

Resistance to *Bacillus thuringiensis* in Colonies of Indianmeal Moth and Almond Moth (Lepidoptera: Pyralidae)

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ABSTRACT Colonies of Indianmeal moth, *Plodia interpunctella* (Hübner), and almond moth, *Cadra cautella* (Walker), reared in the laboratory on diet treated with *Bacillus thuringiensis*, became resistant to *B. thuringiensis*. However, resistance did not progress at the same rate or to the same extent in all of the colonies. Resistance in five Indianmeal moth colonies increased from 2- to 29-fold within three generations, and from 15- to 100-fold in ca. 40 generations under relatively low selection pressure. With higher selection pressure, resistance in one colony increased >250-fold. Resistance in an almond moth colony increased only ca. 7-fold in 21 generations of intensive selection. Resistance was stable when selection was discontinued after the resistance levels reached a plateau, but declined if selection was discontinued earlier. The resistance was partially recessive in the five Indianmeal moth colonies, but not to an equal extent. The resistance assorted independently of the recessive genetic markers *copper*, *golden*, and *white-eye*.

KEY WORDS Insecta, biological control, *Bacillus thuringiensis*, Indianmeal moth

FORMULATIONS OF the δ -endotoxin of *Bacillus thuringiensis* subsp. *kurstaki* are registered by the U.S. Environmental Protection Agency for use on stored grain for controlling Indianmeal moths, *Plodia interpunctella* (Hübner), and almond moths, *Cadra cautella* (Walker). In laboratory and pilot scale studies, levels of moth control have been high (McGaughey 1976, 1978, 1980). However, control levels were variable and generally lower in full-size grain bins than in the small-scale tests (McGaughey 1985b). Poorer control in the field studies apparently resulted from rapid development of *B. thuringiensis* resistance in the Indianmeal moth populations. Bioassays on laboratory colonies founded from these populations revealed that colonies from treated bins were significantly more resistant to *B. thuringiensis* than those from untreated bins, and studies on a colony from Oklahoma confirmed that a resistant strain could be selected within only a few generations (McGaughey 1985a). Resistance appeared to be inherited as a recessive trait, and over the short period of the study it was genetically stable.

Subsequently, we have investigated the potential for selecting resistant strains in several Indianmeal moth colonies and one almond moth colony collected from different states in the central United States. Here we present data on the extent of resistance development in these colonies, along with additional data on the stability, fitness, and inheritance of *B. thuringiensis* resistance in Indianmeal moths.

Materials and Methods

Five Indianmeal moth colonies were studied. Two of these were started from infestations in

southwestern Illinois (45-2 and 50-2), one from southeastern Iowa (37-6), one from southcentral Nebraska (21), and one from Oklahoma (343). These colonies had been reared in the laboratory for 18-26 generations before the selection studies began. The colony from Oklahoma was used in our earlier study (McGaughey 1985a). The almond moth colony (WAM) was started from an infestation in southeastern Texas 10 yr (ca. 135 generations) before selection began.

Insects were collected from infested bins as larvae or pupae in strips of corrugated paper placed on the grain surface to serve as pupation sites. The paper containing insects was returned to the laboratory and placed in jars, where adults emerged. Eggs were collected from these adults and placed in jars on the standard laboratory larval diet of cracked wheat (1,000 g), wheat shorts (1,000 g), wheat germ (100 g), brewers' yeast (80 g), sorbic acid (4 g), and methyl-*p*-hydroxybenzoate (4 g) moistened with a mixture of glycerin (240 ml), honey (240 ml), and water (120 ml). Spools of corrugated paper were placed in these rearing jars to provide pupation sites and to facilitate transfer of pupae to clean jars for adult emergence and oviposition. Oviposition jars were capped with a screen lid and inverted over a dish to collect the eggs. Thirty-five to 50 mg of eggs (2,500-3,500 eggs) were placed on ca. 150 g of diet in Mason jars (0.946 liter) for subsequent generations. If adult emergence or egg production were low, the available eggs were placed on a smaller amount of diet. Rearing conditions were controlled at 25°C and 60-70% RH.

Each of the colonies was subcultured for several successive generations on diet treated with *B. thu-*

ringiensis to evaluate its potential for developing resistance. The dosage of *B. thuringiensis* selected for the diet of each colony was based on the results of preliminary bioassays and was expected to produce 70–90% larval mortality in the first generation. The dosage was incorporated into 500- or 1,000-g samples of diet as an aqueous suspension at 100 ml/kg with a household food mixer. Depending upon the level of survival expected, either 35 or 70 mg of eggs (2,500 or 5,000) were used in each culture jar containing 137 g of diet. For each of these colonies, percentage survival was estimated for each generation by counting the adults that emerged from 30-g samples of treated diet that had been infested with 50 eggs at the same time the culture jars were set up for the colony.

