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RESPONSE OF A EUROPEAN CORN BORER, *OSTRINIA NUBILALIS*, POPULATION TO SELECTION WITH AN ECDYSONE AGONIST, TEBUFENOZIDE

TANGGAPAN POPULASI PENGGEREK BATANG JAGUNG, OSTRINIA NUBILALIS, *TERHADAP SELEKSI DENGAN AGONIS EKDISON TEBUFENOZIDE*

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INTISARI

Penelitian ini bertujuan untuk mengetahui tanggapan dari populasi penggerek batang jagung, Ostrinia nubilalis *(Hübner), yang diseleksi setiap generasi dengan agonis ekdison* (ecdysone agonist*) tebufenozide (RH-5992). Seleksi dilakukan terhadap larva yang baru menetas dengan cara melepas larva tersebut pada pakan buatan yang mengandung 0.285 ppm tebufenozide selama empat hari. Seleksi terus-menerus selama tujuh generasi menyebabkan penurunan mortalitas larva, meningkatkan mortalitas pupa, dan menurunkan rata-rata jumlah keturunan per induk. Perbedaan tingkat kepekaan terhadap tebufenozide antara larva* O. nubilalis *yang tidak diseleksi dan yang diseleksi selama empat generasi tidak nyata. Efek akut dan kronis dari tebufenozide menyebabkan musnahnya populasi* O. nubilalis *setelah diseleksi selama tujuh generasi.*

Kata kunci: Ostrinia nubilalis, *tebufenozide, seleksi*

ABSTRACT

Response of a population of the European corn borer, *Ostrinia nubilalis* (Hübner), to selection with an ecdysone agonist, tebufenozide (RH-5992), was investigated. The selection was carried out by exposing, newly hatched larvae of *O. nubilalis* on an artificial diet containing 0.285 ppm tebufenozide for four days. Throughout seven generations of selection, a slight decrease in the larval mortality, an increase in the pupal mortality, and a decrease in the number of offspring produced per female were noticed. After four generations of selection, a shift in susceptibility of the selected *O. nubilalis* larvae to tebufenozide was not observed. The acute and chronic effects of tebufenozide caused the loss of *O. nubilalis* population after seven generations of selection.

Key words: *Ostrinia nubilalis*, tebufenozide, selection

INTRODUCTION

The incidence of insect resistance to insecticides has increased in the last forty years. Georghiou (1990) reported that more than 500 species of arthropods have become resistant to one or more pesticides caused by either a single factor or combination of genetic, biological, ecological, behavior, and operational factors (Georghiou & Taylor, 1976). Rotation of insecticides with different modes of action is a relatively simple approach that has been used to manage resistance (Pimentel & Burgess, 1985; Comins, 1986; Tabashnik, 1989). Therefore, development of selective insecticides that target other sites besides the nervous system has been an important objective of insecticide discovery over the last two decades.

The ecdysone agonists represent a new class of insect growth regulators (IGRs) with novel modes of action. The ecdysone agonists mimic the action of 20-hydroxyecdysone causing the treated larvae to enter a premature and lethal molting cycle (Wing, 1988; Wing *et al.*, 1988). Tebufenozide (RH-5992), an ecdysone agonist, is selective for Lepidoptera (Oakes, 1994; Dhadialla *et al.*, 1998). This compound has potential for controlling the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), a major pest of maize in many countries (Seymour *et al.*, 1996; Trisyono & Chippendale, 1997). Trisyono & Chippendale (1997) reported that tebufenozide is toxic to egg and larvae of *O. nubilalis*. In addition to its acute effects, tebufenozide applied at sublethal concentrations to the larvae of *O. nubilalis* shows chronic effects such as delayed pupation, reduced pupal weight, and reduced adult emergence. Considering the potential of tebufenozide for controlling *O. nubilalis* and the risk of selection toward resistance, the effects of continuous selection with tebufenozide on a population of *O. nubilalis* were investigated.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Insect Physiology, Department of Entomology, University of Missouri, Columbia, MO, USA.

