

Pro-active Management of Beet Armyworm (*Spodoptera exigua*) Resistance to the IGRs, Tebufenozide and Methoxyfenozide

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Abstract

Susceptibility to tebufenozide and methoxyfenozide of beet armyworm (Spodoptera exigua) from the southern United States and Thailand was determined through exposure of first and third instar larvae to dipped cotton leaves. LC₅₀ estimates of first instar larvae ranged from 0.377 to 32.7 micrograms of tebufenozide per milliliter and 0.034 to 11.5 micrograms of methoxyfenozide per milliliter. LC₅₀ estimates of third instar larvae ranged from 4.37 to 715 micrograms of tebufenozide per milliliter and 0.393 to 47.4 micrograms of methoxyfenozide per milliliter. These estimates translated into 87-fold and 164-fold decreases in susceptibility to tebufenozide and 338-fold and 121-fold decreases in susceptibility to methoxyfenozide of first and third instar larvae from a Thailand strain when compared to the most susceptible of eight United States populations evaluated. Among the United States field populations evaluated, a collection from Belle Glade, Florida was the most susceptible and one taken near Parker, Arizona was the least susceptible. Selection of the Thailand population with tebufenozide or methoxyfenozide resulted in significant reductions in susceptibility to both analogs, indicating a common mechanism of resistance. Isolation and characterization of resistance will provide information that will be helpful for pro-active management of resistance for this valuable group of insecticides in the United States.

Introduction

During the past 15 years, a new class of insect growth regulators emerged from the discovery that substituted dibenzoyl hydrazines, or biasacylhydrazines, act as agonists of 20-hydroxyecdysone (Wing 1988, Wing et al. 1988). Acute doses induce a prompt cessation of feeding followed by eventual death through induction of a premature larval molt (Wing et al. 1988; Rohm & Haas Company 1989; Smaghe & Degheele 1994a, b). Chronic doses have a chemosterilizing effect by disrupting both oogenesis and spermatogenesis (Smaghe & Degheele 1994a, b). Signs of acute poisoning include, but are not limited to, double head capsule formation, blackening of the cuticle, stunted growth, extrusion of the hindgut, and loss of hemolymph. The primary route of entry is ingestion.

The sentinel member of this chemical class was discovered by Rohm and Haas Company scientists in 1983 (Hsu 1991). A more potent analog, RH-5849, was discovered later that same year. RH-5849 showed insecticidal activity against certain Lepidoptera, Diptera, and Coleoptera pest species but was not developed commercially due to discoveries of more potent and selective analogs. Most of the early physiological and toxicological studies on the biasacylhydrazines were performed with this compound.

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Tebufenozide (RH-5992) was discovered in 1986. It was found to be much more potent against and selective towards larval Lepidoptera than was RH-5849 (Carlson et al. 1994; Dhadialla et al. 1998). Under the trade names Confirm®, Mimic®, and Romdan®, it is now widely used in several crops throughout the world. The first uses of tebufenozide in the United States occurred in Alabama and Mississippi in 1994 under Section 18 exemptions. Section 18 exemptions have since been granted for specific crop uses in North Carolina, Georgia, Florida, Mississippi, Louisiana, Texas, New Mexico, California, and Arizona. It currently has Section 3 (full) registrations in the United States for use in cotton, cole crops, leafy vegetables, fruiting vegetables, turnip, cranberries, sugar cane, pecan, and walnut.

Methoxyfenozide, or RH-2485, was discovered in 1990 and is the latest compound in this class to be developed commercially. First announced in 1996, it is the most potent analog to date against larval Lepidoptera (Ishaaya et al. 1995, Trisyono & Chippendale 1997, Le et al. 1996). The first sales of methoxyfenozide, as Intrepid®/Runner®, occurred in 1999 (R. K. Jansson, personal communication). First registration of methoxyfenozide in the United States is slated to occur in cotton and pome fruits during 2000 (R. K. Jansson, personal communication).

The widely distributed, polyphagous pest, *Spodoptera exigua*, is one of the major targets for development of tebufenozide and methoxyfenozide in agriculture. Coinciding with the first uses of tebufenozide in Thailand and new registrations in the United States, we initiated a pro-active resistance management program aimed at sustaining the efficacy of this class of chemistry. Complementary building blocks of this resistance management program are the determination of baseline susceptibilities to tebufenozide and methoxyfenozide of field and laboratory reference populations, routine monitoring of susceptibility, and selection, isolation, and characterization of resistance. Isolation of resistance to novel compounds like tebufenozide and methoxyfenozide allows critical practical information to be collected thereafter regarding cross-resistance, genetics and stability of resistance, and the underlying physiological mechanism(s) of resistance.

