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Diamondback Moth Resistance to *Bacillus thuringiensis* in Hawaii

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Abstract

Resistance to the microbial insecticide *Bacillus thuringiensis* Berliner has evolved in field populations of diamondback moth, *Plutella xylostella* (L.), in Hawaii. Three field populations that had been treated repeatedly with commercial formulations of the spore-crystal protein complex of *B. thuringiensis* subsp. *kurstaki* developed significant resistance to it. The LC_{50} of the most resistant population was 25-36 times greater than the LC_{50}s of susceptible laboratory strains and 41 times greater than the LC_{50} of the most susceptible field population. Laboratory selection of three strains established from the most resistant field population caused 15-30-fold increases in LC_{50} in nine generations, resulting in 430-820-fold resistance compared with a susceptible laboratory strain. Resistance declined slowly in the absence of treatments. Development of resistance to a commercial formulation containing a mixture of *B. thuringiensis* toxins by field populations of DBM casts doubt on the ability of mixtures to retard evolution of resistance. Slow restoration of susceptibility in the absence of treatments suggests that rotations may not be especially effective for retarding resistance development. We urge judicious use of *B. thuringiensis* to conserve its efficacy.

Introduction

Throughout the world, pests are becoming resistant to pesticides at an alarming rate (NRC 1986; Roush and Tabashnik 1990). The success of conventional pesticides is also threatened by increasing awareness of their toxicity to natural enemies (Johnson and Tabashnik 1991) and their harmful effects on the environment. Microbial insecticides are a promising alternative. The most widely used microbial insecticide, *Bacillus thuringiensis* Berliner (*Bt*), is highly toxic to certain pests, yet it has little or no adverse effect on most nontarget organisms, including humans (Flexner et al. 1986; Wilcox et al. 1986).

Bt is especially useful for control of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), a worldwide pest of cruciferous vegetables. *Bt* does not harm the hymenopterous parasitoids of DBM (Brunner and Stevens 1986), but it is highly effective against DBM that are resistant to conventional insecticides (Sun et al. 1986). In two independent laboratory experiments, selection with *Bt* for 10 and 30 generations did not increase resistance to *Bt* (Devriendt and Martouret 1976; Krieg and Langenbruch 1981). These laboratory results and the lack of well-documented cases of resistance from field populations led some scientists to conclude that DBM was unlikely to develop resistance to *Bt* under field conditions (e.g., see p. 238 in Talekar and Griggs 1986). However, at the First International Workshop on Diamondback Moth in 1985, C. N. Sun suggested that 'if *Bt* were used on a scale comparable to that of the synthetic insecticides, DBM might become just as resistant' (p. 398 in Talekar

and Griggs 1986). Kirsch and Schmutterer (1988) reported low efficacy of *Bt* against DBM that was suggestive of resistance, but they could not exclude alternative explanations.

As part of a long-term study of insecticide resistance in DBM (Tabashnik 1986; Tabashnik et al. 1987; Tabashnik and Cushing 1989), we measured susceptibility to *Bt* in 1986-87 and 1989-90 for several populations in Hawaii. Based on these surveys, we documented, for the first time, evolution of resistance to *Bt* in open field populations (Tabashnik et al. 1990). In this paper, we review the results of our field studies and subsequent experiments that characterized the response of resistant populations to continuation and relaxation of selection (Tabashnik et al. 1991).

Methods

Field surveys: During 1986-87 we sampled 50-300 individuals from each of six field populations in Hawaii (Fig. 1) (Tabashnik et al. 1990). During 1989-90, we resampled four of the same field populations. Two of the populations were treated repeatedly with *Bt* between the first and second sampling (SO on Oahu and KH on the island of Hawaii). These heavily treated sites were compared with sites that received little or no *Bt* between the first and second sampling (WO on Oahu and LH on Hawaii). A watercress farm (NO on Oahu) that had been treated with Bt approximately 50-400 times was sampled in 1989 only (Fig. 1).