Each colony was bioassayed periodically to monitor for any change in LC_{50} or slope of the dose-mortality regression. Bioassays were conducted with Dipel, a WP formulation of the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* containing 16,000 IU of potency per mg of formulation. Powder was suspended in water at a concentration of 5 mg/ml; serial 1:2 dilutions were prepared to provide nine doses ranging from 500 to 1.95 mg/kg when applied to larval diet at a rate of 0.1 ml/g. Eventually, the upper end of the dose range was extended to 4,000 mg/kg as resistance progressed. Each concentration of suspension was applied to a 30-g sample of diet in a small mixing bowl and mixed with an electrically driven polyethylene stirrer. Samples of diet were treated with water to serve as controls.

Treated and control samples of diet were placed in Mason jars (0.473 liter) with filter paper caps; 50 eggs of the appropriate insect colony were added to each jar. Mortality levels were determined by counting the adults that emerged and correcting for control mortality (Abbott 1925). Three replicate bioassays were done for each generation tested. Mortality data from the replicates were pooled and analyzed using the probit procedure of Finney (1971) or that of the Statistical Analysis System (SAS Institute 1982). The parent colonies for the selected strains were maintained on untreated diet; these were tested periodically to provide baseline susceptibility data in the absence of selection.

After several generations of selection, some of the resistant strains were subcultured on diet treated at a higher rate to determine whether the progress of selection could be accelerated. Similarly, some were subcultured on untreated diet to determine whether the level of resistance would decrease if selection pressure was discontinued.

The highly selected resistant strains of Indianmeal moths were crossed to the susceptible parent strains to determine the inheritance of resistance, and the hybrid insects were reared for several generations on untreated diet to determine the stability and fitness of resistance in nonhomozygous populations. Virgin males and females from the two strains were separated as they emerged; mass crosses were made between resistant females and sus-

ceptible males and between susceptible females and resistant males. Equal numbers of eggs from these reciprocal crosses were used to infest culture jars. Eggs from random mating of the progeny were used to perpetuate these crosses through subsequent generations. Bioassays were done on the F_1 through F_3 generations to monitor for any change in susceptibility to *B. thuringiensis*.

Linkage tests were done between *B. thuringiensis* resistance in Indianmeal moths and each of the recessive genetic markers *copper* (*cu*) (Brower 1983), *golden* (*g*) (Beeman 1983), and *white-eye* (*w*) (Brower 1982). Exhaustively selected (presumably homozygous resistant) 343 males were mass-crossed to virgin *cu*, *g*, or *w* females. Both marker strains were previously shown to be highly susceptible to Dipel. F_1 adults from each of these crosses were allowed to self-cross en masse. A sample of the resulting F_2 eggs from each cross was placed on medium containing 500 ppm Dipel. This concentration killed heterozygous resistant, but not all homozygous resistant moths. All emerging adults were scored for visible mutant phenotypes. Chi-square analysis of data was done as described by Brower (1972).

Results and Discussion

Resistance progressed rapidly in all five Indianmeal moth colonies. Survival of these colonies on diet treated with *B. thuringiensis* ranged from 19 to 37% in the first generation, but within four to seven generations was >70%, which is the usual survival rate on untreated diet in our laboratory (Fig. 1). When selection pressure was increased 8-fold against the resistant strains of colonies 343 and 37-6, survival decreased to 30–40%. However, within seven generations, survival again increased to the 70–80% range.

The LC_{50} 's for these Indianmeal moth colonies also increased markedly. The LC_{50} of colony 343 increased faster than that of the other colonies, probably because colony 343 was selected using a higher *B. thuringiensis* dosage (62.5 mg/kg versus 31.25 mg/kg for the other colonies). Within three generations at a dose of 62.5 mg/kg, the LC_{50} of colony 343 increased 29-fold (Table 1). The level of resistance continued to increase to slightly more than 100-fold in 16 generations. Thereafter, the LC_{50} remained essentially constant at ca. 1,400 mg/kg through 43 generations of selection. However, when selection pressure was increased 8-fold—to 500 mg/kg at generation nine—the progress of resistance was accelerated. After 36 generations of selection (the last 27 at the higher dosage), the LC_{50} was >3,000 mg/kg, which is more than 250-fold the LC_{50} of the unselected parent colony.

In colony 37-6, resistance progressed at a slower rate but followed the same general pattern as in colony 343 (Table 2). The LC_{50} increased 2-fold in three generations and reached a plateau of ca. 25-fold after 15 generations. The LC_{50} of this colony

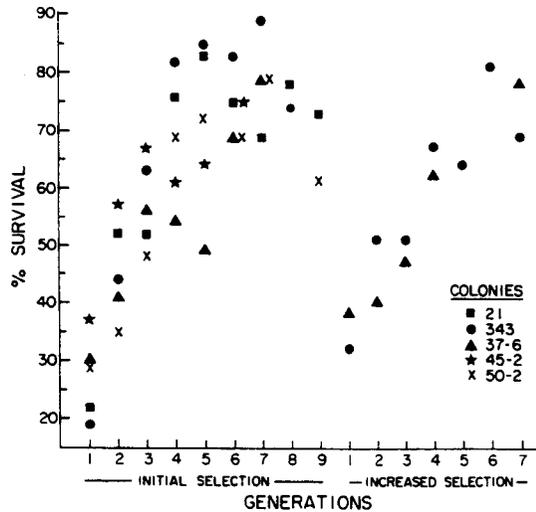


Fig. 1. Percentage survival in successive generations of colonies selected for resistance to *B. thuringiensis* by rearing on treated diet. In the initial selection, the diet for colony 343 was treated at 62.5 mg/kg and for the other colonies at 31.25 mg/kg. Selection pressure on colonies 343 and 37-6 was increased after nine generations to 500 and 250 mg/kg *B. thuringiensis*, respectively.

