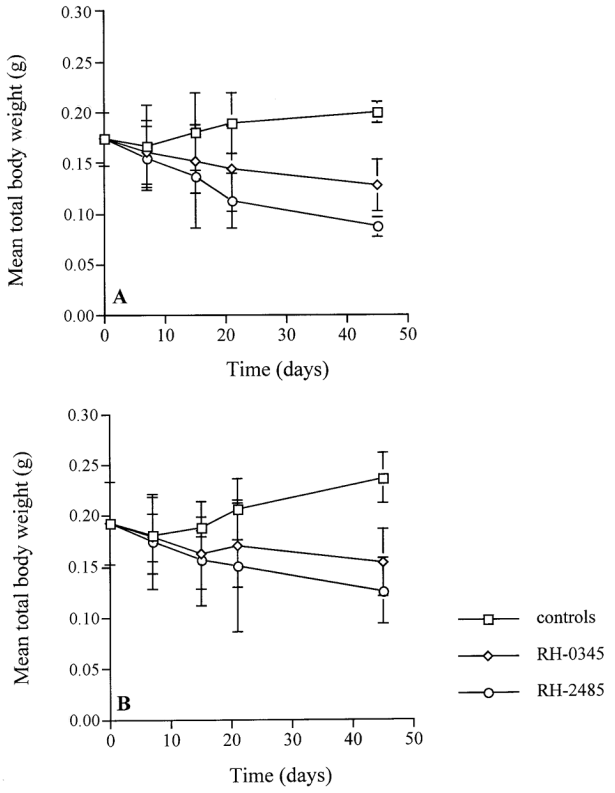

Fig. 1. – Influence of RH-0345 (halofenozide) and RH-2485 (methoxyfenozide) on *Eupolybothrus nudicornis* mean total body weight. A, females collected in spring, B, females collected in autumn. 3 µl of a solution of 10 µg/µl of bisacylhydrazine was injected per animal. Means ± S.D. of five replicates.

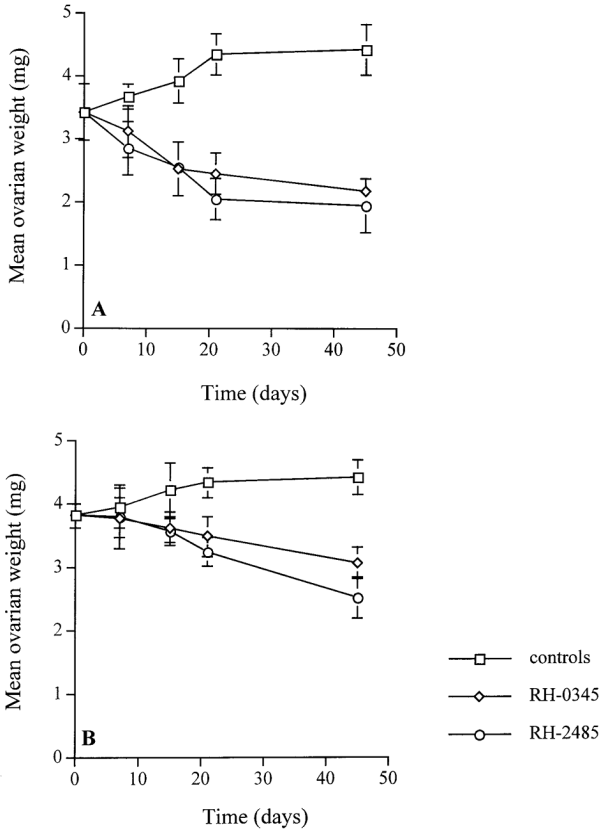

Fig. 2. – Influence of RH-0345 (halofenozide) and RH-2485 (methoxyfenozide) on *Eupolybothrus nudicornis* mean ovarian weight. A, females collected in spring, B, females collected in autumn. 3 µl of a solution of 10 µg/µl of bisacylhydrazine was injected per animal. Means ± S.D. of five replicates.