In January 1996 we began receiving eggs of beet armyworm from several regions in Thailand, three years after use of tebufenozide had commenced in that country. From 1996-1997, a total of ten Thailand populations were evaluated for susceptibility to tebufenozide. Two of these populations displayed significant reductions in susceptibility to tebufenozide in diet incorporation bioassays (JKM, DAP, and TJD, unpublished observations).

In this paper we present data on the baseline susceptibility of *Spodoptera exigua* to tebufenozide and methoxyfenozide and results from laboratory selection of a Thailand strain exhibiting reduced susceptibility to both compounds. It comprises the first of a series of increasingly more in-depth investigations aimed towards providing a better understanding of this resistance. Ultimately, we hope these data will provide insight that will aid in sustaining the efficacy of these valuable new compounds against beet armyworm.

Materials and Methods

Cultures

Field populations were established from samples collected by members of our laboratory and Rohm and Haas Company. Arizona field populations were established from larvae collected from cotton, brought to the Extension Arthropod Resistance Management Laboratory (EARML) in Tucson, AZ, and placed onto artificial diet (*Heliothis* Premix, Stonefly Industries, Bryan, TX) to complete development. Field populations from elsewhere were shipped to EARML as surface sterilized eggs under the terms of USDA-APHIS permit number 39094, and the resulting neonates were allowed to complete development by placing them on the aforementioned artificial diet. The susceptible reference strain was established from eggs shipped to EARML from the USDA-Western Cotton Research Laboratory (WCRL) in Phoenix, AZ.

Rearing

All cultures of *Spodoptera exigua* larvae were reared on Stonefly Industry's *Heliothis* Premix diet, to which was added 5 ml of formalin and 1.5 g of aureomycin (chlortetracycline HCL, Fort Dodge Animal Health, Fort Dodge, IA) to each 2000 g of prepared diet (500 g diet, 1500 ml water) to prevent pathogen growth. Forty to fifty neonates were placed into 5.5 oz plastic cups and incubated at 27°C (16 h photoperiod). Pupae were collected from these cups after 14-21 days and placed into one gallon glass jars with wire mesh lids for adult emergence. Adults were provided 10% sucrose solution and wax paper sheets on which to oviposit. Egg sheets were collected daily, washed in 10% formalin for 10 min., and rinsed in tap water for 10 min.

Leaf-dip Bioassays

First instar larvae. Fully expanded, first true leaves of 2-3 week old cotton plants were dipped for 5 sec. in deionized water solutions containing tebufenozide or methoxyfenozide and 0.01% surfactant (Latron CS-7, Rohm & Haas Co., Philadelphia, PA). Ten replicates were established for each of six to seven concentrations, plus a control (surfactant only). After drying, one leaf each was placed into 1 oz plastic cups containing 10 ml of solidified 2% agar solution. Ten neonates were placed into each plastic cup and incubated at 27°C (16 h photoperiod) for 120 h. Missing and dead larvae were scored as affected. Larvae were scored as dead if no movement was observed after their having been prodded with a dissecting needle. Bioassay data were analyzed using probit analysis (POLO-PC) (LeOra 1987).

Third instar larvae. Leaves were selected and dipped as detailed above in the assay of first instar larvae. Ten to twenty replicates were established for each of six to seven concentrations, plus a control (surfactant only). After drying, pairs of leaves were placed into 100 x 15 mm Petri plates. Two to three ca. 1.5 cm (5-7 day old) third instar larvae were then placed into each Petri plate. The Petri plates were subsequently sealed inside one-gallon plastic zipper bags, each containing a damp paper towel, and incubated at 27°C (16 h photoperiod) for 96 h. Larvae were scored as dead if they exhibited noticeably blackened or malformed cuticle, including a double or slipped head capsule. Missing larvae were not scored. Bioassay data were analyzed using probit analysis (POLO-PC) (LeOra 1987).

