

## Pro-active Management of Beet Armyworm (Lepidoptera: Noctuidae) Resistance to Tebufenozide and Methoxyfenozide: Baseline Monitoring, Risk Assessment, and Isolation of Resistance

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**ABSTRACT** Susceptibility to tebufenozide and methoxyfenozide of beet armyworm [*Spodoptera exigua* (Hübner)] from the southern United States and Thailand was determined through exposure of first and third instars to dipped cotton leaves. Among the field populations evaluated, tebufenozide LC<sub>50</sub> values for first and third instars, respectively, ranged from 0.377 to 4.41 and 4.37–46.6 µg (AI) / ml of solution. Methoxyfenozide LC<sub>50</sub> values for first and third instars of field populations ranged from 0.058 to 0.487 and 0.601–3.83 µg (AI) / ml of solution. A Thailand field strain exhibiting reduced susceptibility to both compounds was subjected to intense laboratory selection for three nonconsecutive generations. At the LC<sub>50</sub> and LC<sub>90</sub>, selected Thailand strains were 45–68 times and 150–1,500 times less susceptible to tebufenozide and 340–320 times and 120–67 times less susceptible to methoxyfenozide as first and third instars, respectively, when compared with the laboratory reference strain. Among the U.S. field populations evaluated, ones from Belle Glade, FL, and Florence, SC, were generally the most susceptible and ones from Maricopa and Parker, AZ, were the least susceptible. Selection of the Thailand field strain with tebufenozide reduced susceptibility to both compounds, and selection of Thailand strains previously pressured with either compound further reduced susceptibility to both, suggesting at least some commonality of resistance mechanism. Characterization of this resistance will provide information that will be helpful for pro-active management of resistance for this valuable group of insecticides.

**KEY WORDS** beet armyworm, insect growth regulator, insect resistance to insecticides, ecdysone agonist

DURING THE PAST fifteen years, a new class of insect growth regulators (IGRs) emerged from the discovery that substituted dibenzoylhydrazines act as agonists of 20-hydroxyecdysone (Wing 1988, Wing et al. 1988). The sentinel member of this chemical class was discovered in 1983 (Hsu 1991). An analog, tebufenozide (RH-5992), proved to be extremely potent against and selective toward larval Lepidoptera (Carlson et al. 1994, Dhadialla et al. 1998) and is now widely used against lepidopteran pests on several crops throughout the world. In the United States, the first uses of tebufenozide occurred in Alabama and Mississippi in 1994 under Section 18 exemptions (Walton et al. 1995). It currently has Section 3 (full) registrations in the United States for use in cotton, cole crops, leafy vegetables, fruiting vegetables, turnips, cranberries, pome fruits, small fruits, sugar cane, and tree nuts.

Methoxyfenozide (RH-2485) is the latest compound in this class to be developed commercially and is the most potent analog to date against larval Lepi-

doptera (Ishaaya et al. 1995; Le et al. 1996; Trisyono and Chippendale 1997; Smagghe et al. 1998b, 1999). The first sales of methoxyfenozide occurred in 1999, and the first full registrations in the United States were granted for cotton and pome fruits in 2000.

The primary route of intoxication for these IGRs is ingestion. Acute doses induce a prompt cessation of feeding followed by eventual death through induction of a premature larval molt (Wing et al. 1988; Rohm and Haas Company 1989; Smagghe and Degheele 1994a, b). Chronic doses have a chemosterilizing effect by disrupting both oogenesis and spermatogenesis (Smagghe and Degheele 1994a, b). Signs of acute poisoning include, but are not limited to, double head capsule formation, cuticular blackening, stunted growth, hindgut extrusion, and hemolymph loss.

Resistance to tebufenozide has been documented in codling moth, *Cydia pomonella* (L.), in southeastern France (Sauphanor and Bouvier 1995, Sauphanor et al. 1998) and greenheaded leafroller, *Planotortrix octo* Dugdale, in New Zealand (Wearing 1998). Sauphanor et al. (1998) observed a 44-fold reduction in susceptibility to tebufenozide in a *C. pomonella* strain selected for resistance to deltamethrin. Wearing (1998) observed a 269-fold decrease in susceptibility to

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tebufenozide of a tebufenozide-selected strain of *P. octo* and cross-resistance between this compound and azinphosmethyl.

With respect to beet armyworm susceptibility to tebufenozide and/or methoxyfenozide, only low-level differences have been observed to date. Mascarenhas et al. (1996, 1998a) detected less than a three-fold difference in tebufenozide susceptibility using a diet overlay assay of neonates from a laboratory strain (USDA-ARS, Stoneville, MS) and field populations taken from Alabama, California, Mississippi, Louisiana, Texas, and Mexico. Mascarenhas et al. (1998b) observed a  $\approx 10$ -fold difference in tebufenozide and methoxyfenozide susceptibility among Louisiana field populations and a laboratory strain (ECOGEN) in five day diet overlay bioassays of third instars. Similarly, low-level differences in tebufenozide susceptibility have been obtained experimentally through laboratory selection. Smagghe et al. (1998a) were able to decrease susceptibility to tebufenozide of a laboratory strain of beet armyworm by 5- to 10-fold after continuous pressuring of larvae for six or more consecutive generations on treated diet.

The widely distributed, polyphagous pest, beet armyworm [*Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)], is one of the major pests for which tebufenozide and methoxyfenozide were developed for crop protection. 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 were 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. This was 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. Three of these populations displayed significant reductions in susceptibility to tebufenozide in diet incorporation bioassays (JKM, unpublished data). In this paper we present data on the baseline susceptibility of *S. exigua* to tebufenozide and methoxyfenozide and results of laboratory selection of a Thailand strain collected from the Bangkok area in 1998 exhibiting resistance to both compounds. It comprises the first in a series of investigations aimed at understanding this resistance to sustain the efficacy of these valuable new compounds against beet armyworm.

## Materials and Methods

**Insects.** Field populations were established from samples collected by members of our laboratory, Dow Agrosiences, 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. Surface sterilization of these eggs involved a 5 min immersion in 3% sodium hypochlorite solution, a 5 min rinse under tap water, and air drying. Neonates hatching from egg sheets were placed onto artificial diet (see below) and allowed to complete development. United States field populations were designated by state and locality names, e.g., AZ, (Parker). The Thailand field strain was designated by country and district name, e.g., Thailand (Bangbuathong). The susceptible reference strain was established from eggs shipped to EARML from the USDA-Western Cotton Research Laboratory (WCRL) in Phoenix, AZ.

**Rearing.** 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 distilled water) to prevent pathogen growth. Forty to fifty neonates each were placed into 175 ml (5.5 oz) plastic cups containing  $\approx 64$  g of diet and incubated at 27°C (16 h photoperiod). Pupae were collected from these cups after 14–21 d, surface sterilized (10 min immersion in 3% sodium hypochlorite solution, 10 min tap water rinse, air dried) and then placed into 3.79 liter (1 gal) 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 a 3.7% formaldehyde solution for 10 min, and rinsed under tap water for 10 min.

**Chemicals.** The insecticides used were flowable liquid tebufenozide (Confirm 2 F, 23% a.i.) and methoxyfenozide (Intrepid 2 F, 24% a.i.) and several wettable powder formulations of tebufenozide and methoxyfenozide, all of which were obtained from Rohm and Haas Company (Philadelphia, PA). Flowable liquid formulations were diluted with distilled water and wettable powder formulations were mixed into *Heliothis* Premix diet (Stonefly Industries, Bryan, TX). The wettable powder formulations were designed such that they contained equivalent amounts of inert ingredients and would yield treated diet ranging from 0.3 to 320  $\mu\text{g}$  tebufenozide or methoxyfenozide/g. Treated diet was also used to select for increased resistance in the Thailand strain, as discussed below.