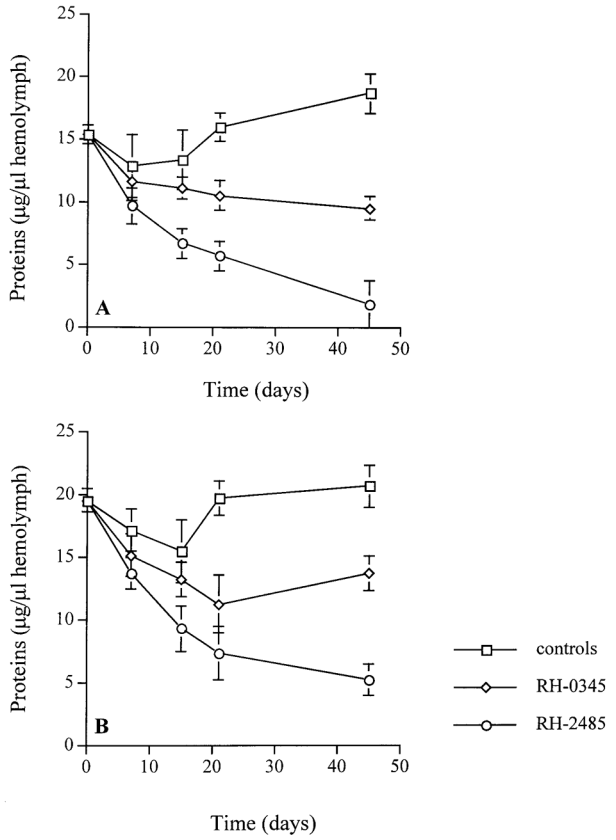

Fig. 3. – Influence of RH-0345 (halofenozide) and RH-2485 (methoxyfenozide) on *Eupolybothrus nudicornis* hemolymph protein concentrations. A, females collected in spring, B,females collected in autumn. 3 µl of a solution of 10 µg/µl of bisacylhydrazine was injected per animal. Means ± S.D. of five replicates.

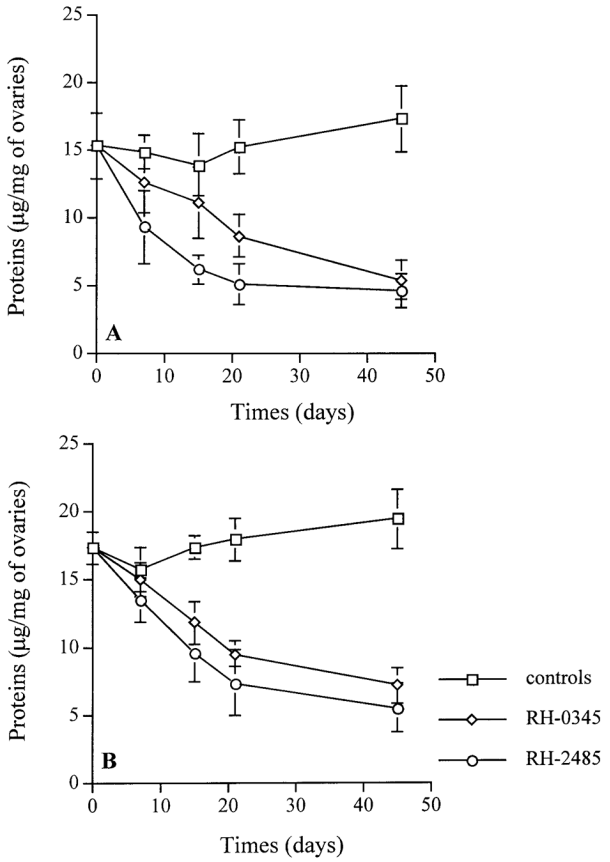

Fig. 4. – Influence of RH-0345 (halofenozide) and RH-2485 (methoxyfenozide) on *Eupolybothrus nudicornis* ovarian protein concentrations. A, females collected in spring, B, females collected in autumn. 3 µl of a solution of 10 µg/µl of bisacylhydrazine was injected per animal. Means ± S.D. of five replicates.

nificant decrease of the mean ovarian weight of females collected in autumn and intoxicated with RH-0345 or RH-2485 was noticed from 21 to 45 days compared to controls. The negative effect was more pronounced with RH-2485 but the differences between the treatments with RH-0345 and RH-2845 were not significant.

The gonadal somatic index (GSI) (the ratio of gonadal tissue weight to total body weight) remained stable (2%) and was not statistically different among controls, RH-0345 and RH-2485 treated females indicating that the decrease in gonadal weight was proportional to the decrease in total body weight.

Hemolymph protein concentrations

The same results were obtained with individuals collected in spring or autumn (Fig. 3A, B). The hemolymph protein concentrations of control females remained stable. No significant differences were detected between the controls and the different treatments 7 days after intoxication. A significant decrease of the hemolymph protein concentrations was noticed from 14 to 45 days and from 21 to 45 days after intoxication with RH-2845 and RH-0345, respectively. The hemolymph protein concentrations of females exposed to RH-2845 were significantly reduced compared to those exposed to RH-0345 from 14 to 45 days after exposure. The hemolymph protein concentrations were significantly higher in females collected in autumn than in females collected in spring.