remained essentially constant at 500-700 mg/kg through 38 generations. Increased selection pressure also accelerated the progression of resistance in this colony. After 36 generations of selection (the last 27 at a dosage of 250 mg/kg), the LC₅₀ was >1,250 mg/kg (ca. 65 times that of the unselected colony).

In colony 21, resistance progressed rapidly under a constant but relatively low selection pressure (Table 3). At a dosage of 31.25 mg/kg, the LC₅₀ increased from 2-fold after three generations to >70-fold in 23 generations. Thereafter, the LC₅₀ remained essentially constant at ca. 2,000 mg/kg through 42 generations.

Resistance in colony 45-2 progressed rapidly to an LC₅₀ of ca. 500 mg/kg in the first four generations, but did not increase much more during 31 generations of selection at a *B. thuringiensis* dosage of 31.25 mg/kg (Table 3). Resistance in colony 50-2 increased slowly but steadily. The LC₅₀ was 265 mg/kg after 39 generations of selection at a *B. thuringiensis* dosage of 31.25 mg/kg (Table 3). We did not attempt to accelerate the progress of resistance in colonies 45-2 or 50-2 by increasing the selection pressure. However, the responses of colonies 343, 37-6, and 21 suggest that these two colonies would have become more resistant if pressured sufficiently.

Selection for a resistant strain in the almond moth colony was less successful than for any of the Indianmeal moth colonies. Using a constant *B. thuringiensis* dosage in the diet, as well as gradually increasing the selection pressure, we were able to achieve only ca. 7-fold resistance levels (Table 4).

Table 1. Dose response of colony 343 to *B. thuringiensis*^a

| Genera- tion | n | Slope ± SE | LC ₅₀ (mg/kg diet) | 95% FL (mg/kg diet) | RF ^b |
|---|-------|------------|-------------------------------------|---------------------------|-----------------|
| Unselected | | | | | |
| 9 | 1,200 | 1.6 ± 0.3 | 16.6 | 6.6-32.2 | |
| 10 | 1,200 | 1.9 ± 0.2 | 14.5 | 10.0-19.8 | |
| 21 | 1,350 | 1.5 ± 0.1 | 11.8 | 10.3-13.5 | |
| 22 | 1,200 | 2.0 ± 0.1 | 12.1 | 10.8-13.5 | |
| 43 | 1,400 | 2.4 ± 0.1 | 12.6 | 11.5-13.7 | |
| 47 | 600 | 2.5 ± 0.3 | 10.6 | 7.6-14.9 | |
| Selected on diet treated at 62.5 mg/kg | | | | | |
| 3 | 750 | 1.7 ± 0.3 | 383 | 234-1,194 | 29 |
| 5 | 600 | 1.9 ± 0.4 | 522 | 269-8,493 | 40 |
| 11 | 900 | 1.7 ± 0.1 | 590 | 520-674 | 45 |
| 13 | 900 | 1.9 ± 0.3 | 813 | 500-1,693 | 63 |
| 15 | 900 | 2.1 ± 0.3 | 1,061 | 744-1,785 | 82 |
| 16 | 600 | 3.4 ± 0.7 | 1,371 | 791-7,278 | 105 |
| 27 | 1,200 | 2.0 ± 0.1 | 1,278 | 1,164-1,404 | 98 |
| 28 | 1,500 | 1.7 ± 0.4 | 1,605 | 903-3,620 | 123 |
| 42 ^c | 800 | 2.2 ± 0.1 | 1,001 | 895-1,115 | 77 |
| 43 ^c | 1,200 | 2.8 ± 0.2 | 1,433 | 1,305-1,571 | 110 |
| Increased selection on diet treated at 500 mg/kg ^d | | | | | |
| 11 | 600 | 3.2 ± 0.3 | 793 | 575-1,113 | 61 |
| 14 | 1,200 | 1.6 ± 0.2 | 597 | 435-875 | 46 |
| 15 | 750 | 2.5 ± 0.3 | 1,034 | 736-1,669 | 80 |
| 16 | 600 | 3.3 ± 0.7 | 1,813 | 1,143-22,924 | 139 |
| 19 | 600 | 2.8 ± 0.3 | 1,964 | 1,721-2,331 | 151 |
| 20 | 750 | 2.4 ± 0.2 | 1,712 | 1,496-2,024 | 132 |
| 35 | 1,350 | 1.0 ± 0.1 | 4,035 | 2,473-9,400 | 310 |
| 36 | 1,200 | 2.3 ± 0.5 | 3,292 | 2,351-6,579 | 253 |
| Discontinued selection ^e | | | | | |
| 2 | 750 | 2.7 ± 0.6 | 812 | 414-2,308 | 62 |
| 3 | 750 | 1.8 ± 0.2 | 636 | 455-936 | 49 |
| 9 | 1,050 | 2.1 ± 0.2 | 850 | 672-1,126 | 65 |
| 10 | 1,200 | 1.8 ± 0.3 | 790 | 510-1,465 | 61 |
| 28 | 1,500 | 1.9 ± 0.1 | 1,125 | 937-1,352 | 87 |
| 29 | 1,350 | 2.1 ± 0.2 | 970 | 806-1,159 | 75 |

