

**Insect Resistance to the Biological Insecticide *Bacillus thuringiensis***

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## Insect Resistance to the Biological Insecticide *Bacillus thuringiensis*

**Abstract.** Resistance to the spore-crystal protein complex of *Bacillus thuringiensis*, the most widely used and intensively studied microbial insecticide, has been presumed to be unlikely to occur. In this study it was found that *Plodia interpunctella*, a major lepidopteran pest of stored grain products, can develop resistance to the insecticide within a few generations. Resistance increased nearly 30-fold in two generations in a strain reared on diet treated with *Bacillus thuringiensis* and after 15 generations reached a plateau 100 times higher than the control level. Resistance was stable when selection was discontinued. The resistance was inherited as a recessive trait. *Plodia interpunctella* strains collected from treated grain bins were more resistant than strains from untreated bins, indicating that the resistance can develop quickly in the field.

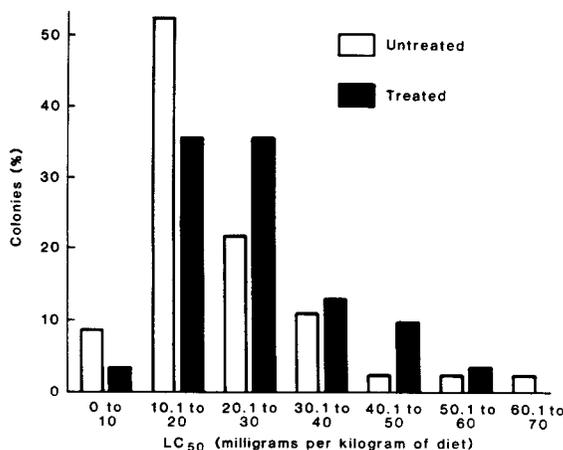
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There have been few reports of insect resistance to microbial insecticides, leading to the presumption that insects are unlikely to become resistant to these agents (1-3). A few investigators have selected laboratory strains of Lepidoptera resistant to insect viruses (1, 2) and of Diptera resistant to the  $\beta$ -exotoxin of *Bacillus thuringiensis* (1, 2, 4). However, attempts to select insects resistant to commercial formulations containing the spores and  $\delta$ -endotoxin complex of *B. thuringiensis* (BT), the most widely used and intensively studied microbial insecticide, have been unsuccessful (1-3). I now report that *Plodia interpunctella*, a lepidopteran pest of stored grain and grain products, can develop resistance to a commercial BT formulation within a few generations and that such resistance has been detected in bins of BT-treated grain.

As part of a large-scale study of the effectiveness of BT in controlling *P. interpunctella* in stored grain, insects were collected from native populations in treated and untreated grain storage facilities over a five-state area. Laboratory

colonies were established from each population, and the susceptibilities of the colonies (strains) to a commercial formulation of BT were determined (5). The median lethal concentration (LC<sub>50</sub>) of BT for 77 colonies ranged from 6.8 to 60.2 mg per kilogram of diet, with a mean of 22.5. The dose-mortality relations were essentially parallel to an average slope of 1.568 (range, 1.030 to 2.132). This range in LC<sub>50</sub>'s is similar to that reported earlier for a smaller number of laboratory colonies (6). However, in my study the colonies established from populations in BT-treated bins were significantly less susceptible than colonies

Fig. 1. Frequency distribution of LC<sub>50</sub> values for *P. interpunctella* colonies collected from untreated and BT-treated bins of grain.



from populations in untreated bins [mean ( $\pm$  standard error) LC<sub>50</sub>'s for treated and untreated bins,  $25.1 \pm 2.0$  ( $n = 31$ ) and  $20.7 \pm 1.7$  ( $n = 46$ ) mg/kg, respectively ( $P = 0.009$ , analysis of variance) (Fig. 1) (7). Slopes of the dose-mortality relations for the two groups of colonies did not differ significantly. Although the difference in mean LC<sub>50</sub>'s is small, it does indicate a selective effect of BT on the insect populations. Large differences would not be expected because the insects were collected within a short time (1 to 5 months) after the grain was treated.

To determine whether a resistant colony could be selected in the laboratory, I selected a colony from a population that was not being effectively controlled by BT. Beginning with the 20th laboratory generation, the colony was subcultured on larval diet treated with BT at 62.5 mg/kg, a dose expected to produce 70 to 90 percent larval mortality (8). Survival in the first generation was 19 percent. In the second generation survival increased to 44 percent, in the third to 63 percent, and in the fourth to 82 percent. Thereafter, survival fluctuated between 68 and 89 percent, comparable to the unselected colony's survival rate of 71 to 89 percent on untreated diet. There was a corresponding rapid increase in the LC<sub>50</sub> for the colony (Fig. 2A). After two generations of selection the LC<sub>50</sub> increased to 27 times the average level for the unselected colony and continued to increase more gradually to 97 times after 15 generations. There was no consistent shift in the slope of the dose-mortality relations between the resistant colony and the unselected colony.