Larvae were reared on cabbage in the laboratory and F_1-F_3 offspring of field-collected DBM were tested for susceptibility to Dipel, a commercial formulation of *Bt*, using leaf residue bioassays. Larvae from two untreated laboratory colonies, LAB-P and LAB-L, were also tested

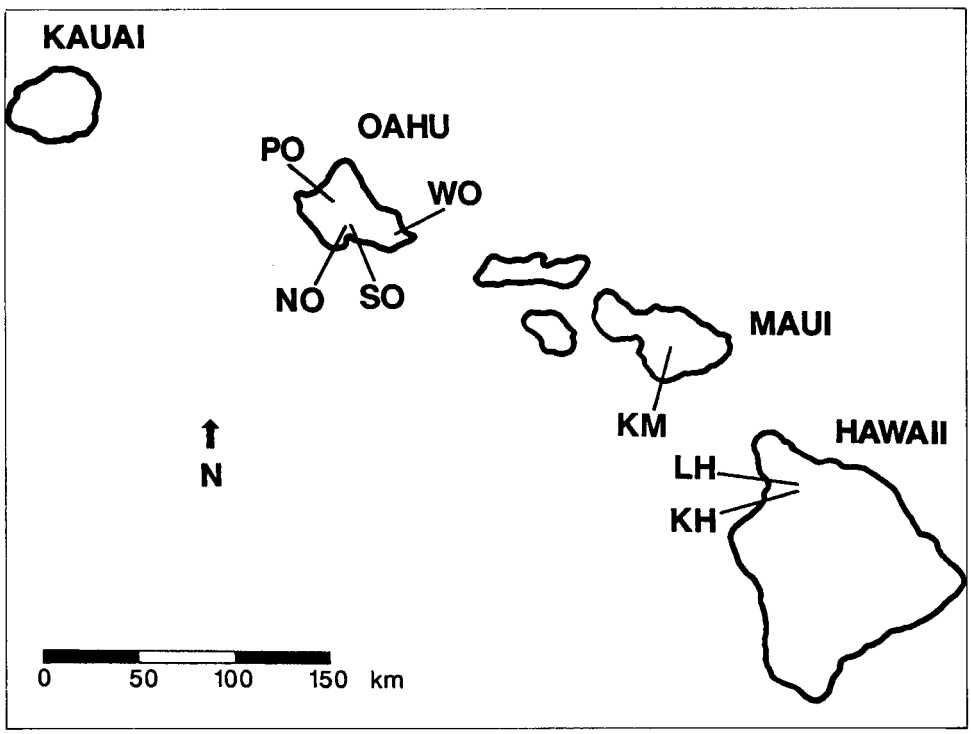

Fig. 1. Study sites (see Tabashnik et al. 1987, 1990 for detailed background information on study sites).

for susceptibility to *Bt* during 1986-87 and 1989-90. Mortality was recorded after 48 hours. All tests were replicated 4-16 times (see Tabashnik et al. 1990 for additional details).

Response to continuation and relaxation of selection: To assess the response of the resistant NO population to continuation and relaxation of selection in the laboratory, we first tested F_1 offspring of field-collected individuals using leaf residue bioassays (Tabashnik et al. 1991). The F_1-F_3 generations were reared without exposure to *Bt*. F_4 offspring were split into three selected subcolonies (NO-P, NO-Q, and NO-R) and one unselected subcolony (NO-U). For each generation of selection, approximately 200 larvae from each subcolony were fed leaf disks dipped in Dipel 2X at 25.6-512 mg active ingredient (AI) per liter which caused approximately 39-94% mortality. Larvae from NO-P, NO-Q, and NO-R were selected for five generations and tested by bioassay. Larvae from NO-P and NO-Q were selected for an additional four generations and then tested by bioassay again. The unselected subcolony (NO-U) was tested by bioassay at the 4th, 6th, 9th and 15th generations.

We also used bioassays to measure the response of the resistant SO population to relaxation of selection in the laboratory and field and to continuation of selection in the field. We collected DBM from SO on 18 May, 30 June, and 15 September 1989. We reared colonies initiated from the field samples of 30 June and 15 September without exposure to *Bt* for three and two generations, respectively, to determine their response to relaxation of selection in the laboratory. The SO field population was not treated with *Bt* between 30 June and 15 September. Response to relaxation of selection in the field was measured by comparing the suceptibility of larvae that were offsprings of individuals collected 30 June versus 15 September. The SO field population was treated with *Bt* five times between 18 May and 30 June. To measure the response to this additional selection, we compared the susceptibility of larvae obtained from individuals collected on 18 May versus 30 June.

Analysis. Median lethal concentrations (LC_{50}s) were estimated using probit analysis (PROC PROBIT, SAS 1985). Two LC_{50}s were considered significantly different if their 95% fiducial limits did not overlap (see Tabashnik et al. 1990 and 1991 for additional details).

