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LABORATORY SELECTION FOR BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) RESISTANCE TO METHOXYFENOZIDE

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ABSTRACT

Beet armyworms, *Spodoptera exigua* (Hübner), were artificially selected in the laboratory for resistance to the insect growth regulator, methoxyfenozide. A field collected beet armyworm colony was separated into three cohorts that were independently selected with three concentrations (0.033 ppm, 0.064 ppm, and 0.125 ppm) of methoxyfenozide incorporated into a meridic diet. These concentrations corresponded closely with the LC_{10} (0.033 ppm), LC_{50} (0.072 ppm), and LC_{90} (0.161 ppm), respectively, for the original colony. After seven generations of continuous exposure to methoxyfenozide, resistance in the colony selected at the low concentration did not increase significantly. In contrast, LC_{50} values increased 9.7- and 9.4-fold for the colonies selected at the moderate and high concentrations, respectively, over that of the original colony. Crosses between resistant and susceptible individuals indicated that the resistance was heritable. At 4 d after exposure, mortality of offspring from the reciprocal crosses was intermediate between mortality for the offspring from the parental crosses. When rated at 10 d, mortality of offspring from the reciprocal crosses was not different significantly from offspring from the cross between susceptible parents. These data will be important for developing a management program for beet armyworm resistance to methoxyfenozide.

Key Words: IPM, Intrepid, *Spodoptera exigua*, Insect Growth Regulator.

RESUMEN

Los gusanos trozadores, *Spodoptera exigua* (Hübner), fueron seleccionados artificialmente en el laboratorio para su resistencia al regulador de crecimiento de insectos, metoxifenozido. Una colonia del gusano trozador recolectada en el campo fue separada en tres cohortes que independientemente fueron seleccionados con tres concentraciones (0.033 ppm, 0.064 ppm, y 0.125 ppm) de metoxifenozido incorporadas en una dieta merídica. Estas concentraciones correspondieron de manera cercana con las concentraciones letales CL_{10} (0.033 ppm), CL_{50} (0.072 ppm), y CL_{90} (0.161 ppm), respectivemente, para la colonia original. Después de siete generaciones de ser expuesta continuamente al metoxifenozido, la resistencia en la colonia seleccionada a la concentración menor no aumentó significativamente. En contraste, los valores de CL_{50} aumentaron por 9.7 y 9.4 veces para las colonias seleccionadas de concentraciones moderadas y altas, respectivamente, sobre las de la colonia original. Los cruzes entre los individuos resistentes y susceptibles indicaron que la resistencia puede ser heredada. A los 4 días después de exponerlos, la mortalidad de la progenie de los cruzes recíprocos fue intermedia entre la mortalidad de la progenie de los cruzes de los padres. Cuando fue calibrada a los 10 dias, la mortalidad de la progenie de los cruzes recíprocos no fue significativamente diferente de la progenie de los cruzes entre los padres susceptibles. Estos datos serán importantes para desarrollar un programa de manejo para la resistencia del gusano trozador al metoxifenozido.

The beet armyworm, *Spodoptera exigua* (Hübner), is an occasional pest of cotton, *Gossypium hirsutum* L., in the southern United States. In general, beet armyworm populations remain low due to actions of natural enemies such as the parasitoid *Cotesia* spp., and various predators and pathogens (Mohaghegh et al. 2001; Bianchi et al. 2002). However, these beneficial insects are inadvertently disturbed with numerous insecticide applications for other pests. Cotton in the southern United States has numerous key pests such as the heliothine complex (tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie)); tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois, and numerous stink bug species (Heteroptera: Pentatomidae) that require multiple applications of broad spectrum insecticides annually to prevent economic losses. The introduction of Bollgard cotton has reduced the numbers of insecticide applications applied for tobacco budworm and bollworm (Williams 1996, 2001). However, Bollgard cotton is typically treated multiple times annually with pyrethroids to control bollworms, and organophosphates to control tarnished plant bugs and stink bugs. Those insecticides provide little control of beet armyworms, but can be damaging to beneficial arthropods. Consequently, beet armyworms generally only reach economically damaging levels after insecticides have been applied to control other pests. There are few insecticides currently labeled in cotton that provide effective, economi-

cal control of beet armyworms. When beet armyworm outbreaks occur, these insecticides are applied over large areas; thereby, providing intense selection for the development of resistance.