Ovarian protein concentrations

The ovarian protein concentrations of control females remained stable during the experiment (Fig. 4A, B). No significant differences were noticed between the controls and the different treatments 7 days after intoxication. The ovarian protein concentrations of females collected in spring and exposed to RH-2845 were significantly reduced compared to those of controls from 14 to 45 days after exposure whereas those of females exposed to RH-0345 were significantly reduced from 21 to 45 days. A significant decrease of the ovarian protein concentrations of females collected in autumn and injected with RH-2485 or RH-0345 compared to controls was noticed from 14 to 45 days after intoxication. No significant differ-

ences were observed between animals exposed to RH-0345 or RH-2485. The ovarian protein concentrations were significantly higher in females collected in autumn than in females collected in spring.

DISCUSSION

The developmental physiology of myriapods as that of insects depends on different hormones and neurohormones but the molting and the juvenile hormones are the principal ones which control different processes such as growth, molt, and metamorphosis (Rees 1995). Ecdysteroids which constitute the molting hormone have been considered as the main key in the control of the molt in immature stages of insects (for review see Gäde *et al.* 1997). Today, it is well accepted that ecdysteroids also play an important role in the processes which regulate the reproduction of insects such as vitellogenesis, ovogenesis and growth of the spermatocytes (Wigglesworth 1984, Hagedorn 1985, Jacob 1992). Hence, Robbins *et al.* (1968, 1970) reported that high concentrations of natural ecdysteroids had chemosterilizing properties in several insects. The goal of our study was to test the effects of ecdysteroid analogs on the growth and on the hemolymphatic and ovarian protein concentrations of other arthropods than insects on which we have only limited evidence. Bisacylhydrazines are non-steroidal agonists of 20-hydroxyecdysone (20E) and exhibit their insecticidal activity via interaction with the ecdysteroid receptor proteins. In cases where these compounds have produced lethal effects, the symptoms have been similar to those expected from a state of ecdysteroid excess, called hyperecdysonisms (Williams 1967).

We showed that sublethal doses of RH-0345 and RH-2485 significantly reduced total body and ovarian weights compared with those of controls. The decrease in ovarian weight was proportional to the decrease in total body weight because the GSI remained constant and was the same among controls and test animals. Previous studies using certain steroid ecdysone analogs have shown that these compounds affected the ovarian growth in *Spodoptera exempta, S. exigua, S. littoralis* and *Leptinotarsa decemlineata* (Smagghe & Degheele 1992, 1994) and inhibited the ovarian development of *Musca domestica* and *Tribolium confusum* (Robbins *et al.* 1968, 1970). It has also been shown that another nonsteroidal ecdysteroid agonist, RH-5849, induced cessation of feeding and weight gain in treated lepidopterous and coleopterous larvae (Smagghe & Degheele 1992, 1994).

Hemolymph and ovarian protein concentrations were higher in females collected in autumn than in females collected in spring. This could be related to oocyte stages reached in these seasons; previtellogenetic oocytes and submature or mature oocytes are found in spring and autumn, respectively (Daas *et al.* 2003). We showed that the two ecdysteroid analogs RH-0345 and RH-2485 reduced the total protein concentrations in both the hemolymph fluid and ovarian tissue of females collected in spring or autumn. These results are in accordance with previous studies of Regier & Kalfatos (1985) and Margaritis (1985) who showed that treatment of *Tenebrio molitor* with RH-0345 reduced the concentrations of proteins stored in the ovary of females.

Taken together, the observed decrease in total body and ovarian weight and total protein production is indicative of a decrease in total growth. These results are consistent with the perceived mode of action of these chemicals. As bisacylhydrazine insecticides exhibit their insecticidal activity via interaction with the ecdysteroid receptor proteins, they elicit their response by interrupting the processes of growth and molting. Several studies have observed similar decreases in total body growth, across a variety of arthropod species, as a result of exposure to bisacylhydrazine insecticides (reviewed in Dhadialla *et al.* 1998).

It is well recognised that ecdysteroid analogs are toxic to insects. The synthetic nonsteroidal ecdysone agonists RH-0345 and RH-2845 are excellent insect control agents because they induce feeding inhibition and precocious incomplete molting, thus causing high larval mortality (Dhadialla *et al.* 1998). RH-0345 has an overall insect control spectrum with accentuated soil-systemic efficacy against scarabid beetle larvae, cutworms, and webworms. Based on its reported narrow pest control spectrum and its structural and mechanistic similarity to the other bisacylhydrazines, it is expected to have low toxicity to non-target arthropods (Dhadialla *et al.* 1998). RH-2485 acts against a wide range of lepidopteran pests of cotton (Ishaaya *et al.* 1995), corn (Trisyono & Chippendale 1997) and other major agronomic crops (Le *et al.* 1996). Our study showed that ecdysteroid analogs may be toxic to other arthropods than insects such as centipedes. We have shown that RH-2485 which was believed to be very selectively toxic to lepidopteran pests (Dhadialla *et al.* 1998) can also exert strong negative effects on total growth of another arthropod, the centipede *E. nudicornis*. In field populations a decrease in total size of a predatory species may affect its ability to compete for limited resources.