**Diet Incorporation Bioassays.** Ten to 20 replicates were established for each of five to seven concentrations, plus a control containing diet only. One or two  $\approx 1.5$  cm (5 to 7-d-old) third instars were placed into each of thirty 28 ml (1 oz) plastic cups containing

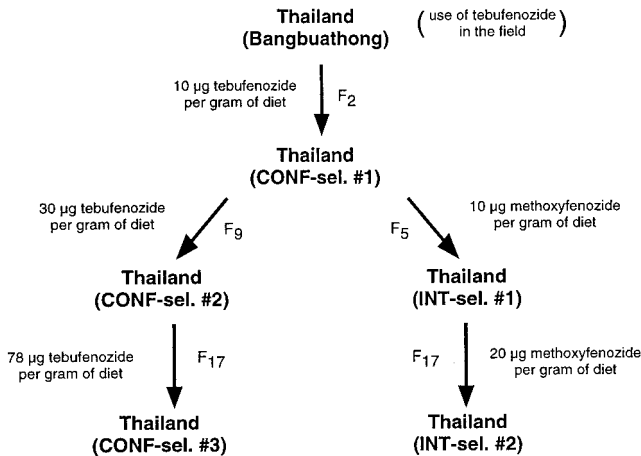


Fig. 1. Selection history of the resistant Thailand strains.

≈10 g of treated or untreated (control group) diet and sealed with plastic lids. The cups were subsequently placed into egg crates and incubated at 27°C (16 h photoperiod) for 96 h. Larvae were scored as dead if they exhibited blackened or malformed cuticle (including a double head capsule), hindgut extrusion, or loss of hemolymph.

**Leaf-dip Bioassays. First Instars.** Fully expanded first true leaves of 2–3 wk old greenhouse grown cotton plants (DPL-5415, Delta Pine and Land Co., Scott, MS) were dipped for five s in deionized water solutions containing tebufenozide or methoxyfenozide and 0.01% surfactant (Latron CS-7™, Rohm and Haas Co., Philadelphia, PA). Ten replicates were established for each of six to seven concentrations, plus a control containing water and surfactant only. After air drying, one leaf each was placed into 28 ml 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 they were prodded with a dissecting needle.

**Third Instars.** Plants were grown and leaves selected and dipped as detailed above for the assay of first instars. Ten to twenty replicates were established for each of six to seven concentrations, plus a control containing water and surfactant only. After drying, pairs of leaves were placed into 100 × 15 mm petri plates. Two to three ≈1.5 cm (5 to 7-d-old) third instars were then placed into each petri plate. The petri plates were subsequently sealed inside 3.79 (1 gal) liter 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 blackened or malformed cuticle (including a double head capsule), hindgut extrusion, or loss of hemolymph. Missing larvae were not scored.

**Selection.** Tebufenozide use in the field in Thailand began in 1994. In December 1997, our collaborators in Thailand treated a field with a high dose of tebufenozide and collected surviving beet armyworm larvae.

They subsequently reared the surviving larvae to adults, and shipped their progeny, as surface sterilized eggs (3% sodium hypochlorite method as described above), to The University of Arizona (EARML). Upon arrival at EARML, selected and unselected substrains of this field strain were established.

Initial laboratory selection of the Thailand field strain involved individual exposure of ≈300 1.5 cm (5 to 7-d-old) third instars each to ca. 10 ml of treated diet in 28 ml (1 oz) plastic cups for 96 h at 27°C (16 h photoperiod). Survivors were transferred in groups of ≈25 larvae into 175 ml (5.5 oz) plastic cups containing untreated diet and allowed to complete development. Subsequent selections were carried out en masse in four liter (17 cup) rectangular plastic containers containing treated diet that was flattened so that pupation chambers would be readily visible. Larvae were exposed until the vast majority had completed or at least initiated the pupal molt. Normally formed pupae were collected and surface sterilized as prescribed above in the section on Rearing.

Progeny of survivors were bioassayed as detailed above to estimate susceptibility to both compounds. Lethal concentration values inferred from dosage-mortality data (see below) from diet incorporation bioassays were used to gauge the intensity of the subsequent selection.

The laboratory selection regime used to increase resistance of the Thailand strains to tebufenozide and methoxyfenozide is depicted in Fig. 1. The initial selection of the Thailand strain occurred at F<sub>2</sub> using 10 µg tebufenozide/g diet. The strain produced by survivors of this selection was named Thailand (CONF-sel. #1). Thailand (CONF-sel. #1) served as the founder of two additional strains that were exposed to different selection regimes, one involving exposure to tebufenozide and the other to methoxyfenozide.

Thailand (CONF-sel. #1) was selected again seven generations after the initial tebufenozide selection, i.e., at F<sub>9</sub>, with 30 µg tebufenozide/g diet. Survivors of this selection founded the strain named Thailand (CONF-sel. #2). Thailand (CONF-sel. #2) was se-

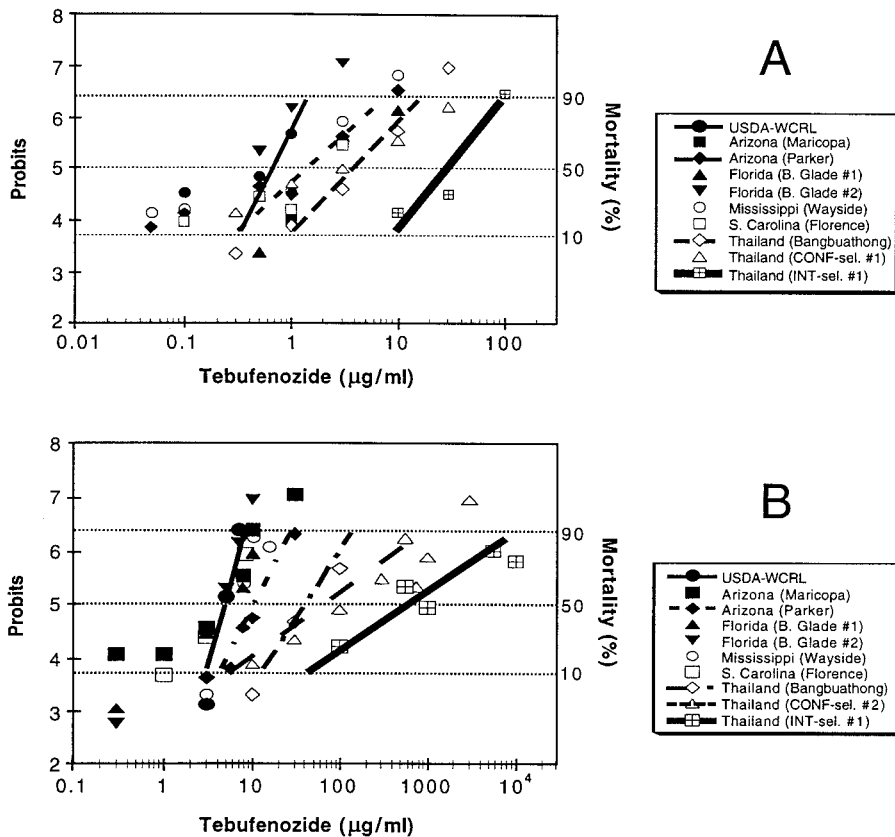


Fig. 2. Susceptibility of resistant and susceptible field populations and a susceptible laboratory population of beet armyworm in cotton leaf-dip bioassays. (A) First instars after 120-h exposure to tebufenozide [probit lines shown for USDA-WCRL, Arizona (Parker), Thailand (Bangbuathong), and Thailand (INT-sel. #1) only]. (B) Third instars after 96-h exposure to tebufenozide [probit lines shown for USDA-WCRL, Arizona (Parker), Thailand (Bangbuathong), Thailand (CONF-sel. #2), and Thailand (INT-sel. #1) only].

lected eight generations later, i.e., at F<sub>17</sub>, with 78 µg tebufenozide/g diet, and the resulting colony was named Thailand (CONF-sel. #3).