^a Three replicate bioassays were done on each generation using 50 insects per dose. Data were pooled (150 insects per dose) for probit analysis.

^b Resistance factor (RF) = LC₅₀ of selected colony ÷ average LC₅₀ of unselected colony.

^c Only two replicates were done, for a total of 100 insects per dose.

^d Resistant colony subcultured on diet treated at 500 mg/kg after selection for nine generations on diet treated at 62.5 mg/kg.

^e Resistant colony subcultured on untreated diet after selection for nine generations on diet treated at 62.5 mg/kg.

This is probably a result of the long time this colony had been maintained in the laboratory before selection began and the associated gradual loss of genetic heterogeneity, rather than any inherent absence of potential for resistance.

We studied the stability of *B. thuringiensis* resistance in two intensively selected strains of the Indianmeal moth (colonies 343 and 37-6) in which susceptible alleles were presumably rare or absent at the selected loci. In the earlier paper on colony 343, McGaughey (1985a) indicated that the level of resistance in that colony remained stable through seven generations of rearing on untreated diet (without selection pressure). In this study, this strain has been reared for 29 generations on untreated diet without any noticeable decrease in resistance

Table

| Genera- tion |
|-----------------|
| 6 |
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to *B. thuringiensis*^a

| FL (mg/kg diet) | RF ^b |
|-----------------|-----------------|
| 32.2 | |
| 19.8 | |
| 13.5 | |
| 13.5 | |
| 13.7 | |
| 14.9 | |
| 1,194 | 29 |
| 8,493 | 40 |
| 674 | 45 |
| 1,693 | 63 |
| 1,785 | 82 |
| 7,278 | 105 |
| 1,404 | 98 |
| 3,620 | 132 |
| 1,115 | 77 |
| 1,571 | 110 |
| 1,113 | 61 |
| 875 | 46 |
| 1,669 | 80 |
| 22,924 | 139 |
| 2,331 | 151 |
| 2,024 | 132 |
| 9,400 | 310 |
| 6,579 | 253 |
| 2,308 | 62 |
| 936 | 49 |
| 1,126 | 65 |
| 1,465 | 61 |
| 1,352 | 87 |
| 1,159 | 75 |

generation using 50 insects per dose) for probit analysis.
 colony ÷ average LC₅₀ of unselected colony.
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Table 2. Dose response of colony 37-6 to *B. thuringiensis*^a

| Genera- tion | n | Slope ± SE | LC ₅₀ (mg/kg diet) | 95% FL (mg/kg diet) | RF ^b |
|---|-------|------------|-------------------------------|---------------------|-----------------|
| Unselected | | | | | |
| 6 | 1,050 | 2.4 ± 0.2 | 21.5 | 17.6-26.2 | |
| 7 | 1,050 | 2.3 ± 0.3 | 13.9 | 9.0-20.2 | |
| 17 | 900 | 2.1 ± 0.2 | 16.5 | 11.8-22.7 | |
| 18 | 1,350 | 2.0 ± 0.1 | 16.4 | 14.8-18.3 | |
| 30 | 1,200 | 2.1 ± 0.1 | 18.2 | 15.9-20.7 | |
| 40 | 1,350 | 1.9 ± 0.2 | 31.7 | 22.2-45.6 | |
| Selected on diet treated at 31.25 mg/kg | | | | | |
| 3 | 1,200 | 2.0 ± 0.1 | 48 | 43-53 | 2 |
| 4 | 1,200 | 1.6 ± 0.1 | 106 | 81-142 | 5 |
| 5 | 900 | 1.4 ± 0.4 | 234 | 104-2,657 | 12 |
| 13 | 1,050 | 2.0 ± 0.2 | 336 | 271-422 | 17 |
| 15 | 1,050 | 2.0 ± 0.3 | 540 | 377-823 | 27 |
| 16 | 900 | 2.0 ± 0.2 | 501 | 353-746 | 25 |
| 20 | 1,200 | 1.7 ± 0.2 | 451 | 300-733 | 23 |
| 37 | 1,500 | 1.8 ± 0.2 | 648 | 487-851 | 33 |
| 38 | 1,350 | 2.4 ± 0.1 | 779 | 713-848 | 40 |
| Increased selection on diet treated at 250 mg/kg ^c | | | | | |
| 11 | 1,050 | 2.4 ± 0.2 | 297 | 240-367 | 15 |
| 12 | 1,200 | 1.5 ± 0.2 | 281 | 192-431 | 14 |
| 13 | 1,050 | 1.7 ± 0.4 | 405 | 198-1,070 | 21 |
| 17 | 900 | 1.8 ± 0.2 | 710 | 537-991 | 36 |
| 18 | 900 | 1.9 ± 0.2 | 801 | 609-1,123 | 41 |
| 36 | 1,200 | 2.1 ± 0.2 | 1,377 | 1,080-1,765 | 70 |
| 37 | 1,050 | 3.1 ± 0.3 | 1,247 | 1,010-1,507 | 63 |
| Discontinued selection ^d | | | | | |
| 3 | 1,200 | 1.5 ± 0.2 | 173 | 126-239 | 9 |
| 4 | 1,200 | 1.4 ± 0.3 | 203 | 100-442 | 10 |
| 10 | 1,500 | 1.6 ± 0.1 | 179 | 134-242 | 9 |
| 11 | 1,350 | 1.4 ± 0.1 | 252 | 200-323 | 13 |
| 28 | 1,050 | 1.4 ± 0.1 | 48 | 39-58 | 2 |
| 29 | 1,500 | 1.5 ± 0.1 | 74 | 63-86 | 4 |