O. nubilalis. A colony of *O. nubilalis*, collected from Fulton, MO, USA in 1995, was maintained on a BioServe artificial diet in 1-oz clear plastic cups using established laboratory procedures (Chippendale & Cassatt, 1985). Larvae were reared individually in plastic cups containing a cube of diet (approximately 12 g) under long days (16 L : 8 D) at 30°C. Pupae were collected twice in a week, and placed into a Petri dish layered with a filter paper. The pupae were then placed in a cage covered with waxed paper on the top of the cage for egg laying and white cloth on the side. Moist paper towel was placed on the waxed paper and moist cotton was placed in the cage to supply moisture for the adults. The waxed paper containing the egg masses was replaced twice in a week, and the paper was placed in a jar supplied with a moist filter paper. The pupae, adults, and eggs were held at 25°C 13 L : 11 D.

Chemical. Technical tebufenozide (RH-5992, *N-tert*-butyl-*N*-3,5-dimethylbenzoyl-*N'*-4-ethylbenzoylhydrazine) containing 95% AI was obtained from Rohm and Haas Co. (Spring House, PA, USA). Analytical grade of acetone was used as a solvent.

Selection of* O. nubilalis *with tebufenozide. Five to ten newly hatched larvae of *O. nubilalis* were transferred into a 1-oz plastic cup containing a cube of diet treated with 0.285 ppm tebufenozide, approximately the LC_{90} (Trisyono & Chippendale, 1997). Four hundreds and forty six larvae were selected initially. After four days of exposure, the surviving larvae that had ecdysed into the second instar were transferred onto fresh control diet for the subsequent development. The larval mortality (%) was determined after four days of exposure on the treated diet. The total larval mortality (%) was determined because additional mortality was observed even after the surviving larvae were fed with control diet. Pupae were collected and sexed daily. The pupal mortality (%) was determined by dividing the number of pupae that did not eclose into adults with the total number of pupae. The colony was maintained using procedures similar to those for the unselected *O. nubilalis*. Selection was carried out every generation until the F_6 generation using the same concentration of tebufenozide.

After two and four generations of selection, the LC_{50}s of tebufenozide against the selected colony were determined on day four after exposure on the treated diet. The resistance ratio (RR) was determined by dividing the LC_{50} of selected colony by the LC_{50} of unselected colony obtained from the previous study (Trisyono & Chippendale, 1997). Probit analyses were carried out using POLO-PC (LeOra Software, 1987).

RESULTS

After four days of exposure, the larval mortality of *O. nubiialis* was >83% for the parent and the F_1. The larval mortality after four days of exposure decreased for the F_2 to the F_6, ranging from 58.5 to 74.3% (Table 1). Additional larval mortality was observed even after the surviving larvae were fed with fresh control diet. The total larval mortality for the parent and F_1 generation was 94.5 and 93.9%, respectively. The total larval mortality became <85% in the following generations (F_2 to F_6). In contrast, the pupal mortality increased with increasing number of selections causing significant decrease in the number of adults obtained after the F_4. Even though ratios between the number of male and female pupae or adults were relatively consistent (approximately 1:1 ratio) throughout the study, the average number of larvae produced per adult female decreased with increasing number of selections. At the last generation (F_6), eight egg masses were laid, but they did not hatch.

The LC_{50} values of tebufenozide on the unselected and selected *O. nubilalis* larvae did not differ significantly (Table 2). Similarly, the slopes of probit lines between the unselected and selected *O. nubilalis* did not differ significantly.

Table 1. Progress toward selection of an *O. nubilalis* population with tebufenozide

Generation	Selected larvae, no	larval mortality, %		Pupae, no		Pupal mortality, %	Adults, no		Total no. larvae produced	Average no. larvae per female
		4d	Total	Male	Female		Male	Female		
Parent	446	87.9	94.5	12	11	13.0	10	10	823	82.3
F_1	823	83.7	93.9	28	22	10.0	24	21	1028	49.0
F_2	878	67.9	80.2	90	84	NA	NA	NA	1200	NA
F_3	1200	58.5	83.7	106	90	21.4	93	61	2268	37.2
F_4	980	71.3	84.4	76	77	52.3	42	31	760	24.5
F_5	760	74.3	81.4	67	74	NA	NA	NA	282	NA
F_6	282	66.7	84.8	28	15	79.1	6	3	0	0

NA = not available. Larvae were exposed on a diet containing 0.285 ppm tebufenozide for four days. Surviving larvae that had ecdysed into the second instar were transferred on to fresh control diet.