Thailand (CONF-sel. #1) was selected with 10 µg methoxyfenozide/g diet three generations after its creation, i.e., at F<sub>5</sub>, and survivors founded the strain named Thailand (INT-sel. #1). Thailand (INT-sel. #1) was selected again 12 generations later, i.e., at F<sub>17</sub>, with 20 µg methoxyfenozide/g diet. The resulting colony was named Thailand (INT-sel. #2). Except as noted above, these selected Thailand strains were not otherwise exposed to pesticides.

**Data Analysis.** Estimations of probit parameters of the concentration-mortality responses from bioassays were calculated by probit analysis using POLO (LeOra 1987). Concentration-mortality responses were entered and analyzed in POLO as individual replicates, rather than as summations of all replicates within a given concentration, hence the larger than typical  $\chi^2$  values and associated degrees of freedom. The median and 90% lethal concentrations (LC<sub>50</sub>s and LC<sub>90</sub>s) and their corresponding 95% CL (CL) were calculated. Resistance, or relative toxicity, ratios and their corresponding 95% CL were calculated using the

method of Robertson and Preisler (1992). The USDA-WCRL strain served as the basis of comparison and was assigned a ratio of 1.0. Lethal concentrations between field populations or between Thailand strains before and after selection were considered significantly different if the 95% CL of their corresponding resistance ratios did not bracket the value of 1.0.

**Results**

**Tebufenozide Leaf-dip Bioassays. First Instars.** Substantial differences were detected in beet armyworm susceptibility to tebufenozide. Estimates of LC<sub>50</sub> values ranged from 0.377 µg/ml for Florida (Belle Glade #2) to 4.41 µg/ml for Thailand (Bangbuathong); estimates of LC<sub>90</sub> values ranged from 1.13 µg/ml for Florida (Belle Glade #2) to 16.9 µg/ml for Thailand (Bangbuathong) (Fig. 2A; Table 1). Comparisons of LC<sub>50</sub> and LC<sub>90</sub> values revealed that the Thailand field strain was 6- to 11-fold less susceptible to tebufenozide than the USDA reference strain.

All U.S. field populations, other than Florida (Belle Glade #2), were less susceptible to tebufenozide than the reference strain at the LC<sub>50</sub> and LC<sub>90</sub> (Table 1).

Table 1. Probit regressions of responses of first-instar beet armyworms to tebufenozide in 120 h cotton leaf-dip bioassays

Population	<i>n</i>	Slope (SE)	LC <sub>50</sub> (95% CL)	LC <sub>90</sub> (95% CL)	χ <sup>2</sup> (df) <sup>a</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain							
USDA-WCRL	700	4.19 (0.913)	0.736 (0.419–0.969)	1.49 (1.11–3.67)	119 (58) <sup>c</sup>	—	—
Field strains							
Florida (Belle Glade #2)	600	2.69 (0.455)	0.377 (0.233–0.503)	1.13 (0.870–1.64)	51.1 (48)	0.51 (0.34–0.76)	0.76 (0.51–1.1)
S. Carolina (Florence)	700	1.94 (0.331)	1.29 (0.778–1.79)	5.90 (4.16–10.7)	61.6 (58)	1.8 (1.2–2.7)	4.0 (2.4–6.6)
Florida (Belle Glade #1)	790	1.77 (0.174)	1.34 (0.828–1.96)	7.11 (4.62–13.3)	142 (68) <sup>c</sup>	1.8 (1.3–2.6)	4.8 (3.0–7.4)
Mississippi (Wayside)	800	2.37 (0.303)	1.59 (0.950–2.25)	5.51 (3.78–10.7)	159 (68) <sup>c</sup>	2.2 (1.5–3.0)	3.7 (2.5–6.0)
Arizona (Parker)	780	1.89 (0.279)	1.63 (0.663–2.61)	7.76 (4.85–19.2)	170 (68) <sup>c</sup>	2.2 (1.5–3.4)	5.2 (3.3–8.3)
Arizona (Maricopa)	700	3.27 (0.520)	1.80 (1.35–2.21)	4.42 (3.52–6.25)	56.6 (58)	2.4 (1.8–3.4)	3.0 (2.0–4.4)
Thailand (Bangbuathong)	690	2.20 (0.279)	4.41 (3.01–5.81)	16.9 (12.7–25.1)	66.4 (57)	6.0 (4.2–8.6)	11 (7.5–17)
Selected strains							
Thailand (CONF-sel. #1)	800	1.25 (0.192)	2.86 (1.13–5.09)	30.2 (19.0–54.0)	64.0 (68)	3.9 (1.8–8.2)	20 (11–36)
Thailand (INT-sel. #1)	800	2.62 (0.504)	32.7 (18.0–44.6)	101 (71.9–218)	111 (68) <sup>c</sup>	45 (31–64)	68 (44–108)

Larvae were scored as dead if they exhibited no movement after prodding. Lethal concentrations are expressed as micrograms of tebufenozide per milliliter of solution.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

<sup>c</sup> Departure from the expected model ( $P < 0.05$ ).

No significant differences were observed among the remaining U.S. field strains evaluated.

**Third Instars.** Differences in susceptibility to tebufenozide were even greater for third instars than they were for first instars. Estimates of LC<sub>50</sub> values ranged from 4.37 μg/ml for the South Carolina strain to 46.6 μg/ml for Thailand (Bangbuathong); estimates of LC<sub>90</sub> values ranged from 7.06 μg/ml for the USDA reference strain to 147 μg/ml for Thailand (Bangbuathong) (Fig. 2B; Table 2). Based upon LC<sub>50</sub> and LC<sub>90</sub> values, the Thailand field strain was 10- to 21-fold less susceptible to tebufenozide than the reference strain.

The Mississippi (Wayside) and Arizona (Parker) strains differed significantly from the reference strain at the LC<sub>50</sub>, and all U.S. field strains, other than South Carolina (Florence) and Florida (Belle Glade #2), differed significantly from the reference strain at the LC<sub>90</sub> (Table 2). The Arizona (Parker) strain, with

LC<sub>50</sub> and LC<sub>90</sub> values of 12.0 and 29.6 μg/ml, respectively, was significantly less susceptible to tebufenozide than was the next least susceptible strain, MS, (Wayside) [Arizona (Parker)/Mississippi (Wayside) RR<sub>50</sub> = 1.9 (95% CL = 1.4–2.5); RR<sub>90</sub> = 2.2 (1.5–3.1)].