^a Three replicate bioassays were done on each generation using 50 insects per dose. Data were pooled (150 insects per dose) for probit analysis.

^b Resistance factor (RF) = LC₅₀ of selected colony ÷ average LC₅₀ of unselected colony.

^c Resistant colony subcultured on diet treated at 250 mg/kg after selection for nine generations on diet treated at 31.25 mg/kg.

^d Resistant colony subcultured on untreated diet after selection for nine generations on diet treated at 31.25 mg/kg.

(Table 1). However, in colony 37-6, resistance eventually decreased when selection pressure was discontinued. The LC₅₀ remained stable at ca. 200-250 mg/kg through 11 generations, but decreased markedly by generations 28 and 29 (Table 2). Because a selection plateau had not been reached in colony 37-6 when selection was discontinued, the presence of susceptible genotypes may account for the instability of resistance in this strain.

The five resistant strains of Indianmeal moths were crossed with their respective susceptible parent strains after 30-40 generations of selection, and the hybrid progeny were reared for five consecutive generations on untreated diet to determine the stability (fitness) of alleles for resistance in the presence of those for susceptibility. No significant reversion to susceptibility occurred in any of the strains (Table 5). Resistance might be expected to revert eventually to susceptibility in view of the delayed partial reversion observed in colony 37-6.

Table 3. Dose response of colonies 21, 45-2, and 50-2 to *B. thuringiensis*^a

| Genera- tion | n | Slope ± SE | LC ₅₀ (mg/kg diet) | 95% FL (mg/kg diet) | RF ^b |
|---|-------|------------|-------------------------------|---------------------|-----------------|
| Colony 21—unselected | | | | | |
| 8 | 1,350 | 1.9 ± 0.3 | 16.2 | 10.5-24.4 | |
| 9 | 1,350 | 1.6 ± 0.2 | 15.8 | 8.8-26.6 | |
| 10 | 1,350 | 2.1 ± 0.3 | 15.4 | 9.6-23.7 | |
| 42 | 1,350 | 1.3 ± 0.1 | 37.3 | 26.6-52.9 | |
| 43 | 1,350 | 1.2 ± 0.1 | 52.5 | 45.1-61.5 | |
| Colony 21—selected on diet treated at 31.25 mg/kg | | | | | |
| 3 | 1,350 | 1.4 ± 0.2 | 65 | 44-99 | 2 |
| 7 | 1,350 | 1.2 ± 0.1 | 105 | 79-145 | 4 |
| 8 | 750 | 1.5 ± 0.1 | 208 | 179-247 | 8 |
| 22 | 1,200 | 1.2 ± 0.1 | 941 | 642-1,591 | 34 |
| 23 | 1,350 | 0.8 ± 0.1 | 1,974 | 1,128-4,651 | 72 |
| 41 | 1,200 | 1.2 ± 0.1 | 2,870 | 2,112-4,470 | 105 |
| 42 | 1,350 | 1.7 ± 0.2 | 2,013 | 1,595-2,670 | 73 |
| Colony 45-2—unselected | | | | | |
| 2 | 1,200 | 1.4 ± 0.2 | 17.6 | 11.7-25.0 | |
| 3 | 1,200 | 1.5 ± 0.1 | 15.2 | 15.9-20.2 | |
| 4 | 1,200 | 1.7 ± 0.1 | 12.6 | 10.1-15.6 | |
| 36 | 1,350 | 1.6 ± 0.1 | 20.8 | 16.4-26.4 | |
| 37 | 1,200 | 1.7 ± 0.1 | 27.1 | 24.0-30.6 | |
| 38 | 1,350 | 1.9 ± 0.1 | 18.2 | 16.3-20.3 | |
| Colony 45-2—selected on diet treated at 31.25 mg/kg | | | | | |
| 2 | 1,350 | 1.0 ± 0.2 | 167 | 84-519 | 9 |
| 3 | 750 | 1.8 ± 0.5 | 274 | 105-5,643 | 15 |
| 4 | 750 | 1.0 ± 0.2 | 523 | 241-18,228 | 28 |
| 5 | 900 | 1.2 ± 0.2 | 453 | 230-2,739 | 24 |
| 30 | 1,200 | 2.1 ± 0.2 | 715 | 589-854 | 38 |
| 31 | 1,350 | 1.3 ± 0.2 | 610 | 377-939 | 33 |
| Colony 50-2—unselected | | | | | |
| 6 | 1,200 | 2.2 ± 0.2 | 16.2 | 13.1-19.8 | |
| 7 | 900 | 2.1 ± 0.1 | 8.5 | 7.6-9.4 | |
| 8 | 900 | 2.6 ± 0.1 | 8.1 | 7.4-9.0 | |
| 40 | 1,350 | 1.6 ± 0.2 | 22.2 | 16.1-30.5 | |
| 41 | 1,350 | 1.5 ± 0.1 | 30.3 | 23.1-39.7 | |
| Colony 50-2—selected on diet treated at 31.25 mg/kg | | | | | |
| 3 | 1,050 | 2.0 ± 0.4 | 43 | 24-81 | 3 |
| 4 | 1,200 | 1.9 ± 0.3 | 64 | 41-103 | 4 |
| 7 | 1,050 | 2.2 ± 0.4 | 101 | 64-170 | 6 |
| 20 | 1,200 | 2.7 ± 0.3 | 140 | 112-175 | 8 |
| 21 | 1,200 | 2.8 ± 0.3 | 142 | 116-174 | 8 |
| 38 | 1,200 | 2.7 ± 0.4 | 220 | 150-324 | 13 |
| 39 | 1,050 | 2.7 ± 0.1 | 265 | 241-290 | 15 |