Table 2. The LC_{50} values of tebufenozide on he unselected and selected population of *O. nubilalis*

Population[a]	No.larvae	Control mortality, %	Slope ± SE[b]	LC_{50} (95% CL), ppm	Resistance ratio (95%CL)[c]
Unselected[d]	449	0	0.257 ± 0.30a	0.243 (0.205-0.307)	1.0
Selected, F_2	150	3.3	3.19 ± 0.61a	0.227 (0.175-0.287)	1.0 (0.7-1.4)
Selected, F_4	249	0	3.68 ± 0.45a	0.263 (0.229-0.307)	1.2 (0.9-1.6)

LC_{50}s were determined after newly hatched larvae of *O. nubilalis* were exposed for four days on control or treated diet.

[a] Larvae were selected every generation by exposing them on to a diet containing 0.285 ppm tebufenozide for four days.

[b] Slopes followed by the same letter are not significantly different.

[c] Response of the selected population compared with that of the unselected population. If the lower and upper 95% CLs bracket the value 1.0, the two LC_{50}s are not significantly different (Robertson & Preisler, 1992).

[d] Trisyono & Chippendale, 1997

DISCUSSION

A continuous selection of *O. nubilalis* larvae with 0.285 ppm tebufenozide (the expected LC_{90} of seven days after treatment) over four generations did not cause a shift in susceptibility of this species to tebufenozide based on the LC_{50} values. A slight increase in the LC_{50} and the slopes value at the sixth generation compared to those of the parent was due to a decrease in mortality for the larvae receiving low concentrations of tebufenozide. For example, larval mortality after four days of exposure on diet containing 0.125 ppm tebufenozide was 25, 21, and 16% for the parent, the F_2, and the F_4, respectively.

The population of selected *O. nubilalis* was lost because an increase in the pupal mortality and a decrease in the number of offspring produced per female. Even though the number of offspring produced per female decreased since the F_1, the number of larvae for selection was sufficient because relatively large number of adults were available up to the fourth selection. A significant increase in the pupal mortality and a decrease in the adult fertility from the F_3 to the F_4 caused a significant decrease in the total number of larvae produced by the F_4. This decrease may be due to the chronic effects of tebufenozide on the larval growth and development (Trisyono & Chippendale, 1997; 1998). The population was lost at the F_6 because eight egg masses laid by the F_6 females did not hatch. A similar result was reported on the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Smagghe & Degheele, 1997) and on the beet armyworm, *S. exigua* (Hübner) (Smagghe *et al.*, 1998). They found that the LC_{50} values of tebufenozide against larvae of *S. littoralis* did not change after four generations of selection. In contrast, larvae of the beet armyworm, *S. exigua*, selected with tebufenozide over ten generations decreased ten times in its susceptibility to this compound. The population of both species was lost due to a significant decrease in adult fertility (Smagghe & Degheele, 1997; Smagghe *et al.*, 1998). Furthermore, Salem *et al.* (1997) reported that application of tebufenozide to the Indian meal moth, *Plodia interpunctella* (Hübner), caused inhibition of the formation of new oocytes and degeneration of the ovaries.

Even though a continuous selection (six generations) of *O. nubilalis* with tebufenozide did not cause a shift in susceptibility of this species to tebufenozide, insect resistance management programs should be implemented when this material will be used to control a targeted pest. Cross resistance between conventional insecticides or chitin synthesis inhibitors and the ecdysone agonists has been reported in the codling moth, *Cydia pomonella* L. (Sauphanor & Bouvier, 1995), and *S. littoralis* (Smagghe & Degheele, 1997). In addition, Ishaaya *et al.* (1995) reported that a field population of *S. littoralis*, >100 times more resistant to cypermethrin than was the laboratory strain, showed three times less susceptible to tebufenozide or methoxyfenozide than was the laboratory strain when these compounds were applied to the first instar larvae. These results suggest that previous exposure to insecticides may contribute in the adaptation of an insect to a new insecticide with different mode of action, such as ecdysone agonists. The acute and chronic effects of tebufenozide could provide controls for the multivoltine lepidopteran pests and may slow the development of resistance to this compound.

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