Results

Field development of resistance. Our results showed that repeated treatments with *Bt* increased resistance to *Bt* in field populations of DBM (Fig. 2). The initial survey of field populations during 1986-87 showed that the SO population, which had been treated with *Bt* approximately 50-100 times from 1978 to 1982, was significantly more resistant to *Bt* than either of the susceptible laboratory strains (LAB-P and LAB-L). The LC_{50} of the SO population was about six times greater than the LC_{50}s of the LAB-P strain and the most susceptible field population, KM, a minimally treated population from Maui (Fig. 2). However, the LC_{50} of the SO population was not significantly greater than the LC_{50} of the two minimally treated populations on Oahu (WO and PO).

Between our initial survey and subsequent sampling during 1989-90, the SO population was treated with *Bt* 15 times and the KH population was treated repeatedly (number of treatments not known). Resistance to *Bt* in both of these populations increased significantly. The LC_{50} for SO doubled and the LC_{50} for KH quadrupled (Fig. 2). In contrast, during the same period, resistance to *Bt* did not increase significantly in two minimally treated field populations (WO and LH) nor in the two untreated laboratory populations (LAB-P and LAB-L). Results from 1989-90 showed that resistance in SO and KH, the two heavily treated populations, was significantly greater than resistance in WO and LH, the minimally treated populations.

The heavily treated NO population, which was sampled only in 1989, was highly resistant to *Bt* (Fig. 2). The LC_{50} for NO was 28 times greater than the average LC_{50} of the two laboratory colonies and 41 times greater than the LC_{50} of the most susceptible field population (KM).

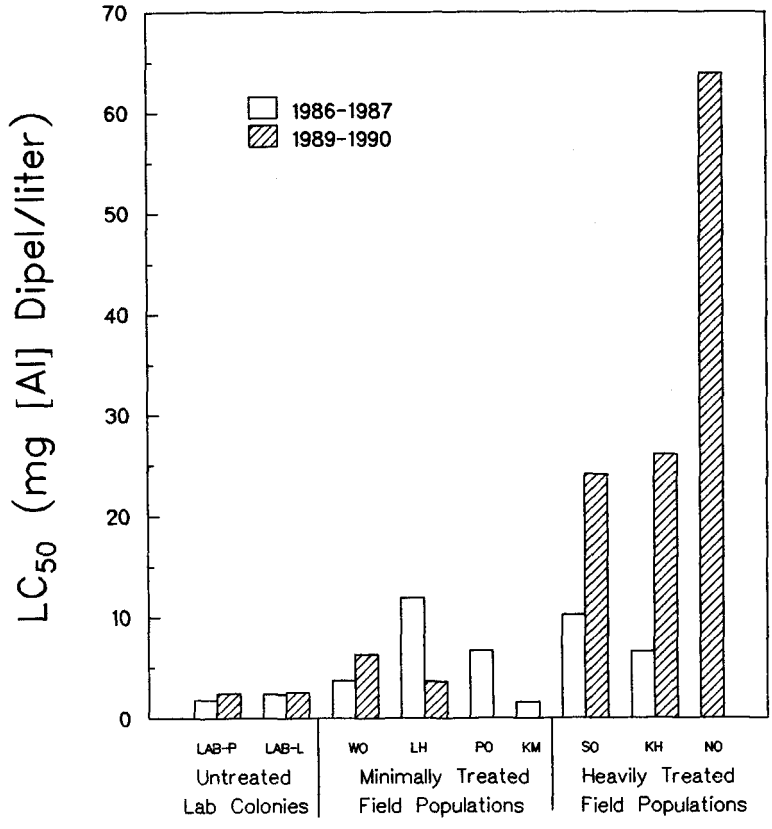

Fig. 2. Susceptibility of DBM larvae to *Bt* (Dipel). PO and KM were sampled only during 1986-87. NO was sampled only during 1989. KH received few Bt treatments before 1986, but was treated repeatedly between 1987 and 1990. The average slope of the concentration-mortality lines was 1.45 (range: 1.08 + 0.21 SE to 2.66 + 0.52 SE) (expanded from Tabashnik et al. 1990).

Response to continuation of selection. Laboratory selection with *Bt* rapidly increased resistance to *Bt* in each of the selected subcolonies (NO-P, NO-Q, and NO-R) (Fig. 3). Five generations of selection caused 5-7-fold increases in LC_{50}; nine generations of selection increased LC_{50}s by 15-30-fold. After nine generations of selection, LC_{50}s were 430-820 times greater than the LC_{50} of the susceptible LAB-P colony. Mortality at the field rate of *Bt* (25.6 mg (AI)/l) was less than 2% for the selected subcolonies compared with 90-100% for susceptible laboratory colonies.