Incorporation of Tebufenozide and Methoxyfenozide into Diet

Pre-mixed wettable powder formulations of tebufenozide and methoxyfenozide were obtained from Rohm & Haas Company. These formulations were then further diluted into *Heliothis* Premix diet to obtain mixtures that, upon the addition of three parts water, would yield treated diet ranging in concentration from 0.3 to 320 µg tebufenozide or methoxyfenozide/ml. Some of this treated diet was used to select for increased resistance in the Thailand strain.

Selection for Resistance

The Thailand strain was initially selected on diet containing 10 µg/ml tebufenozide. Approximately 300 third instar larvae were placed individually into 1 oz plastic cups containing approximately 10 ml of treated diet and incubated at 27°C (16 hr. photoperiod) for 96 h. Survivors were transferred into 5.5 oz plastic cups containing untreated diet and allowed to complete development. Progeny of survivors were bioassayed as detailed above to estimate susceptibility to both compounds. The resulting colony was subsequently divided into two strains that were subjected to different selection pressures. One was pressured with 30 µg/ml tebufenozide in diet (= Confirm-selected #1) and again with 78 µg/ml tebufenozide in diet (= Confirm-selected #2). The other was pressured with 10 µg/ml methoxyfenozide in diet (= Intrepid-selected #1). Subsequent generations were not pressured.

Results

Susceptibility to tebufenozide in leaf-dip bioassays

First instar larvae. LC₅₀ estimates ranged from 0.377 µg/ml for Florida (Belle Glade 2) to 32.7 µg/ml for Thailand (Intrepid-selected #1) (Table 1). LC₉₀ values ranged from 1.13 µg/ml for Florida (Belle Glade 2) to 101 µg/ml for Thailand (Intrepid-selected #1) (Table 2). Based upon comparisons of LC₅₀ and LC₉₀ estimates, the Intrepid-selected Thailand strain was 44- to 68-fold less susceptible than the reference strain (Tables 1, 2).

The Florida (Belle Glade 2) strain was the most susceptible to tebufenozide of the U.S. field populations evaluated. In fact, this population was twice as susceptible as the reference strain at the LC₅₀, although their 95% confidence limits overlap slightly at the low end. The Arizona (Parker) strain was the least susceptible U.S. field population evaluated, having LC₅₀ and LC₉₀ estimates of 1.63 and 7.76 µg/ml, respectively. These values were 4.3 and 6.9-fold greater than those of the most susceptible field populations evaluated.

The non-selected Thailand strain exhibited LC₅₀ and LC₉₀ values of 4.41 and 16.9 µg tebufenozide/ml. These values were approximately twice those of the least susceptible U.S. field population, Arizona (Parker). After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 10 µg methoxyfenozide/ml diet, tebufenozide LC₅₀ and LC₉₀ values increased to 32.7 and 101 µg/ml, a 6- to 7-fold further reduction in susceptibility.

Third instar larvae. LC₅₀ values ranged from 4.37 µg/ml for the South Carolina strain to 715 µg/ml for Thailand (Intrepid-selected #1) (Table 1). LC₉₀ values ranged from 7.06 µg/ml for the USDA reference strain to 10,500 µg/ml for Thailand (Intrepid-selected #1) (Table 3). Thailand (Intrepid-selected #1) was 150- to 1500-fold less susceptible to tebufenozide than the reference strain (Tables 3, 4).

The Arizona (Parker) strain, with LC₅₀ and LC₉₀ estimates of 12.0 and 29.6 µg/ml, respectively, was the least susceptible of the U.S. field strains evaluated. The LC₅₀ value for the Parker strain was significantly different from those of the other U.S. field strains evaluated. All U.S. field strains, other than Arizona (Parker), were no more than two-fold less susceptible to tebufenozide than was the reference strain at both the LC₅₀ and LC₉₀ (Tables 3, 4).

After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 78 µg tebufenozide/ml diet, tebufenozide LC₅₀ and LC₉₀ estimates increased from 46.6 and 147 µg/ml to 127 and 2030 µg/ml. These values corresponded to a 2.7- to 13.8-fold further reduction in susceptibility. After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 10 µg methoxyfenozide/ml diet, tebufenozide LC₅₀ and LC₉₀ estimates increased from 46.6 and 147 µg/ml to 715 and 10,500 µg/ml. These values corresponded to a 15- to 71-fold further reduction in susceptibility.