Methoxyfenozide (Intrepid 2F, Dow AgroSciences, Indianapolis, IN) is one of a few insecticides that provides effective control of beet armyworms in cotton. This insecticide is an insect growth regulator that acts as an agonist of 20-hydroxyecdysone, a key hormone in the molting process (Wing 1988). Methoxyfenozide affects only the larval stage of Lepidoptera (Wing et al. 1988). Intoxicated larvae cease feeding soon after ingestion and eventually die due to a premature molt. Although methoxyfenozide is highly effective against beet armyworm larvae, this compound has little impact on beneficial arthropods (Wing et al. 1988). Therefore, this insecticide is beneficial in integrated pest management systems and preservation of the insect growth regulators is an important concern.

Beet armyworms are inherently tolerant to many insecticides and have a high propensity for developing resistance to insecticides (Wolfenbarger 2002). Much of the research reported on beet armyworm resistance has represented compounds from the organochlorine, organophosphate, carbamate, and pyrethroid classes of insecticides. Little work has been conducted on beet armyworm resistance to insect growth regulators. However, Moulton et al. (2002) demonstrated that the necessary genetic variability needed for beet armyworms to develop resistance to the insect growth regulators, tebufenozide and methoxyfenozide, was present in populations from Thailand, and may be present in field populations from the United States. Because of this, proactive resistance management plans should be in place to ensure the sustainability of this insecticide. Baseline data are currently available for beet armyworm susceptibility to methoxyfenozide (Mascarenhas et al. 1998a, b). Those data are important for resistance management; however, information concerning the genetic heritability of resistance is not available. Understanding the heritability of beet armyworm resistance to methoxyfenozide will be necessary to design appropriate resistance management strategies. The current study was conducted to determine inheritance patterns of beet armyworm resistance to methoxyfenozide as a proactive step in the development of an effective resistance management plan.

Materials and Methods

A colony of beet armyworms was established from larvae collected on pigweed, *Amaranthus* spp., collected during the summer of 2002. This colony was maintained in the laboratory for five generations before the initiation of the experiment. A concentration-mortality bioassay was conducted with the commercial formulation of Intrepid 2F. The insecticide was incorporated into a meridic diet to determine the susceptibility of the colony before selection. Serial dilutions of Intrepid 2F and distilled water were made from a stock solution with a concentration of 1000 ppm of methoxyfenozide active ingredient. Dilutions ranged from 1.6 ppm to 100 ppm plus a non-treated control for initial bioassays. One ml of each solution was incorporated into 100 ml of meridic diet to obtain eight concentrations of treated diet that ranged from 0.016 ppm to 1.0 ppm. Treated and non-treated diet was dispensed into 29.5-ml plastic cups in 2.5-ml aliquots with a repeat pipetter. A total of 60 cups for each concentration was used for the bioassay. A single neonate beet armyworm was placed in each cup, and mortality was rated after 96 h. Mortality was scored as inability of larvae to move after being touched with a blunt probe. A concentration-mortality curve was generated with Probit Analysis and the LC_{50} and LC_{90} values were determined (PROC PROBIT, SAS Institute 1989).

The selection experiments were initiated in November of 2002. Three colonies were independently selected for resistance to methoxyfenozide with three different levels of selection pressure (low, moderate, and high). The concentrations included 0.032 ppm, 0.064 ppm, and 0.125 ppm for the low, moderate, and high levels of selection, respectively. These concentrations corresponded closely with LC_{10} (0.033 ppm), LC_{50} (0.072 ppm), and LC_{90} (0.161 ppm) values, respectively, obtained from the concentration-mortality curve of the original colony. Larvae were exposed to the insecticide by incorporating formulated Intrepid 2F into meridic diet as previously described. However, instead of using 29.5-ml plastic cups, approximately 20 ml of treated diet was dispensed into 236 ml cardboard cups. This facilitated the selection of a greater number of individuals on each concentration compared to making selections to an individual larva in small cups. Approximately 100 neonates were placed into each cup and allowed to feed for 96 h. After 96 h, surviving larvae were transferred individually onto non-treated diet in 29.5-ml cups. Only larvae that appeared to be developing normally (larvae that molted to the second instar) were selected. Dead and moribund larvae were discarded. Larvae were allowed to complete development on the non-treated diet. After pupation, beet armyworms from each colony (i.e., selection pressure) were mass mated (20 males and 20 females) in 3.79-L cardboard containers. At least ten mating containers were established for each colony.