^a Three replicate bioassays were done on each generation using 50 insects per dose. Data were pooled (150 insects per dose) for probit analysis.

^b Resistance factor (RF) = LC₅₀ of selected colony ÷ average LC₅₀ of unselected colony.

The F₁ hybrid progeny of colony 343 were almost as susceptible as the unselected parent colony (i.e., resistance was recessive), which is similar to the result obtained earlier when the resistant strain of this colony was crossed with the susceptible laboratory colony (McGaughey 1985a). Progeny from the crosses of the other four colonies were somewhat more resistant than the colony 343 cross. Thus, resistance in these four colonies was less recessive (more dominant) than in colony 343.

Linkage analysis between *B. thuringiensis* resistance in colony 343 and the recessive genetic markers *cu*, *g*, and *w* indicated that resistance assorted independently of all three (Table 6). Like most lepidopterans, the Indianmeal moth has a

Table 4. Dose response of almond moths to *B. thuringiensis*^a

| Genera- tion | n | Slope ± SE | LC ₅₀ (mg/kg diet) | 95% FL (mg/kg diet) | RF ^b |
|--|-------|------------|-------------------------------------|------------------------|-----------------|
| Unselected | | | | | |
| 4 | 1,350 | 2.3 ± 0.3 | 24.3 | 17.9-33.0 | |
| 5 | 1,050 | 2.6 ± 0.2 | 18.6 | 14.8-23.2 | |
| 24 | 1,200 | 1.7 ± 0.2 | 15.2 | 11.0-20.8 | |
| 25 | 1,050 | 2.3 ± 0.1 | 31.2 | 28.1-34.6 | |
| Selected on diet treated at 15.625 mg/kg | | | | | |
| 2 | 1,050 | 2.7 ± 0.3 | 19.1 | 15.2-23.9 | — |
| 3 | 1,200 | 2.2 ± 0.1 | 19.4 | 17.4-21.6 | — |
| 4 | 1,350 | 2.0 ± 0.1 | 16.9 | 15.2-18.8 | — |
| 5 | 1,350 | 2.3 ± 0.3 | 42.4 | 30.7-58.8 | 1.9 |
| 9 | 900 | 2.8 ± 0.3 | 66.2 | 48.7-89.1 | 3.0 |
| 22 | 900 | 3.1 ± 0.2 | 69.3 | 63.5-75.5 | 3.1 |
| 24 | 1,050 | 2.7 ± 0.4 | 73.3 | 49.2-109.4 | 3.3 |
| Increased selection on diet treated at 62.5 mg/kg ^c | | | | | |
| 10 | 1,350 | 1.8 ± 0.5 | 48.0 | 16.6-152.3 | 2.2 |
| 11 | 1,350 | 2.3 ± 0.4 | 69.7 | 42.7-116.1 | 3.1 |
| 17 | 1,350 | 2.0 ± 0.4 | 90.8 | 49.8-176.2 | 4.1 |
| 21 | 1,350 | 2.7 ± 0.5 | 106.4 | 66.9-169.8 | 4.8 |
| 22 | 750 | 4.1 ± 0.3 | 95.7 | 88.7-103.2 | 4.3 |
| 23 | 1,500 | 1.8 ± 0.3 | 121.2 | 66.4-233.5 | 5.4 |
| Increased selection on diet treated at 125 mg/kg ^d | | | | | |
| 15 | 900 | 2.5 ± 0.2 | 94.3 | 73.9-120.6 | 4.2 |
| 16 | 900 | 2.9 ± 0.3 | 102.7 | 78.8-134.4 | 4.6 |
| 21 | 900 | 2.9 ± 0.3 | 146.3 | 111.0-192.2 | 6.6 |
| 22 | 900 | 4.7 ± 0.3 | 139.3 | 129.8-149.3 | 6.2 |
| 23 | 900 | 2.8 ± 0.2 | 174.2 | 159.1-190.7 | 7.8 |

^a Three replicate bioassays were done on each generation using 50 insects per dose. Data were pooled (150 insects per dose) for probit analysis.