**Methoxyfenozide Leaf-dip Bioassays. First Instars.** Differences in susceptibility of first instars to methoxyfenozide were even greater than for tebufenozide. Estimates of LC<sub>50</sub> values ranged from 0.0339 μg/ml for the USDA reference strain to 0.487 μg/ml for Arizona (Maricopa); estimates of LC<sub>90</sub> values ranged from 0.0631 μg/ml for the USDA reference strain to 1.74 μg/ml for Thailand (Bangbuathong) (Fig. 3A; Table 3). Comparisons of LC<sub>50</sub> and LC<sub>90</sub> values revealed that the Thailand field strain was 9.2- to 28-fold less susceptible to methoxyfenozide than the reference strain. The neonate leaf-dip bioassay for Thailand (Bangbuathong) was conducted at F<sub>16</sub>,

Table 2. Probit regressions of responses of third-instar beet armyworms to tebufenozide in 96 h cotton leaf-dip bioassays

Population	<i>n</i>	Slope (SE)	LC <sub>50</sub> (95% CL)	LC <sub>90</sub> (95% CL)	χ <sup>2</sup> (df) <sup>a</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain							
USDA-WCRL	509	7.39 (1.18)	4.73 (3.97–5.32)	7.06 (6.29–8.37)	276 (218)	—	—
Field strains							
S. Carolina (Florence)	484	3.57 (0.605)	4.37 (3.29–5.24)	9.98 (8.32–13.2)	179 (218)	0.93 (0.73–1.2)	1.4 (1.1–1.8)
Florida (Belle Glade #2)	280	5.72 (1.342)	4.45 (3.23–5.14)	7.45 (6.59–9.32)	126 (238)	0.94 (0.75–1.2)	1.1 (0.87–1.3)
AZ (Maricopa)	346	2.84 (0.564)	4.58 (2.28–6.36)	12.9 (9.46–24.3)	268 (165) <sup>c</sup>	0.97 (0.68–1.4)	1.8 (1.3–2.5)
Florida (Belle Glade #1)	368	3.54 (0.762)	5.65 (3.72–6.94)	13.0 (10.9–18.1)	166 (178)	1.2 (0.89–1.6)	1.8 (1.4–2.3)
Mississippi (Wayside)	210	3.85 (0.695)	6.39 (4.78–7.72)	13.8 (11.3–18.9)	45.1 (68)	1.3 (1.1–1.7)	1.9 (1.5–2.5)
Arizona (Parker)	374	3.27 (0.493)	12.0 (9.74–14.5)	29.6 (23.1–43.9)	117 (118)	2.5 (2.0–3.2)	4.2 (3.1–5.7)
Thailand (Bangbuathong)	164	2.57 (0.499)	46.6 (31.2–67.2)	147 (95.8–321)	28.2 (48)	9.9 (6.8–14)	21 (12–36)
Selected strains							
Thailand (CONF-sel. #2)	604	1.10 (0.121)	127 (60.7–218)	2,030 (1,200–4,120)	381 (294) <sup>c</sup>	26 (15–45)	280 (165–464)
Thailand (INT-sel. #2)	364	1.66 (0.185)	177 (115–250)	1,046 (725–1,690)	149 (138)	37 (25–55)	148 (97–225)
Thailand (INT-sel. #1)	160	1.10 (0.164)	715 (366–1,280)	10,500 (5,080–32,700)	125 (138)	150 (82–280)	1,500 (610–3580)

Larvae were scored as dead if they exhibited double head capsule formation, blackened cuticle, hindgut extrusion, or loss of hemolymph. Lethal concentrations are expressed as micrograms of tebufenozide per milliliter of solution.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

<sup>c</sup> Departure from the expected model ( $P < 0.05$ ).

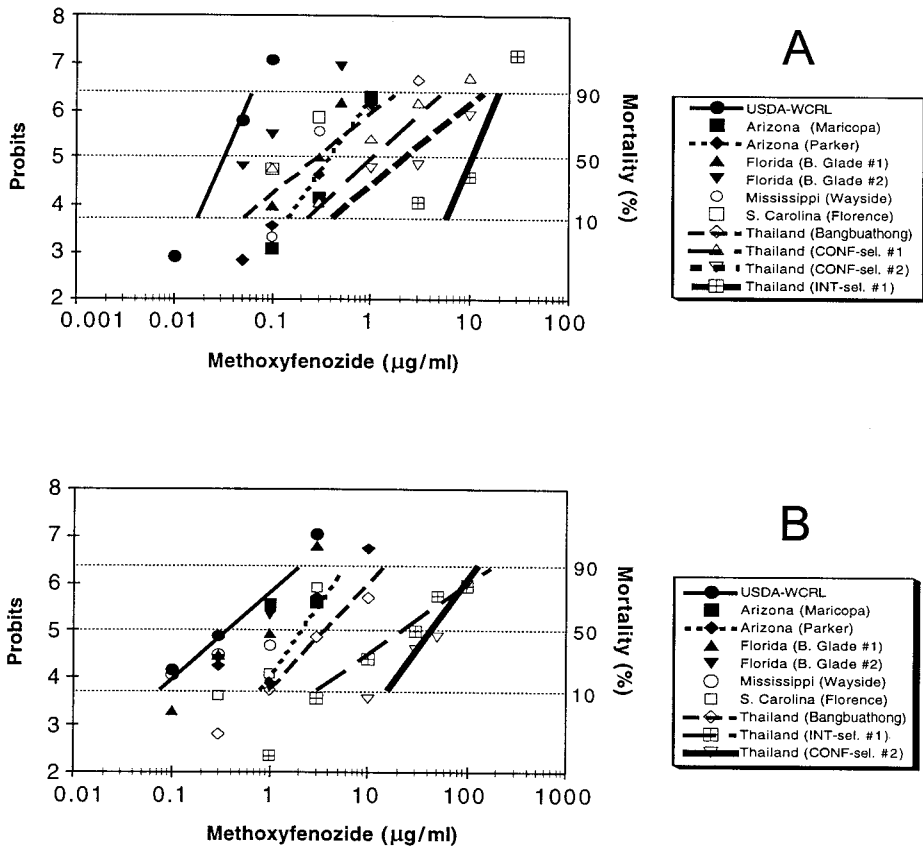


Fig. 3. Susceptibility of resistant and susceptible field populations and a susceptible laboratory population of beet armyworm in cotton leaf-dip bioassays. (A) First instars after 120-h exposure to methoxyfenozide [probit lines shown for USDA-WCRL, Arizona (Parker), Thailand (Bangbuathong), Thailand (CONF-sel. #1), Thailand (CONF-sel. #2), and Thailand (INT-sel. #1) only]. (B) Third instars after 96-h exposure to methoxyfenozide [probit lines are shown for USDA-WCRL, Arizona (Parker), Thailand (Bangbuathong), Thailand (INT-sel. #1), and Thailand (CONF-sel. #2) only].

Table 3. Probit regressions of responses of first-instar beet armyworms to methoxyfenozide in 120 h cotton leaf-dip bioassays

Population	n	Slope (SE)	LC <sub>50</sub> (95% CL) <sup>a</sup>	LC <sub>90</sub> (95% CL) <sup>a</sup>	χ <sup>2</sup> (df) <sup>b</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain							
USDA-WCRL	700	4.76 (1.17)	0.0339 (0.019–0.042)	0.0631 (0.053–0.082)	60.4 (58)	—	—
Field strains							
Florida (Belle Glade #2)	700	1.83 (0.353)	0.058 (0.025–0.096)	0.290 (0.181–0.572)	52.1 (58)	1.7 (0.85–3.4)	4.6 (2.6–8.1)
S. Carolina (Florence)	700	3.15 (0.365)	0.149 (0.119–0.182)	0.380 (0.300–0.528)	65.4 (59)	4.4 (3.0–6.3)	6.0 (4.3–8.3)
Mississippi (Wayside)	700	4.39 (1.02)	0.218 (0.150–0.263)	0.427 (0.353–0.628)	58.9 (58)	6.5 (4.4–9.5)	6.8 (4.9–9.3)
Florida (Belle Glade #1)	500	2.41 (0.347)	0.303 (0.185–0.419)	1.03 (0.750–1.64)	51.3 (38)	9.0 (5.7–14)	16 (11–24)
Thailand (Bangbuathong)	700	1.72 (0.308)	0.313 (0.125–0.519)	1.74 (1.14–3.05)	62.7 (58)	9.2 (4.7–18)	28 (17–45)
Arizona (Parker)	700	3.02 (0.485)	0.407 (0.286–0.518)	1.08 (0.849–1.55)	58.8 (58)	12 (7.9–18)	17 (12–24)
Arizona (Maricopa)	600	4.28 (0.625)	0.487 (0.385–0.583)	0.971 (0.808–1.25)	37.7 (48)	14 (9.9–21)	15 (12–21)
Selected strains							
Thailand (CONF-sel. #1)	790	1.97 (0.336)	1.11 (0.401–1.85)	4.98 (3.04–12.1)	134 (67) <sup>b</sup>	33 (19–57)	79 (50–125)
Thailand (CONF-sel. #2)	700	1.73 (0.247)	2.53 (1.50–3.66)	13.9 (9.66–23.3)	59.9 (58)	75 (45–130)	220 (140–350)
Thailand (INT-sel. #1)	700	5.14 (1.00)	11.5 (8.79–14.0)	20.4 (16.3–33.4)	87.0 (58) <sup>b</sup>	340 (240–480)	320 (240–440)

Larvae were scored as dead if they exhibited no movement after prodding. Lethal concentrations are expressed as micrograms of methoxyfenozide/milliliter of solution.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Departure from the expected model ( $P < 0.05$ ).