Tebufenozide resistance in the Thailand strains declined in the laboratory in the absence of selection pressure. LC₅₀ estimates for third instar larvae from Thailand (Intrepid-selected #1) declined from 715 µg/ml five months post selection to 351 µg/ml eleven months post selection (Tables 3, 4). LC₉₀ estimates for this strain declined from 10,500 µg/ml to 3,137 µg/ml during this time. Nine months after laboratory colonization, the non-selected Thailand strain exhibited LC₅₀ and LC₉₀ estimates of 46.6 and 147 µg tebufenozide/ml. These values were 4- to 5-fold greater than those of the least susceptible U.S. field population evaluated, Arizona (Parker). After 14 months in the laboratory, LC₅₀ and LC₉₀ values for the non-selected Thailand strain declined to 14.4 and 76.5 µg tebufenozide/ml, only 1.2- and 2.6-fold greater than those observed in Arizona (Parker) (Tables 3, 4).

Methoxyfenozide leaf-dip bioassays

First instar larvae. LC₅₀ values ranged from 0.034 µg/ml for the USDA reference strain to 11.5 µg/ml for Thailand (Intrepid-selected #1) (Table 5). LC₉₀ values ranged from 0.0631 µg/ml for the USDA reference strain to 20.4 µg/ml for Thailand (Intrepid-selected #1) (Table 6). These values corresponded to a 320- to 340-fold reduction in susceptibility of Thailand (Intrepid-selected #1) to methoxyfenozide.

Arizona (Parker), with LC₅₀ and LC₉₀ values of 0.407 and 1.08 µg/ml, respectively, was the least susceptible U.S. field strain evaluated. Based upon these values, the Arizona (Parker) strain was 3.7 to 7.0-fold less susceptible than the most susceptible field population evaluated, Florida (Belle Glade 2). Florida (Belle Glade 2) was the only field strain evaluated that was not significantly different than the reference strain at both the LC₅₀ and LC₉₀.

At the time it was first assayed, the non-selected Thailand strain exhibited LC₅₀ and LC₉₀ values of 0.313 and 1.74 µg tebufenozide/ml. This LC₅₀ value was less than those observed in the two Arizona strains evaluated. After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 10 µg methoxyfenozide/ml diet, methoxyfenozide LC₅₀ and LC₉₀ values increased to 11.5 and 20.4 µg/ml, a 12- to 37-fold further reduction in susceptibility.

Third instar larvae. LC₅₀ values ranged from 0.393 µg/ml for the USDA reference strain to 47.4 µg/ml for Thailand (Confirm-selected #2) (Table 7). LC₉₀ values ranged from 1.83 µg/ml for Florida (Belle Glade 2) to 169 µg/ml for Thailand (Intrepid-selected #1) (Table 8). The most highly resistant Thailand strains were 87- to 120-fold less susceptible to methoxyfenozide than the reference strain (Tables 7, 8).

Arizona (Parker), with LC₅₀ and LC₉₀ values of 2.23 and 5.89 µg/ml, respectively, was the least susceptible U.S. field strain evaluated. These estimates were 3.7- and 3.2-fold greater than those of the most susceptible field populations. The South Carolina and Arizona (Parker) strains were the only ones that differed significantly from the reference strain at the LC₅₀. All U.S. field strains, other than Arizona (Parker) were no more than 5-fold less susceptible to methoxyfenozide than the reference strain (Tables 7, 8).

LC₅₀ and LC₉₀ values for the non-selected Thailand population were 3.83 and 14.1 µg methoxyfenozide/ml, only 0.6 and 2.4 times that observed in the Arizona (Parker) strain. After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 78 µg tebufenozide/ml diet, methoxyfenozide LC₅₀ and LC₉₀ values increased to 47.4 and 130 µg/ml, a 9.2- to 12.4-fold further reduction in susceptibility. After selection of the Thailand strain with 10 µg tebufenozide/ml diet and 10 µg methoxyfenozide/ml diet, methoxyfenozide LC₅₀ and LC₉₀ values increased to 23.5 and 169 µg/ml, a 6.1 to 12-fold further reduction in susceptibility.

Discussion

A Thailand population of beet armyworm collected from the Bangbuathong District, Thailand, an intensive vegetable production area near Bangkok, has been confirmed to be highly resistant to tebufenozide and methoxyfenozide. Susceptibility to tebufenozide of this Thailand population compared to that of the laboratory reference strain, as inferred from leaf-dip bioassays, ranged from 44- to 68-fold for first instar larvae to 150- to 1500-fold for third instar larvae. For methoxyfenozide, these resistance ratios were 320- to 340-fold and 87- to 120-fold, respectively.