^b Resistance factor (RF) = LC₅₀ of selected colony ÷ average LC₅₀ of unselected colony.

^c Resistant colony subcultured on diet treated at 62.5 mg/kg after selection for eight generations on diet treated at 15.625 mg/kg.

^d Colony selected for eight generations on diet treated at 15.625 mg/kg, five generations on diet treated at 62.5 mg/kg, then on diet treated at 125 mg/kg.

haploid chromosome number of ca. 30 (Robinson 1971, Lynn & Oberlander 1983). Markers for the remaining (ca. 27) chromosomes are not yet available. Our attempts to determine whether a single major gene for *B. thuringiensis* resistance could be segregated were inconclusive because heterozygous and homozygous genotypes could not be discriminated with the available bioassay methods.

Although the Indianmeal moth data do not necessarily show the maximum levels of resistance that populations could attain, the LC₅₀ of colony 343 reared on diet treated with Dipel at 500 mg/kg was >3,000 mg/kg. This is 250 times the stable response level of the parent colony reared continuously on untreated diet. Resistance appeared within two or three generations in all the colonies but did not progress at the same rate or reach the same level in all colonies. The selection pressure (i.e., *B. thuringiensis* dosage) or the initial frequency of the gene or genes in the populations may have influenced the rates of progression of resistance. Failure of the colonies to reach similar levels of resistance within a few generations sug-

Table 5. Dose response of progeny from resistant × susceptible crosses of Indianmeal moth colonies^a

| Genera- tion | n | Slope ± SE | LC ₅₀ (mg/kg diet) | 95% FL (mg/kg diet) |
|-----------------------------|-------|------------|-------------------------------------|------------------------|
| Colony 343 ^b | | | | |
| F ₁ | 1,200 | 2.4 ± 0.4 | 86.3 | 56.6-131.4 |
| F ₂ | 2,100 | 1.5 ± 0.1 | 105.0 | 85.2-128.8 |
| F ₃ | 1,950 | 1.6 ± 0.1 | 59.4 | 48.7-72.1 |
| F ₄ | 1,500 | 1.6 ± 0.1 | 63.7 | 53.4-74.4 |
| F ₅ | 1,200 | 1.7 ± 0.1 | 48.5 | 40.1-57.1 |
| Colony 37-6 ^c | | | | |
| F ₁ | 1,200 | 2.5 ± 0.1 | 91.1 | 82.8-100.4 |
| F ₂ | 1,950 | 1.6 ± 0.1 | 97.2 | 86.4-109.1 |
| F ₃ | 1,200 | 1.8 ± 0.2 | 75.5 | 56.7-95.9 |
| F ₄ | 1,500 | 1.5 ± 0.1 | 126.2 | 109.2-144.3 |
| F ₅ | 1,200 | 2.0 ± 0.1 | 131.7 | 118.0-146.9 |
| Colony 21 ^d | | | | |
| F ₁ | 1,500 | 1.0 ± 0.1 | 282.9 | 233.2-351.0 |
| F ₂ | 1,950 | 1.2 ± 0.1 | 400.5 | 312.4-514.4 |
| F ₃ | 1,800 | 1.2 ± 0.1 | 409.1 | 323.6-516.6 |
| F ₄ | 1,500 | 1.1 ± 0.1 | 183.5 | 112.3-271.5 |
| F ₅ ^e | 1,000 | 1.0 ± 0.1 | 203.0 | 136.5-283.0 |
| Colony 45-2 ^f | | | | |
| F ₁ | 1,500 | 1.5 ± 0.2 | 100.2 | 68.1-151.5 |
| F ₂ | 2,100 | 1.2 ± 0.1 | 87.7 | 67.1-113.7 |
| F ₃ | 1,500 | 1.5 ± 0.1 | 134.0 | 118.0-151.9 |
| F ₄ | 1,350 | 1.4 ± 0.1 | 83.8 | 70.0-98.3 |
| F ₅ | 1,500 | 1.7 ± 0.1 | 138.9 | 122.2-156.7 |
| Colony 50-2 ^g | | | | |
| F ₁ | 1,500 | 2.0 ± 0.2 | 67.9 | 54.0-85.6 |
| F ₂ | 1,200 | 1.9 ± 0.2 | 105.6 | 76.9-142.6 |
| F ₃ | 900 | 2.4 ± 0.2 | 71.5 | 63.9-79.5 |
| F ₄ | 900 | 2.6 ± 0.2 | 84.0 | 75.7-92.7 |
| F ₅ | 1,350 | 2.0 ± 0.1 | 139.3 | 125.0-155.2 |

^a Progeny were reared on untreated diet. Three replicate bioassays were done on each generation using 50 insects per dose. Data were pooled (150 insects per dose) for probit analysis.