<sup>c</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

Table 4. Probit regressions of responses of third-instar beet armyworms to methoxyfenozide in 96 h cotton leaf-dip bioassays

Population	<i>n</i>	Slope (SE)	LC <sub>50</sub> (95% CL)	LC <sub>90</sub> (95% CL)	χ <sup>2</sup> (df) <sup>a</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain							
USDA-WCRL	359	1.85 (0.300)	0.393 (0.211–0.592)	1.94 (1.33–3.29)	81.1 (118)	—	—
Field strains							
Mississippi (Wayside)	188	1.72 (0.234)	0.601 (0.409–0.866)	3.35 (2.10–6.83)	49.0 (58)	1.5 (0.84–2.8)	1.7 (0.84–3.5)
Florida (Belle Glade #2)	280	2.67 (0.462)	0.605 (0.420–0.800)	1.83 (1.33–3.09)	94.5 (238)	1.5 (0.87–2.7)	0.94 (0.52–1.7)
Florida (Belle Glade #1)	181	2.11 (0.307)	0.656 (0.463–0.919)	2.65 (1.74–5.12)	56.9 (57)	1.7 (0.93–3.0)	1.4 (0.69–2.7)
Arizona (Maricopa)	148	2.02 (0.535)	0.935 (0.272–1.78)	4.04 (2.13–12.6)	43.5 (47)	2.4 (0.95–5.9)	2.1 (0.88–4.9)
S. Carolina (Florence)	180	4.09 (1.04)	1.80 (1.18–2.36)	3.71 (2.79–6.79)	39.7 (58)	4.6 (2.6–8.1)	1.9 (1.1–3.4)
Arizona (Parker)	196	3.04 (0.785)	2.23 (1.17–3.29)	5.89 (3.95–13.2)	41.7 (58)	5.7 (2.9–11)	3.0 (1.6–5.9)
Thailand (Bangbuathong)	240	2.26 (0.426)	3.83 (2.25–5.54)	14.1 (9.57–26.7)	103 (118)	9.7 (5.1–18)	7.3 (3.8–14)
Selected strains							
Thailand (INT-sel. #1)	681	1.50 (0.146)	23.5 (16.5–31.2)	169 (122–266)	382 (298) <sup>c</sup>	59 (34–100)	86 (49–150)
Thailand (INT-sel. #2)	376	2.86 (0.358)	26.7 (19.3–33.6)	75.0 (59.7–103)	181 (138)	68 (40–120)	39 (24–63)
Thailand (CONF-sel. #2)	200	2.94 (0.737)	47.4 (29.7–62.4)	130 (93.0–284)	47.8 (58)	120 (69–210)	67 (36–130)

Larvae were scored as dead if they exhibited double head capsule formation, blackened cuticle, hindgut extrusion, or loss of hemolymph. Lethal concentrations are expressed as micrograms of methoxyfenozide/milliliter of solution.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Departure from the expected model ( $P < 0.05$ ).

<sup>c</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

which was several generations later than similar assays of other field populations. This discrepancy might be the reason why it exhibited a lower LC<sub>50</sub> value than did the two Arizona strains evaluated (Table 3).

All U.S. field strains, except Florida (Belle Glade #2), were less susceptible than the reference strain at the LC<sub>50</sub> and all U.S. field strains were less susceptible than the reference strain at the LC<sub>90</sub> (Table 3). The Arizona (Maricopa) strain differed significantly from the least susceptible non-Arizona field strain, FL, (Belle Glade #1), at the LC<sub>50</sub> [RR<sub>50</sub> = 1.6 (1.1–2.3)] but not at the LC<sub>90</sub> [RR<sub>90</sub> = 0.94 (0.65–1.4)]. Thus, we detected a significant difference in susceptibility of first instars from U.S. populations to methoxyfenozide.

**Third Instars.** The magnitude of differences in susceptibility to methoxyfenozide of third instars was very comparable to that observed for tebufenozide. Estimates of LC<sub>50</sub> values ranged from 0.393 μg/ml for the USDA reference strain to 3.83 μg/ml for Thailand (Bangbuathong); estimates of LC<sub>90</sub> values ranged

from 1.83 μg/ml for Florida (Belle Glade #2) to 14.1 μg/ml for Thailand (Bangbuathong) (Fig. 3B; Table 4). Based upon these values, the Thailand field strain was 7.3- to 9.7-fold less susceptible to methoxyfenozide than the reference strain.

The South Carolina (Florence) and Arizona (Parker) strains were less susceptible than the reference strain at the LC<sub>50</sub> and LC<sub>90</sub> (Table 4). The South Carolina (Florence) and Arizona (Parker) strains were significantly less susceptible than the most susceptible field strain evaluated, MS, (Wayside), at the LC<sub>50</sub> [SC(Florence)/m(Wayside) RR<sub>50</sub> = 3.0 (95% CL = 1.9–4.8); AZ(Parker)/m(Wayside) RR<sub>50</sub> = 3.7 (2.1–6.6)] but not the LC<sub>90</sub> [SC(Florence)/m(Wayside) RR<sub>90</sub> = 1.1 (0.57–2.2); AZ(Parker)/m(Wayside) RR<sub>90</sub> = 1.8 (0.83–3.7)].

**Diet Incorporation Bioassays. Tebufenozide.** Estimates of LC<sub>50</sub> and LC<sub>90</sub> values were 1.36 and 3.30 μg/g for the USDA reference strain and 6.60 and 18.4 μg/g for Thailand (Bangbuathong). These values repre-

Table 5. Probit regressions of responses of third instars of a laboratory susceptible and selected and unselected Thailand strains to tebufenozide in 96 h diet incorporation bioassays

Population	<i>n</i>	Slope (SE)	LC <sub>50</sub> (95% CL)	LC <sub>90</sub> (95% CL)	χ <sup>2</sup> (df) <sup>a</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain							
USDA-WCRL	610	3.34 (0.263)	1.36 (1.04–1.83)	3.30 (2.36–5.85)	160 (38) <sup>c</sup>	—	—
Field strain							
Thailand (Bangbuathong)	510	2.79 (0.230)	6.40 (5.54–7.35)	18.4 (15.3–23.3)	133 (134)	4.7 (3.9–5.7)	5.6 (4.2–7.5)
Selected strains							
Thailand (CONF-sel. #1)	120	4.94 (1.05)	23.5 (19.0–29.1)	42.7 (33.4–70.3)	20.2 (28)	17 (14–22)	13 (8.9–19)
Thailand (CONF-sel. #3)	462	1.60 (0.139)	43.8 (34.8–54.7)	277 (199–430)	178 (178)	32 (25–41)	83 (54–130)
Thailand (INT-sel. #1)	100	2.41 (0.452)	26.0 (17.5–35.7)	88.4 (60.4–175)	12.7 (18)	19 (13–28)	27 (16–46)
Thailand (INT-sel. #2)	320	2.28 (0.621)	63.5 (24.6–91.1)	232 (167–503)	127 (138)	47 (28–78)	70 (44–110)

Larvae were scored as dead if they exhibited double head capsule formation, blackened cuticle, hindgut extrusion, or loss of hemolymph. Lethal concentrations are expressed as micrograms of tebufenozide/gram of diet.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Departure from the expected model ( $P < 0.05$ ).