Each of the beet armyworm colonies were exposed to selection at the respective concentrations for seven generations. After seven generations, reciprocal crosses were made with individuals from colonies selected at moderate

and high concentrations to individuals from another laboratory susceptible (LC_{50} = 0.08, slope = 0.72, df = 4, χ^2 = 1.13, P = 0.89) colony. This colony was originally collected from pigweed during 2001. Male beet armyworms from pheromone traps were incorporated into this colony during the summer of 2002. Reciprocal crosses between the laboratory selected colonies and laboratory susceptible colony were conducted during May of 2003. At least ten mating pairs were established for each of the parental colonies and their reciprocal crosses. The laboratory selected colonies were only crossed with the laboratory susceptible colony. Reciprocal crosses were not done between the colonies selected at the moderate and high concentrations.

Offspring from each mating pair (90 per pair) were exposed to a discriminating concentration (0.125 ppm) of formulated Intrepid 2F in meridic diet that corresponded to an LC_{90} for the original colony. Data for percent mortality of offspring was corrected for control mortality by Abbott's Formula (Abbott 1925) and analyzed with analysis of variance (PROC MIXED, Littell et al. 1996).

Results and Discussion

Beet armyworms exposed to moderate and high selection pressures developed 9.7- and 9.4-fold levels of resistance compared to the original colony within seven generations (Fig. 1). The LC_{50} value (95% fiducial limits) for the original colony was 0.07 (0.064-0.082) ppm (slope = 1.60, df = 4, χ^2 = 1.18, P = 0.88). The susceptibility of the colony at the low selection pressure did not change after seven generations of selection (LC_{50} = 0.07, slope = 0.95, df = 5, χ^2 = 8.55, P=0.13). Within seven generations, the LC_{50} value (95% fiducial limits) increased to 0.68 (0.427-1.106) ppm for the colony selected at the moderate selection pressure (slope = 0.93, df = 4, χ^2 =10.25, P = 0.04). Similarly, the

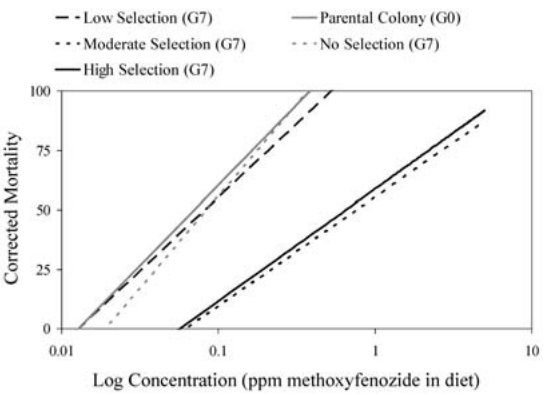

Fig. 1. Results of probit analysis for concentration-mortality curves of beet armyworm susceptibility to methoxyfenozide before and after laboratory selections.

LC_{50} value (95% fiducial limits) for the colony at the high selection pressure increased to 0.66 (0.554-0.809) ppm. The concentration-mortality curves and corresponding LC_{50} values were similar between the colonies selected at the moderate and high concentrations based on overlap of 95% fiducial limits. This may be an indication that the same mechanism of resistance was isolated from the two different selection pressures. Although the two selection pressures yielded similar LC_{50} values, the colony exposed to the high selection pressure appeared to be less variable in its response to the methoxyfenozide treated diet than the colony exposed to the moderate selection pressure. This is evidenced by the wide range of 95% fiducial limits observed with the colony selected at the moderate concentration. Also, the colony at the high selection pressure had a lower χ^2 value than the colony selected at the moderate concentration indicating that they were more homogeneous in their response to methoxyfenozide. This would be expected because the higher selection pressure should create a greater genetic bottleneck; whereas, the moderate selection pressure may have resulted in selection of other factors such as overall health and vigor in addition to the resistance trait.