^b Cross between resistant strain selected for 39 generations at a dosage of 62.5 mg/kg and the 43rd generation of the unselected strain (Table 1).

^c Cross between resistant strain selected for 40 generations at a dosage of 31.25 mg/kg and the 40th generation of the unselected strain (Table 2).

^d Cross between resistant strain selected for 41 generations at a dosage of 31.25 mg/kg and the 42nd generation of the unselected strain (Table 3).

^e Only two replications were done, for 100 insects per dose.

^f Cross between resistant strain selected for 30 generations at a dosage of 31.25 mg/kg and the 36th generation of the unselected strain (Table 3).

^g Cross between resistant strain selected for 40 generations at a dosage of 31.25 mg/kg and the 41st generation of the unselected strain (Table 3).

gests that the colonies represent different biotypes, that resistance is controlled by multiple alleles or multiple loci, or that various modifying genes influence the expression of resistance in the populations. Nevertheless, the rapid appearance of the partially recessive trait under laboratory selection with a relatively low dosage of the insecticide suggests that a substantial portion of the resistance is due to a single major factor that occurs with a high frequency (Wood & Mani 1981). The earlier report by Kinsinger & McCaughey (1979) of a wide range in susceptibility of Indianmeal moth and almond moth colonies with no known prior exposure to *B. thuringiensis* supports the notion that native pop-

from resistant × colonies^a

| 95% FL (mg/kg diet) |
|------------------------|
| 56.6-131.4 |
| 85.2-128.8 |
| 48.7-72.1 |
| 53.4-74.4 |
| 40.1-57.1 |
| 82.8-100.4 |
| 86.4-109.1 |
| 56.7-95.9 |
| 109.2-144.3 |
| 118.0-146.9 |
| 233.2-351.0 |
| 312.4-514.4 |
| 323.6-516.6 |
| 112.3-271.5 |
| 136.5-283.0 |
| 68.1-151.5 |
| 67.1-113.7 |
| 118.0-151.9 |
| 70.0-98.3 |
| 122.2-156.7 |
| 54.0-85.6 |
| 76.9-142.6 |
| 63.9-79.5 |
| 75.7-92.7 |
| 125.0-155.2 |

three replicate bioassays per dose. Data analysis.

39 generations at a ratio of the unselected

40 generations at a ratio of the unselected

41 generations at a ratio of the unselected

insects per dose. 30 generations at a ratio of the unselected

40 generations at a ratio of the unselected

different biotypes, multiple alleles or modifying genes in the population. The appearance of the laboratory selection insecticide suggests the resistance is associated with a high level. The earlier report of a wide range of resistance in almond and almond exposure to *B. thuringiensis* that native pop-

Table 6. Segregation of phenotypes in crosses between *B. thuringiensis*-R and golden (*g*), copper (*cu*), and white-eye (*w*) mutants of Indianmeal moths

| Phenotypes of <i>B.t.</i> -resistant progeny | | | | | | | | χ^2 |
|--|----------|--------------|-----|--------------------|----------|--------------|-----|-------------------|
| Total no. observed | | | | Total no. expected | | | | |
| <i>cu</i> | <i>g</i> | <i>cu, g</i> | + | <i>cu</i> | <i>g</i> | <i>cu, g</i> | + | |
| 41 | 44 | 19 | 138 | 45 | 45 | 15 | 136 | 1.47 ^a |
| <i>w</i> | | | + | <i>w</i> | | | + | |
| 44 | | | 181 | 56 | | | 169 | 3.42 ^b |

^a 0.90 > *P* > 0.50 for independent assortment.

^b 0.10 > *P* > 0.05 for independent assortment.

ulations may be naturally polymorphic for resistance genotypes.

These data demonstrate that high levels of resistance to *B. thuringiensis* can occur within two or three generations in colonies of Indianmeal moths. This rate is sufficiently rapid to occur in bins of grain treated with *B. thuringiensis* within a single season and could cause failure of the recommended dosage to provide control during the fall when infestations usually reach their peak. Although the resistance appears to be a recessive trait, reversion of a population to a susceptible state would depend on immigration of susceptible adults or some reproductive or developmental advantage of susceptible individuals. No reproductive or developmental disadvantages were observed in the laboratory rearing of these resistant colonies, with the exception of delayed, partial reversion of resistance in colony 37-6. Moreover, even though the progeny of crosses between resistant and susceptible populations were much more sensitive to *B. thuringiensis* than were the resistant strains, they were resistant enough to render questionable the efficacy of currently recommended dosages.

Those who use *B. thuringiensis* moth control in stored grain should be alert to the possibility of resistance. If resistance occurs, studies should be conducted to evaluate its impact on control programs.

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