Selection of the Thailand population with either analog significantly increased resistance to both, indicating at least some level of cross-resistance. The Thailand strain selected with methoxyfenozide was somewhat more resistant to both compounds than was the one selected entirely with tebufenozide. However, because the Intrepid®-selected strain was initially selected with tebufenozide, it is not possible to discern whether there are some differences in mechanisms of resistance to these analogs or whether the greater toxicity of methoxyfenozide resulted in more effective amplification of resistance. Experiments are currently underway to distinguish between these hypotheses.

This report is the first of tebufenozide and methoxyfenozide resistance in beet armyworm, or any species of *Spodoptera*. Smagghe et al. (1998) were able to decrease susceptibility to tebufenozide of a laboratory strain of beet armyworm after continuous pressuring of larvae for ten consecutive generations on treated diet. However, the shift in susceptibility they observed was only 5- to 10-fold at the LC_{50} and LC_{90} , and no significant shifts were observed until the sixth generation. They also found that piperonyl butoxide synergized tebufenozide activity in this laboratory strain when both compounds were incorporated into the diet. Synergism ratios were 3.4-fold for the non-selected strain and 6-fold for the tenth and eleventh generations of the selected strain. In a similar study, Smagghe and Degheele (1997) were unable to reduce susceptibility to tebufenozide of a laboratory strain of cotton leafworm, *Spodoptera littoralis*, by more than 4- to 5-fold after more than one dozen continuous generations of selection.

The Arizona (Parker) strain was the least susceptible U.S. field strain evaluated. It had the highest LC_{90} estimates in first and third instar larvae leaf-dip assays of both analogs and the highest LC_{50} estimates in assays of both analogs against third instar larvae. Compared to the most susceptible field population, resistance ratios for the Arizona (Parker) population ranged from 2.7-7.1 for all assays conducted. Selection of this Arizona strain in a manner similar to that used for the Thailand strain is currently underway and will hopefully shed light on the mechanism attributable for its decreased sensitivity to these compounds. Because tebufenozide now has Section 3 registrations in cotton and vegetables, it is of vital interest that we carefully monitor sensitivity to it and methoxyfenozide in the intensive vegetable/cotton production system of southwestern Arizona.

Many insecticides, including the benzoylureas, chlorfluazuron (Atabron®), triflumuron (Alsystin®), and teflubenzuron (Nomolt®), have been rendered ineffective in the Bangbuathong District of Thailand due to ill-advised agricultural practices, most notably dilution of insecticide residues on leaves by drench irrigation (Wirojchewan Tawatchai, in. litt.). This practice is likely to blame for the high incidence of insecticide resistance development in this area and the highly accelerated rate of tebufenozide resistance development in beet armyworm. The first signs of tebufenozide resistance occurred there after only three years of use and by the fourth year tebufenozide had been rendered completely ineffective.

We cannot be certain that our findings of tebufenozide and methoxyfenozide resistance in Thailand reflect the eventual path that evolution will take in other populations of beet armyworm around the world. Similarly, we cannot be certain that the reduced susceptibilities we observed in some domestic U.S. populations are representative of the potential for or earliest stages of resistance development to these compounds. However, with the isolation of the strains described herein, we now have the ability to test these and related questions, the results of which will improve our ability to react to beet armyworm resistance, once it occurs in Arizona, and help sustain this valuable new technology. Lastly, our findings of resistance to tebufenozide and methoxyfenozide in Thailand should serve as a warning of the need to steward this extremely valuable new class of insecticides.