<sup>c</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

**Table 6.** Probit regressions of responses of third instars of a laboratory susceptible and selected and unselected Thailand strains to methoxyfenozide in 96 h diet incorporation bioassays

Population	<i>n</i>	Slope (SE)	LC <sub>50</sub> (95% CL) <sup>a</sup>	LC <sub>90</sub> (95% CL) <sup>a</sup>	χ <sup>2</sup> (df) <sup>b</sup>	RR <sub>50</sub> (95% CL) <sup>b</sup>	RR <sub>90</sub> (95% CL) <sup>b</sup>
Laboratory strain USDA-WCRL	150	3.31 (0.941)	0.282 (0.153–0.381)	0.689 (0.499–1.55)	60.9 (118)	—	—
Field strain Thailand (Bangbuathong)	441	2.46 (0.260)	1.52 (1.25–1.81)	5.03 (4.00–6.91)	52.5 (83)	5.4 (3.4–8.6)	7.3 (3.7–14)
Selected strains Thailand (CONF-sel. #1)	442	1.74 (0.153)	5.04 (3.82–6.73)	27.4 (18.0–50.9)	144 (84) <sup>b</sup>	18 (12–27)	40 (22–71)
Thailand (INT-sel. #1)	150	2.33 (0.359)	9.41 (5.62–14.2)	33.4 (21.2–76.1)	61.2 (38)	33 (21–54)	49 (26–89)
Thailand (INT-sel. #2)	132	2.52 (0.421)	7.12 (5.05–10.1)	23.0 (15.1–46.8)	54.8 (97)	25 (15–41)	33 (17–65)

Larvae were scored as dead if they exhibited double head capsule formation, blackened cuticle, hindgut extrusion, or loss of hemolymph. Lethal concentrations are expressed as micrograms of methoxyfenozide/gram of diet.

<sup>a</sup> Pearson chi-square statistic (degrees of freedom).

<sup>b</sup> Departure from the expected model ( $P < 0.05$ ).

<sup>c</sup> Resistance ratio with 95% confidence limits as calculated by the method of Robertson and Preisler (1992) using the USDA-WCRL strain as the ratio divisor.

sented significant 4.7 (3.9–5.7)- to 5.6 (4.2–7.5)-fold differences in susceptibility to tebufenozide (Table 5).

**Methoxyfenozide.** Estimates of LC<sub>50</sub> and LC<sub>90</sub> values were 0.282 and 0.689 μg/g for the USDA reference strain and 1.52 and 5.03 μg/g diet for Thailand (Bangbuathong). These values represented significant 5.4 (3.4–8.6)- to 7.3 (3.7–14)-fold differences in susceptibility to methoxyfenozide (Table 6).

**Selection. Tebufenozide Leaf-dip Bioassays of First Instars.** The Thailand field strain, Thailand (Bangbuathong), exhibited LC<sub>50</sub> and LC<sub>90</sub> values of 4.41 and 16.9 μg/ml (Table 1) before exposure to tebufenozide in the laboratory. A single selection with 10 μg tebufenozide/g diet at F<sub>2</sub> left LC<sub>50</sub> and LC<sub>90</sub> values for the resulting strain, Thailand (CONF-sel. #1), virtually unchanged at 2.86 and 30.2 μg/ml [RR<sub>50</sub> = 0.65 (0.30–1.4); RR<sub>90</sub> = 1.8 (1.0–3.2)] (Table 1).

Further selection of Thailand (CONF-sel. #1) with 10 μg methoxyfenozide/g diet at F<sub>5</sub> yielded Thailand (INT-sel. #1), which exhibited LC<sub>50</sub> and LC<sub>90</sub> values of 32.7 and 101 μg/ml. Thus, with this bioassay Thailand (INT-sel. #1) was 45- to 68-fold less susceptible to tebufenozide when compared with the USDA reference strain (Table 1).

**Tebufenozide Leaf-dip Bioassays of Third Instars.** Selection of Thailand (Bangbuathong) with 10 μg tebufenozide/g diet at F<sub>2</sub> and 30 μg tebufenozide/g diet at F<sub>9</sub> increased tebufenozide LC<sub>50</sub> and LC<sub>90</sub> values from 46.6 and 147 μg/ml to 127 and 2030 μg/ml for Thailand (CONF-sel. #2) (Table 2). These values represented a 2.7 (1.4–5.1)- to 13 (6.3–28)-fold significant further reduction in susceptibility to tebufenozide.

Selection of the Thailand strain initially pressured with 10 μg tebufenozide/g diet, i.e., Thailand (CONF-sel. #1), with 10 μg methoxyfenozide/g diet at F<sub>5</sub> yielded Thailand (INT-sel. #1), which exhibited LC<sub>50</sub> and LC<sub>90</sub> values of 715 and 10,500 μg/ml. These values corresponded to a 15 (7.6–31)- to 71 (25–200)-fold further reduction in susceptibility to tebufenozide compared with the unselected field strain. Thailand (INT-sel. #1) was 150- to 1500-fold less susceptible to tebufenozide than the USDA reference strain in this

bioassay (Table 2). Selection of Thailand (INT-sel. #1) with 20 μg methoxyfenozide/g diet at F<sub>17</sub> yielded a strain, Thailand (INT-sel. #2), that when compared with Thailand (INT-sel. #1) exhibited decreased resistance to tebufenozide in this bioassay [RR<sub>50</sub> = 0.25 (0.12–0.50); RR<sub>90</sub> = 0.10 (0.038–0.26)] (Table 2) but increased resistance to tebufenozide in the diet incorporation bioassay [RR<sub>50</sub> = 2.4 (1.3–4.4); RR<sub>90</sub> = 2.6 (1.4–5.0)] (Table 5).

**Methoxyfenozide Leaf-dip Bioassays of First Instars.** After a single selection of Thailand (Bangbuathong) with 10 μg tebufenozide/g diet at F<sub>2</sub>, methoxyfenozide LC<sub>50</sub> and LC<sub>90</sub> values increased from 0.313 and 1.74 μg/ml to 1.11 and 4.98 μg/ml (Table 3). The difference in LC values before and after this selection was significant [RR<sub>50</sub> = 3.6 (1.7–7.6); RR<sub>90</sub> = 2.9 (1.6–5.2)]. Selection of Thailand (CONF-sel. #1) with 30 μg tebufenozide/g diet at F<sub>9</sub> yielded Thailand (CONF-sel. #2), which exhibited methoxyfenozide LC<sub>50</sub> and LC<sub>90</sub> values of 2.53 and 13.9 μg/ml. These differences represented a 2.2 (1.2–4.2)- to 2.8 (1.6–5.0)-fold significant further reduction in susceptibility. Thailand (CONF-sel. #2) was eight-fold [RR<sub>50</sub> = 8.1 (3.9–17)]; RR<sub>90</sub> = 8.0 (4.4–15)] less susceptible to methoxyfenozide than the unselected field strain.