This study will be completed by analyses of toxicological endpoints such as reproduction or vitellogenin (biomarker of exposure) measurements investigating the dose-effect relationships.

LITERATURE CITED

Bradford MM 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.

Daas T, Bouzerna N, Descamps M 1996. Développement post-embryonnaire et cycle de développement de *Eupolybothrus elongatus* (Newport) dans l'Est algérien. *Mém Mus Natl Hist Nat* 169: 365-370.

Daas O, Daas T, Descamps M 2003. Étude de paramètres biologiques chez *Eupolybothrus elongatus* en vue de leur utilisation pour l'évaluation de la toxicité de composés chimiques utilisés en agriculture. *Bull Soc Zool Fr* 128: 239-246.

Daas O, Daas T, Scaps P, Descamps M 2005. Compared toxicity of two ecdysteroid analogs (RH-0345 and RH-5992) and of a JH mimic (pyriproxyfen) on *Eupolybothrus nudicornis* (Myriapoda Chilopoda) hemolymph components. *Bull Soc Zool Fr* 130: 303-313.

Dhadialla TS, Carlson GR, Le DP 1998. New insecticides with ecdysteroidal and juvenile hormone activity. *Annu Rev Ento - mol* 43: 545-569.

Fabre MC, Descamps M, Baert JL 1990. Identification and partial characterization of vitellin and vitellogenin from *Scolopendra cingulata* Latreille (Myriapoda Chilopoda). *Ber Nat Med Verein Innsbruck* 10: 117-121.

Gäde G, Hoffmann KH, Spring JH 1997. Hormonal regulation in insects: facts, gaps, and future directions. *Physiol Rev* 77: 963-1032.

Hagedorn HH 1985. The role of ecdysteroids in reproduction. *In* Kerkut GA & Gilbert LI eds, Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol 8, Pergamon Press, Oxford: 205-262.

Hoffmann KH, Lorenz MW 1998. Recent advances in hormones in insect pest control. *Phytoparasitica* 26: 323-330.

Ishaaya I, Yablonski S, Horowitz AR 1995. Comparative toxicology of two ecdysteroid agonists, RH-2485 and RH-5992, on susceptible and pyrethroid resistant strains of the Egyptian cotton leafworm, *Spodoptera littoralis*. *Phytoparasitica* 23: 139-145.

Jacob M 1992. *In vitro* spermatogenesis in *Oryctes rhinoceros* (Coleoptera, Scarabaeidae): the role of ecdysone and juvenile hormone. *Zool Sci* 9: 457-461.

Le DP, Thirugnanam M, Lidert Z, Carlson GR, Ryan JB 1996. RH-2485: a new selective insecticide for caterpillar control. *Proc Brighton Crop Protect Conf Pests Dis* 2: 481-486.

Maragaritis LH 1985. Structure and physiology of the eggshell. *In* Kerkut GA & Gilbert LI eds, Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol 1, Pergamon Press, Oxford: 153-230.

Rees HH 1995. Ecdysteroid biosynthesis and inactivation in relation to function. *Eur J Entomol* 92: 9-39.

Regier JC, Kalfatos FC 1985. Molecular aspects of chorion formation. *In* Kerkut GA & Gilbert LI eds, Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol 1, Pergamon Press, Oxford: 141-151.

Robbins WE, Kaplanis JN, Thompson JN, Shortino TJ, Cohen CF, Joyner SC 1968. Ecdysones and analogs: effects on development and reproduction of insects. *Science* 1611: 1158-1160.

Robbins WE, Kaplanis JN, Thompson JN, Shortino TJ, Joyner SC 1970. Ecdysones and synthetic analogs: molting hormone activity and inhibitive effects on insect growth, metamorphosis and reproduction. *Steroids* 16: 105-125.

Smagghe G, Degheele D 1992. Effects of RH 5849, the first nonsteroidal ecdysteroid agonist, on larvae *Spodoptera lit - toralis* (Boisd.) (Lepidoptera: Noctuidae). *Arch Insect Biochem Physiol* 21: 119-128.

Smagghe G, Degheele D 1994. Action of the nonsteroidal ecdysteroid mimic RH 5849 on larval development and adult reproduction of insects of different orders. *Invert Reprod Develop* 25: 227-236.

Trisyono A, Chippendale M 1997. Effect of the nonsteroidal ecdysone agonists, methoxyfenozide and tebufenozide, on the European corn borer (Lepidoptera: Pyralidae). *J Econ Entomol* 90: 1486-1492.

Wigglesworth VB 1984. Insect Physiology. Chapman & Hall, London, 191 p.

Williams CM 1967. Third-generation pesticides. *Sci Am* 17: 13-17.

Received May 23, 2005
Accepted October 5, 2005