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Table 1. Probit regression analysis of responses of first instar BAW larvae to tebufenozide in leaf-dip bioassays. LC_{50} s expressed as μg tebufenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{50} (95% FL)	RR
USDA-WCRL	700	4.19	0.736 (0.419-0.969)	1.0
Florida-Belle Glade2	600	2.69	0.377 (0.233-0.503)	0.51
Florida-Belle Glade1	790	1.77	1.34 (0.828-1.96)	1.8
S. Carolina	700	1.94	1.29 (0.778-1.79)	1.8
AZ-Parker	780	1.89	1.63 (0.663-2.61)	2.2
Mississippi	800	2.37	1.59 (0.950-2.25)	2.2
AZ-Maricopa	700	3.27	1.80 (1.35-2.21)	2.4
Thailand (non-selected)	690	2.20	4.41 (3.01-5.81)	6.0
Thailand (INT-sel.#1)	800	2.62	32.7 (18.0-44.6)	44

Table 2. Probit regression analysis of responses of first instar BAW larvae to tebufenozide in leaf-dip bioassays. LC_{90} s expressed as μg tebufenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{90} (95% FL)	RR
USDA-WCRL	700	4.19	1.49 (1.11-3.67)	1.0
Florida-Belle Glade2	600	2.69	1.13 (0.870-1.64)	0.76
AZ-Maricopa	700	3.27	4.42 (3.52-6.25)	3.0
Mississippi	800	2.37	5.51 (3.78-10.7)	3.7
S. Carolina	700	1.94	5.90 (4.16-10.7)	4.0
Florida-Belle Glade1	790	1.77	7.11 (4.62-13.3)	4.8
AZ-Parker	780	1.89	7.76 (4.85-19.2)	5.2
Thailand (non-selected)	690	2.20	16.9 (12.7-25.1)	11
Thailand (INT-sel.#1)	800	2.62	101 (71.9-218)	68

Table 3. Probit regression analysis of responses of third instar BAW larvae to tebufenozide in leaf-dip bioassays. LC_{50} s expressed as μg tebufenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain. "A" and "B" are results from assays conducted on Intrepid-selected Thailand strain five and 11 months, respectively, after second round of selection. "C" and "D" are results from assays conducted on non-selected Thailand strain 9 and 14 months, respectively, after its colonization in the laboratory.

Population	n	Slope	LC_{50} (95% FL)	RR
USDA-WCRL	509	7.39	4.73 (3.97-5.32)	1.0
S. Carolina	484	3.57	4.37 (3.29-5.24)	0.92
Florida-Belle Glade2	280	5.72	4.45 (3.23-5.14)	0.94
AZ-Maricopa	346	2.84	4.58 (2.28-6.36)	0.97
Florida-Belle Glade1	368	3.54	5.65 (3.72-6.94)	1.2
Mississippi	210	3.85	6.39 (4.78-7.72)	1.4
AZ-Parker	374	3.27	12.0 (9.74-14.5)	2.5
Thailand (non-selected) ^D	227	1.77	14.4 (10.0-21.0)	3.0
Thailand (non-selected) ^C	164	2.57	46.6 (31.2-67.2)	9.9
Thailand (CONF-sel.#2)	604	1.10	127 (60.7-218)	27
Thailand (INT-sel.#1) ^B	232	1.35	351 (219-516)	74
Thailand (INT-sel.#1) ^A	160	1.10	715 (366-1280)	150

Table 4. Probit regression analysis of responses of third instar BAW larvae to tebufenozide in leaf-dip bioassays. LC_{90} s expressed as μg tebufenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain. "A" and "B" represent results from assays conducted on Intrepid-selected Thailand strain five and 11 months, respectively, after second round of selection. "C" and "D" are results from assays conducted on non-selected Thailand strain 9 and 14 months, respectively, after being placed in the laboratory.

Population	n	Slope	LC_{90} (95% FL)	RR
USDA-WCRL	509	7.39	7.06 (6.29-8.37)	1.0
Florida-Belle Glade2	280	5.72	7.45 (6.59-9.32)	1.1
S. Carolina	484	3.57	9.98 (8.32-13.2)	1.4
AZ-Maricopa	346	2.84	12.9 (9.46-24.3)	1.8
Florida-Belle Glade1	368	3.54	13.0 (10.9-18.1)	1.8
Mississippi	210	3.85	13.8 (11.3-18.9)	2.0
AZ-Parker	374	3.27	29.6 (23.1-43.9)	4.2
Thailand (non-selected) ^D	227	1.77	76.5 (74.4-156)	11
Thailand (non-selected) ^C	164	2.57	147 (95.8-321)	21
Thailand (CONF-sel.#2)	604	1.10	2030 (1198-4120)	290
Thailand (INT-sel.#1) ^B	232	1.35	3137 (1895-6884)	440
Thailand (INT-sel.#1) ^A	160	1.10	10500 (5080-32700)	1500