Selection of Thailand (CONF-sel. #1) with 10 μg methoxyfenozide/g diet at F<sub>5</sub> increased methoxyfenozide LC<sub>50</sub> and LC<sub>90</sub> values from 1.11 and 4.98 μg/ml to 11.5 and 20.4 μg/ml (Table 3). These differences represented a 10 (5.9–18)- to 4.1 (1.5–11)-fold significant further reduction in susceptibility to methoxyfenozide before and after selection. Thailand (INT-sel. #1) was 37 (20–69)- to 12 (7.2–19)-fold further reduced in susceptibility to methoxyfenozide compared with the unselected field strain and 340- to 320-fold less susceptible than the USDA reference strain with this bioassay (Table 3).

**Methoxyfenozide Leaf-dip Bioassays of Third Instars.** After selection of Thailand (Bangbuathong) with 10 μg tebufenozide/g diet at F<sub>2</sub> and 30 μg tebufenozide/g diet at F<sub>9</sub>, methoxyfenozide, LC<sub>50</sub> and LC<sub>90</sub> values for the resulting strain, Thailand (CONF-sel. #2), increased from 3.83 and 14.1 μg/ml to 47.4

and 130  $\mu\text{g}/\text{ml}$  (Table 4). These differences represented a 12 (7.4–21)- to 9.2 (4.8–18)-fold significant further reduction in susceptibility compared with the unselected field strain.

After selection of Thailand (Bangbuathong) with 10  $\mu\text{g}$  tebufenozide/g diet at  $F_2$  and 10  $\mu\text{g}$  methoxyfenozide/g diet at  $F_5$ , methoxyfenozide  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values increased from 3.83 and 14.1  $\mu\text{g}/\text{ml}$  (unselected field strain) to 23.5 and 169  $\mu\text{g}/\text{ml}$  (Thailand-INT sel. #1). These differences represented a 6.1 (3.7–10) to 12 (6.6–21)-fold significant further reduction in susceptibility compared with the unselected field strain. Selection of Thailand (INT-sel. #1) with 20  $\mu\text{g}$  methoxyfenozide/g diet at  $F_{17}$  yielded Thailand (INT-sel. #2). This strain did not exhibit a further reduction in susceptibility to methoxyfenozide in this bioassay [ $\text{RR}_{50} = 1.1$  (0.80–1.6);  $\text{RR}_{90} = 0.45$  (0.30–0.67)] (Table 4) nor in the diet bioassay [ $\text{RR}_{50} = 1.1$  (0.81–1.6);  $\text{RR}_{90} = 0.45$  (0.30–0.67)] (Table 6). Thailand (CONF-sel. #2) and Thailand (INT-sel. #1), respectively, were 120- to 67-fold and 59- to 86-fold less susceptible than the USDA reference strain with this bioassay (Table 4).

**Tebufenozide Diet Incorporation Bioassays.** Before selection Thailand (Bangbuathong) exhibited  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 6.40 and 18.4  $\mu\text{g}/\text{g}$  (Table 5). These values increased significantly [ $\text{RR}_{50} = 3.7$  (2.9–4.6);  $\text{RR}_{90} = 2.3$  (1.6–3.4)] to 23.5 and 42.7 after a single selection at  $F_2$  with 10  $\mu\text{g}$  tebufenozide/g of diet [= Thailand (CONF-sel. #1)]. Selection of Thailand (CONF-sel. #1) with 30  $\mu\text{g}$  tebufenozide/g of diet at  $F_9$  and 78  $\mu\text{g}$  tebufenozide/g of diet at  $F_{17}$  resulted in a colony, Thailand (CONF-sel. #3), that exhibited  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 43.8 and 277  $\mu\text{g}/\text{g}$ . The  $\text{LC}_{90}$  values before and after this selection were also significantly different [ $\text{RR}_{50} = 1.8$  (1.4–2.5);  $\text{RR}_{90} = 6.4$  (3.9–11)]. Thailand (CONF-sel. #3) was 6.8 (5.2–8.9)- to 15 (9.6–23)-fold reduced in susceptibility to tebufenozide when compared with the unselected field strain.

Selection of Thailand (CONF-sel. #1) with 10  $\mu\text{g}$  methoxyfenozide/g of diet at  $F_5$  yielded a strain, Thailand (INT-sel. #1), that exhibited tebufenozide  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 26.0 and 88.4  $\mu\text{g}/\text{g}$  (Table 5) versus 23.5 and 42.7  $\mu\text{g}/\text{g}$  for Thailand (CONF-sel. #1), a slight response to selection [ $\text{RR}_{50} = 1.1$  (0.75–1.6);  $\text{RR}_{90} = 2.1$  (1.2–3.7)]. Thailand (INT-sel. #1) was 4.1 (2.8–5.9)- to 4.8 (2.8–8.3)-fold reduced in susceptibility to tebufenozide compared with the unselected field strain with this bioassay. Selection of Thailand (INT-sel. #1) with 20  $\mu\text{g}$  methoxyfenozide/g of diet at  $F_{17}$  yielded Thailand (INT-sel. #2), which exhibited  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 63.5 and 232  $\mu\text{g}/\text{g}$ . This change represented a 2.4 (1.3–4.4)- to 2.6 (1.4–5.0)-fold significant reduction in susceptibility to tebufenozide. The most highly resistant Thailand strains, Thailand (CONF-sel. #3) and Thailand (INT-sel. #2) were 32- to 83-fold and 47- to 70-fold less susceptible to tebufenozide than the USDA reference strain with this bioassay (Table 5).

**Methoxyfenozide Diet Incorporation Bioassays.** Before selection Thailand (Bangbuathong) exhibited

$\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 1.52 and 5.03  $\mu\text{g}/\text{g}$  (Table 6). These values increased to 5.04 and 27.4  $\mu\text{g}/\text{g}$  after a single selection with 10  $\mu\text{g}$  tebufenozide/g diet at  $F_2$  [= Thailand (CONF-sel. #1)]. This difference represented a 3.3 (2.3–4.7)- to 5.4 (2.9–10)-fold significant further reduction in susceptibility to methoxyfenozide.

Selection of Thailand (CONF-sel. #1) with 10  $\mu\text{g}$  methoxyfenozide/g diet at  $F_5$  yielded Thailand (INT-sel. #1), which exhibited methoxyfenozide  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 9.41 and 33.4  $\mu\text{g}/\text{g}$  (Table 6). These values represented only a slight increase from the 5.04 and 27.4  $\mu\text{g}/\text{g}$  values for Thailand (CONF-sel. #1) [ $\text{RR}_{50} = 1.9$  (1.3–2.8);  $\text{RR}_{90} = 1.22$  (0.69–2.2)], but a 6- [ $\text{RR}_{50} = 6.2$  (4.0–9.6)] to seven-fold [ $\text{RR}_{90} = 6.6$  (3.4–13)] significant reduction in susceptibility to methoxyfenozide compared with the unselected field strain from which it was derived. Selection of Thailand (INT-sel. #1) with 20  $\mu\text{g}$  methoxyfenozide/g of diet at  $F_{17}$  yielded a strain, Thailand (INT-sel. #2), which exhibited  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 7.12 and 23.0  $\mu\text{g}/\text{g}$ . Lethal concentration values before and after this selection were not statistically different [ $\text{RR}_{50} = 0.75$  (0.47–1.2);  $\text{RR}_{90} = 0.68$  (0.34–1.3)]. The most highly resistant Thailand strain in this bioassay, Thailand (INT-sel. #1), was 33- to 49-fold less susceptible to methoxyfenozide than the USDA reference strain (Table 6).

## Discussion

**Confirmation of Tebufenozide Resistance in a Thailand Population of Beet Armyworm.** Our findings indicate that severe resistance to the insect growth regulators tebufenozide and methoxyfenozide occurred in a few growing seasons when these insecticides were used excessively. A 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 (Fig. 2) and methoxyfenozide (Fig. 3). This is the first report of a major resistance to tebufenozide and methoxyfenozide in the beet armyworm or any other species of *Spodoptera*.