After nine generations of selection at a BT dose of 62.5 mg/kg, selection pressure was increased by subculturing the resistant colony on a diet treated with BT at 500 mg/kg. In the first generation survival was 32 percent, in the second

and third it was 51 percent, and thereafter it fluctuated between 64 and 81 percent. The  $LC_{50}$  increased, but not much faster than in the parent colony, which continued to be maintained on diet treated with BT at 62.5 mg/kg (Fig. 2B). Resistance leveled off at about 100 times the  $LC_{50}$  for the parent colony.

After nine generations of selection the resistant colony was subcultured back to untreated diet to determine the stability of its resistance. Resistance did not decrease during seven subsequent generations on untreated diet (Fig. 2C), indicating that a pure resistant strain had been selected or that there was not a significant reproductive disadvantage associated with the resistance.

The resistant strain (R) was crossed with a susceptible laboratory colony (S) to determine the dominance of the resistance. Virgin males and females were separated as they emerged and mass crosses were made between R females and S males and between S females and R males. Progeny from both crosses were susceptible to BT, indicating that the resistance was recessive ( $LC_{50}$  for R females  $\times$  S males,  $26.2 \pm 0.8$  mg/kg;  $n = 1600$ ; slope,  $1.941 \pm 0.085$  and  $LC_{50}$  for S females  $\times$  R males,  $43.1 \pm 1.5$  mg/

kg;  $n = 1500$ ; slope,  $2.618 \pm 0.133$ ). There was a small difference in susceptibility between progeny of the two reciprocal crosses, but their susceptibilities were closer to that of the susceptible laboratory colony (27 mg/kg) than to that of the resistant strain ( $\sim 400$  mg/kg at the time of the crosses) and appear to be too close to suggest sex linkage. There was no sex-related difference in susceptibility among the progeny.

A mixed bioassay was done with the tenth selected generation of the resistant strain and a known susceptible strain distinguishable by its golden wing scales (9, 10). Upon emergence the insects were sorted visually and the  $LC_{50}$  was calculated for each strain. The resistant strain had an  $LC_{50}$  of  $453.4 \pm 34.5$  mg/kg (slope,  $2.202 \pm 0.150$ ;  $n = 600$ ) and the susceptible golden-wing strain had an  $LC_{50}$  of  $14.9 \pm 0.8$  mg/kg (slope,  $3.307 \pm 0.457$ ,  $n = 700$ ), strongly suggesting that the wide differences in susceptibility did not arise from an error in the bioassay.

I also attempted to select for resistance to BT in four other colonies beginning 18 to 26 generations after colonization. All developed resistance within two or three generations and became pro-

gressively more resistant, confirming that the test colony was not unique. No selection attempts failed to demonstrate resistance.

For more than 20 years BT has been used to control phytophagous insects, and to my knowledge development of significant resistance in the field or laboratory has not been reported (1, 2). Most have considered such resistance to be possible but unlikely, perhaps because of the apparently complex mode of action of BT formulations in lepidopteran species (1, 3). These species apparently respond to the crystal protein ( $\delta$ -endotoxin), spores,  $\beta$ -exotoxin, or a combination thereof. *Plodia interpunctella* responds to the crystal protein but is most susceptible to spore-crystal mixtures (11). Notwithstanding the probable complexity of the mode of action of BT, these results and an earlier report (6) show that there is already extensive diversity in the susceptibility of populations of *P. interpunctella* to the insecticide. This diversity and the somewhat higher resistance in colonies collected from BT-treated bins reflect the capacity of *P. interpunctella* to develop high levels of resistance within a few generations. The rapid appearance of the recessive trait under laboratory selection with a relatively low dose of the insecticide also suggests that the resistance may be due to a single major factor that occurs with a high frequency (12).

The stored grain environment is ideal for development of resistance because BT is stable on stored grain (13, 14) and because the environment may remain undisturbed for long periods, permitting the insects to breed for successive generations in contact with the bacterial spores and toxins. In field crop situations the instability of foliarly applied BT and the transitory nature of plant-pest interactions decrease the probability of resistance. Repeated applications over a wide geographic area for several years would be required to expose the pests for many successive generations.