Table 5. Probit regression analysis of responses of first instar BAW larvae to methoxyfenozide in leaf-dip bioassays. LC_{50} s expressed as μg methoxyfenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{50} (95% FL)	RR
USDA-WCRL	700	4.76	0.034 (0.019-0.042)	1.0
Florida-Belle Glade2	700	1.83	0.058 (0.025-0.096)	1.7
S. Carolina	700	3.15	0.149 (0.119-0.182)	4.4
Mississippi	700	4.39	0.218 (0.150-0.263)	6.4
Florida-Belle Glade1	500	2.41	0.303 (0.185-0.419)	8.9
Thailand (non-selected)	700	1.72	0.313 (0.125-0.519)	9.2
AZ-Parker	700	3.02	0.407 (0.286-0.518)	12
AZ-Maricopa	600	4.28	0.487 (0.385-0.583)	14
Thailand (CONF-sel.#2)	700	1.73	2.53 (1.50-3.66)	74
Thailand (INT-sel.#1)	700	5.14	11.5 (8.79-14.0)	340

Table 6. Probit regression analysis of responses of first instar BAW larvae to methoxyfenozide in leaf-dip bioassays. LC_{90} s expressed as μg methoxyfenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{90} (95% FL)	RR
USDA-WCRL	700	4.76	0.0631 (0.053-0.082)	1.0
Florida-Belle Glade2	700	1.83	0.290 (0.181-0.572)	4.6
S. Carolina	700	3.15	0.380 (0.300-0.528)	6.0
Mississippi	700	4.39	0.427 (0.353-0.628)	6.8
AZ-Maricopa	600	4.28	0.971 (0.808-1.25)	15
Florida-Belle Glade1	500	2.41	1.03 (0.750-1.64)	16
AZ-Parker	700	3.02	1.08 (0.849-1.55)	17
Thailand (non-selected)	700	1.72	1.74 (1.14-3.05)	28
Thailand (CONF-sel.#2)	700	1.73	13.9 (9.66-23.3)	220
Thailand (INT-sel.#1)	700	5.14	20.4 (16.3-33.4)	320

Table 7. Probit regression analysis of responses of third instar BAW larvae to methoxyfenozide in leaf-dip bioassays. LC_{50} s expressed as μg methoxyfenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{50} (95% FL)	RR
USDA-WCRL	359	1.85	0.393 (0.211-0.592)	1.0
Mississippi	188	1.72	0.601 (0.409-0.866)	1.5
Florida-Belle Glade2	280	2.67	0.605 (0.420-0.800)	1.5
Florida-Belle Glade1	181	2.11	0.656 (0.463-0.919)	1.7
AZ-Maricopa	148	2.02	0.935 (0.272-1.78)	2.4
S. Carolina	180	4.09	1.80 (1.18-2.36)	4.6
AZ-Parker	196	3.04	2.23 (1.17-3.29)	5.7
Thailand (non-selected)	240	2.26	3.83 (2.25-5.54)	9.8
Thailand (INT-sel.#1)	681	1.50	23.5 (16.5-31.2)	60
Thailand (CONF-sel.#2)	200	2.94	47.4 (29.7-62.4)	120

Table 8. Probit regression analysis of responses of third instar BAW larvae to methoxyfenozide in leaf-dip bioassays. LC_{90} s expressed as μg methoxyfenozide/milliliter. Resistance ratios based upon comparisons to USDA-WCRL strain.

Population	n	Slope	LC_{90} (95% FL)	RR
USDA-WCRL	359	1.85	1.94 (1.33-3.29)	1.0
Florida-Belle Glade2	280	2.67	1.83 (1.33-3.09)	0.94
Florida-Belle Glade1	181	2.11	2.65 (1.74-5.12)	1.4
Mississippi	188	1.72	3.35 (2.10-6.83)	1.7
S. Carolina	180	4.09	3.71 (2.79-6.79)	1.9
AZ-Maricopa	148	2.02	4.04 (2.13-12.6)	2.1
AZ-Parker	196	3.04	5.89 (3.95-13.2)	3.0
Thailand (non-selected)	240	2.26	14.1 (9.57-26.7)	7.3
Thailand (CONF-sel.#2)	200	2.94	130 (93.0-284)	67
Thailand (INT-sel.#1)	681	1.5	169 (122-266)	87