Based on results from crosses between individuals from the colonies selected at the moderate and high selection pressures with individuals from the susceptible colony, the selected trait was determined to be heritable. However, the mode of inheritance is difficult to determine from these data. Mortality of larvae was significantly different among the different crosses for the colonies selected at the moderate (F = 60.46; df = 3, 22.2; P < 0.01) and high (F = 16.05; df = 3, 29; P < 0.01) pressures at 4 d (Fig. 2). When rated at 4 d, mortality of offspring from the parental crosses for the susceptible and resistant colonies averaged (SEM) 92.8 (2.53) percent and <29.0 (<8.85) percent, respectively. Offspring from the reciprocal crosses had intermediate levels of mortality.

Similar to mortality at 4 d, mortality at 10 d was significantly different among the crosses for the moderate (F = 12.25; df = 3, 32; P < 0.01) and high (F = 8.16; df = 3, 29; P < 0.01) selection pressures (Fig. 2). At 10 d; however, mortality was not significantly different among offspring from the reciprocal crosses and offspring from the parental cross for the susceptible colony. Mortality of offspring from the reciprocal crosses and the parental cross for the susceptible colony ranged from 82.9 (3.67) to 99.1 (0.38) percent for the colony selected at the moderate pressure. Mortality of offspring from the cross between resistant parents averaged 54.3 (9.7) percent. For the colony selected at the high pressure, mortality of offspring from the reciprocal crosses and the parental cross for the susceptible colony ranged from 88.4 (3.75) to 99.1 (0.38) percent; while, mortality of off-

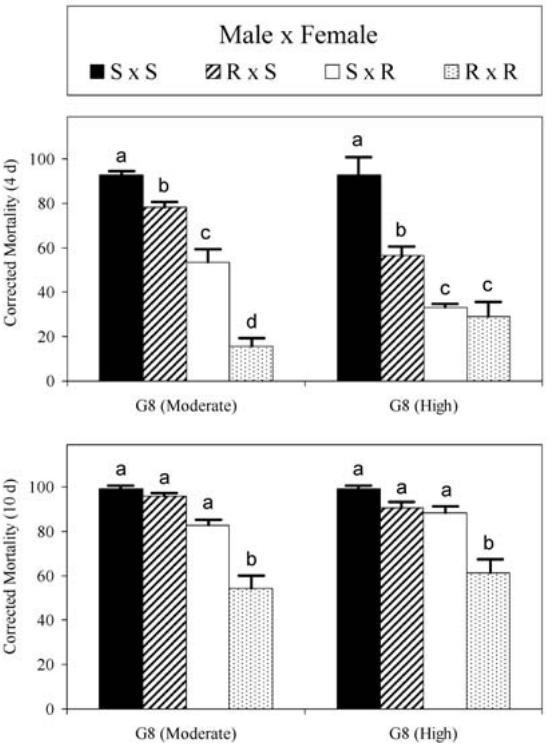

Fig. 2. Mortality of offspring from reciprocal crosses on a discriminating concentration of methoxyfenozide.

spring from the parental cross of the resistant colony averaged 61.4 (8.6) percent.

Based on results of these laboratory selections, the potential for beet armyworms to develop resistance to methoxyfenozide does exist. Beet armyworm larvae from a field-collected colony developed approximately 10-fold level of resistance in a relatively short period of time (seven generations) in the current study. Moulton et al. (2002) isolated a beet armyworm strain from Thailand with 320- to 340-fold level of resistance to methoxyfenozide compared to a laboratory strain. In a similar study, a sex linkage was determined for a beet armyworm strain exhibiting resistance to several insecticides (Wolfenbarger 2002). In that experiment, resistance to fenvalerate and methomyl was associated with the two X chromosomes of the male resistant strain and not the Y chromosome of the female (Wolfenbarger 2002). In contrast, ratings at 4 d from crosses in the current study suggest that the Y chromosome of the female may be important in resistance development. However, when mortality was rated at 10 d, sex-linkage of the trait was not apparent; therefore, no definitive conclusions can be made concerning inheritance of the trait selected in these experiments. More research is necessary to isolate strains of beet armyworms with varying levels of resistance to methoxyfenozide so that they can be crossed with susceptible individuals for multiple generations to determine inheritance of resistance. This information will be important for proactive management of beet armyworm resistance to methoxyfenozide.

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