Susceptibility to tebufenozide of this field population compared with that of a laboratory reference strain, as inferred from leaf-dip bioassays, differed at the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  from 6.0- to 11-fold for first instars (Table 1) to 9.9- to 21-fold for third instars (Table 2). For methoxyfenozide, resistance ratios were 14- to 15-fold (Table 3) and 9.7- to 7.3-fold (Table 4), respectively, at the  $\text{LC}_{50}$  and  $\text{LC}_{90}$ . These levels of resistance appear to understate field observations in Thailand. Tebufenozide use in the Bangbuathong District began in 1994, and the first signs of resistance occurred in 1997. By 1998, after only four years of use, tebufenozide had been rendered completely ineffective (Wirojchewan Tawatchai, personal communication).

Laboratory selection of the Thailand field strain better demonstrated the magnitude of this resistance. After two to three intensive selections upon noncon-

secutive generations with tebufenozide or methoxyfenozide, resistance in this Thailand population at the  $LC_{50}$  and  $LC_{90}$  increased to 45- to 68-fold for first instars and 150- to 1500-fold for third instars. For methoxyfenozide, these resistance ratios were 340- to 320-fold and 120- to 67-fold, at the  $LC_{50}$  and  $LC_{90}$ , respectively.

Many insecticides, including organophosphates, pyrethroids, benzoylureas, *Bacillus thuringiensis*, and now dibenzoylhydrazines, 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 overhead drench irrigation. This practice, coupled with intense reliance on insecticides, is likely to blame for the high incidence of insecticide resistance in the area and the highly accelerated rate of tebufenozide resistance development in beet armyworm (Wirojchewan Tawatchai, personal communication).

**Resistance Threat in U.S. Populations of Beet Armyworm.** No cases of field resistance of *Spodoptera exigua* to tebufenozide and methoxyfenozide have been reported in the U.S. Among the U.S. field strains evaluated, only the Arizona (Parker) strain exhibited LC values that were significantly greater than those of any other population. The  $LC_{50}$  and  $LC_{90}$  values for Arizona (Parker) in tebufenozide leaf-dip bioassays of third instars, 12.0 and 29.6  $\mu\text{g}/\text{ml}$ , respectively, were 1.9 (1.4–2.5)- and 2.2 (1.5–3.1)-fold greater than those of Mississippi (Wayside), the second least susceptible U.S. field strain evaluated (Figs. 2 and 3). For methoxyfenozide, the Arizona (Maricopa) strain was significantly less susceptible at the  $LC_{50}$  [ $RR_{50} = 1.6$  (1.1–2.3)], though not the  $LC_{90}$  [ $RR_{90} = 0.94$  (0.65–1.4)], than was the most susceptible non-Arizona field strain, South Carolina, (Florence), in leaf-dip bioassays of first instars. Compared with the most susceptible U.S. field populations evaluated, Arizona, (Parker) was 2.7 (2.1–3.7)- to 3.0 (2.0–4.3)-fold less susceptible to tebufenozide in the leaf-dip bioassay of third instars, and Arizona (Maricopa) was 8.5 (4.4)- to 3.4 (1.9–6.0)-fold less susceptible to methoxyfenozide in the leaf-dip bioassay of first instars. These differences are similar in magnitude to the 4.4- and nine-fold differences in tebufenozide and methoxyfenozide susceptibility, respectively, that Mascarenhas et al. (1998a) observed among southeastern U.S. field populations.

Selection of the Arizona (Parker) strain in a manner similar to that which the Thailand strain was subjected did not result in an appreciable magnification of resistance (data not shown). This outcome suggests that the mechanism conferring high levels of resistance to these compounds in the Thailand strains is either not present in the Parker strain or occurs at a very low frequency. Now that tebufenozide has Section 3 registrations in cotton, leafy greens, and vegetables, and methoxyfenozide has Section 3 registration in cotton, we are intensively monitoring beet armyworm susceptibility to both compounds in the vegetable/cotton production system of southwestern Arizona to determine whether the slight reductions in tebufenozide

and methoxyfenozide susceptibility we observed in Arizona populations will result in more rapid resistance development under field conditions.

**Cross-resistance Between Tebufenozide and Methoxyfenozide.** Exposure of the Thailand population to tebufenozide under field conditions yielded beet armyworm with significantly decreased sensitivity to methoxyfenozide (Table 4). Subsequent selection of the Thailand field population in the laboratory with tebufenozide-impregnated diet (10  $\mu\text{g}/\text{g}$ ) yielded Thailand (CONF-sel. #1), a strain exhibiting further decreased susceptibility to methoxyfenozide. This conclusion was based upon significant  $RR_{50}$  [Thailand (CONF-sel. #1)/Thailand (Bangbuathong)  $RR_{50} = 3.6$  (95%CL = 1.7–7.6)] and  $RR_{90}$  [ $RR_{90} = 2.9$  (1.6–5.2)] values for neonates with the leaf-dip bioassay (Table 3) and  $RR_{50}$  [ $RR_{50} = 3.3$  (2.3–4.7)] and  $RR_{90}$  [ $RR_{90} = 5.4$  (2.9–10)] values for third instars with the diet incorporation bioassay (Table 6). Selection of a tebufenozide-selected Thailand substrain, Thailand (CONF-sel. #1), with 10  $\mu\text{g}$  methoxyfenozide/g diet at  $F_5$  resulted in strain, Thailand (INT-sel. #1), exhibiting a significant decline in susceptibility to tebufenozide based upon  $RR_{50}$  [Thailand (INT-sel. #1)/Thailand (CONF-sel. #1)  $RR_{50} = 12$  (5.3–25)] and  $RR_{90}$  [ $RR_{90} = 3.4$  (1.8–6.2)] values for first instars with the leaf-dip bioassay (Table 1). Thus, we have demonstrated cross-resistance between tebufenozide and methoxyfenozide in this Thailand population.

**Monitoring Beet Armyworm Susceptibility to Tebufenozide and Methoxyfenozide. Sensitivity of Bioassays.** Based upon comparisons of  $RR_{50}$ s derived from dose-responses of Thailand (Bangbuathong) and Thailand (INT-sel. #1) versus the USDA reference strain, the leaf-dip assay of tebufenozide against third instars was two-fold [ $RR_{50} = 9.9$  (Table 2) versus 4.7 (Table 5) for Thailand (Bangbuathong)] to eight-fold [ $RR_{50} = 150$  (Table 2) versus 19 (Table 5) for Thailand (INT-sel. #1)] more sensitive in detecting differences in susceptibility than was the diet incorporation assay. For methoxyfenozide against third instars, the leaf-dip assay was also  $\approx$ two-fold [ $RR_{50} = 9.7$  (Table 4) versus 5.4 (Table 6) for Thailand (Bangbuathong);  $RR_{50} = 59$  (Table 4) versus 33 (Table 6) for Thailand (INT-sel. #1)] more sensitive than the diet incorporation assay. Thus, the leaf-dip bioassay appeared to be the more discriminating resistance detection tool for monitoring beet armyworm susceptibility to tebufenozide and methoxyfenozide in the strains we contrasted. In response to this finding, we changed from diet incorporation to leaf-dip bioassays in 1998.

**Pro-active Management of Resistance to Tebufenozide and Methoxyfenozide.** We cannot be certain that our findings regarding 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 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 monitor and manage beet armyworm resistance, once it occurs in the U.S. Lastly, the fact that resistance to tebufenozide and methoxyfenozide in Thailand developed in less than three years should serve as a warning for the need for careful stewardship of this valuable new class of selective insecticides.

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