The speed at which *P. interpunctella* developed resistance to BT in this study suggests that it could do so within a single storage season in bins of treated grain.

#### References and Notes

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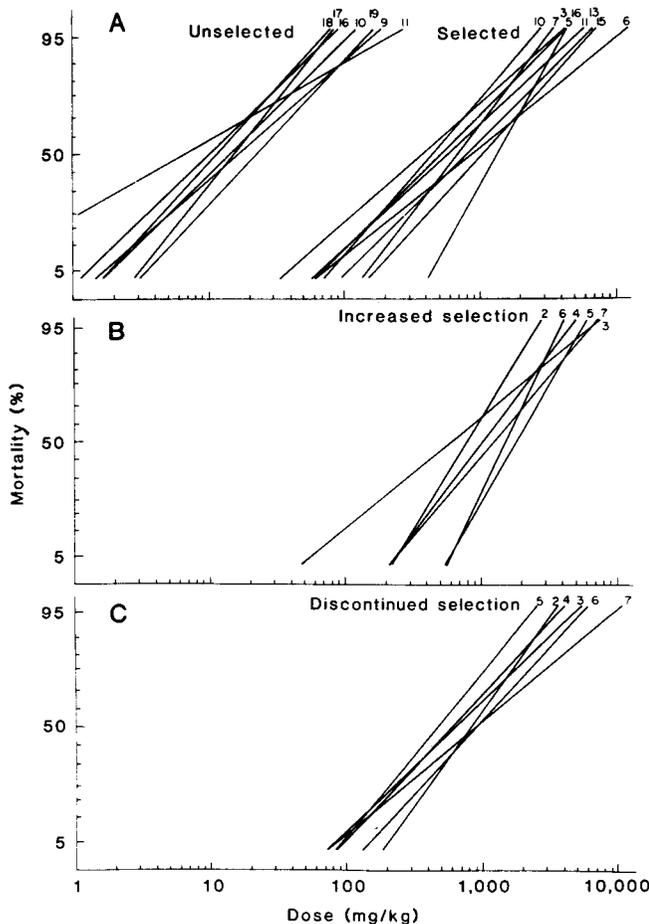


Fig. 2. Dose-mortality responses of a *P. interpunctella* colony selected for resistance to BT by rearing on BT-treated diet. (A) Responses of several generations of the unselected parent colony and the colony reared (selected) on diet treated at 62.5 mg/kg. (B) Responses of several generations of the resistant colony when reared (selected) on diet treated at 500 mg/kg after nine generations of selection at 62.5 mg/kg. (C) Responses of several generations of the resistant colony when reared on untreated diet after nine generations of selection at 62.5 mg/kg.

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  5. Insects were collected from infested bins as larvae or pupae in strips of corrugated paper placed on the grain surface. The paper containing insects was returned to the laboratory and placed in jars, where adults emerged. Eggs were collected from these adults and used to start colonies on the standard laboratory larval diet of cracked wheat fortified with wheat germ, yeast, honey, and glycerol. All insect rearing and testing was done at 25°C and 60 to 70 percent relative humidity. Bioassays were conducted with Dipel, a wettable powder formulation of *B. thuringiensis* subsp. *kurstaki* containing 16,000 IU of potency per milligram of formulation. Powder was suspended in water at 5 mg/ml and serial 1:2 dilutions were prepared to provide nine doses ranging from 500 to 1.95 mg/kg when applied to larval diet at 0.1 ml/g. Each concentration of suspension was thoroughly mixed into a 30-g sample of diet. Samples of diet were treated with water to serve as controls. Samples of diet were placed in Mason jars with filter-paper caps and 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. Three to six bioassays (replicates) were done on different generations of each colony. The  $LC_{50}$  and the slope of the dose-mortality relation were calculated for each bioassay with the probit analysis procedure of the Statistical Analysis System, SAS Institute, Cary, N.C.
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  7. The  $LC_{50}$ 's of colonies from treated and untreated bins, weighted by the inverse of their variances, were compared by analysis of variance by using the general linear models procedure of the Statistical Analysis System.
  8. The colony was bioassayed periodically to monitor for changes in susceptibility. The bioassay procedure was similar to that used on the original colonies except that three replicate bioassays were done on each generation tested, and eventually the upper dose was raised to 2000 mg/kg. Data from the three replicates were pooled for calculating dose-mortality regressions.
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  15. I thank R. W. Beeman for advice on genetic aspects of the study; E. B. Dicke, R. E. Schulze, and L. H. Hendricks for assistance in conducting the study; and G. A. Milliken for the statistical analyses.

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