In contrast to the rapid response to laboratory selection, five treatments of *Bt* applied to the SO field population caused no detectable increase in LC_{50}. The LC_{50} estimated from the 18 May field collection (before treatments) was 24 mg AI/l; the 30 June LC_{50} (after treatments) was 11 mg AI/l.

Response to relaxation of selection. Field-selected resistance to *Bt* declined slowly when treatment with *Bt* was stopped. In the laboratory, the LC_{50}s of the unselected subcolony initiated from NO (NO-U) were 64, 29, 18, 38, and 9.5 mg AI/l at the F_1, F_4, F_6, F_9, and F_{15} generations, respectively (Fig. 4). LC_{50}s for the F_6 and F_{15} were significantly lower than the initial LC_{50}. LC_{50}s for the F_4 and F_9 did not differ significantly from the original estimate.

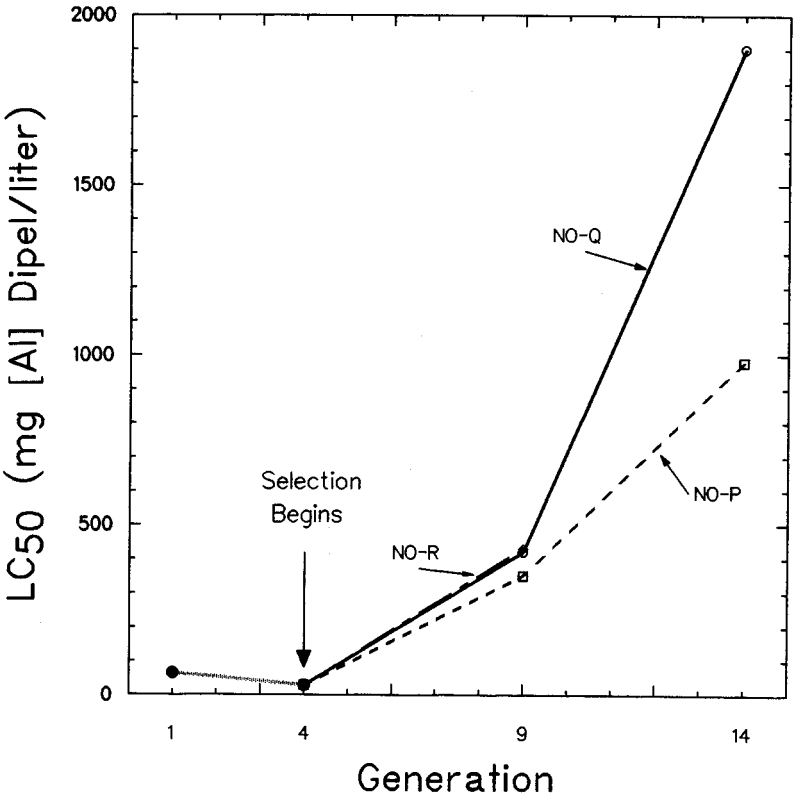

Fig. 3. Response of a field-selected DBM population to additional selection for resistance to *Bt* in the laboratory (adapted from Tabashnik et al. 1991).

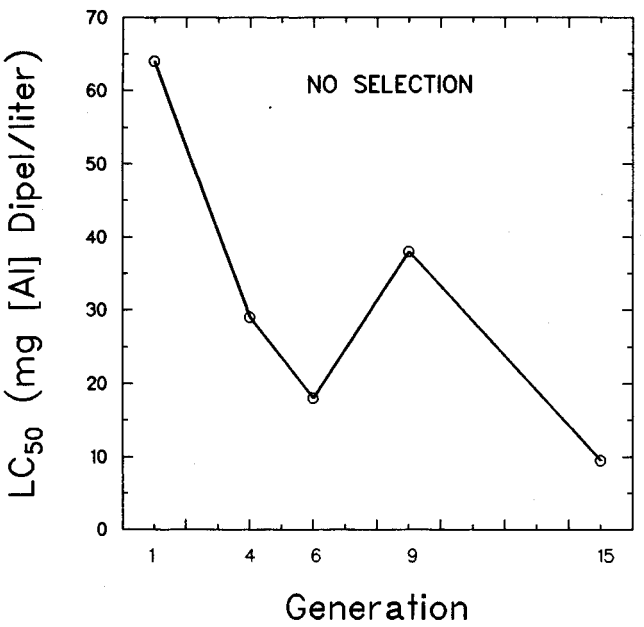

Fig. 4.
Response of a field-selected DBM population to relaxation of selection in the laboratory (adapted from Tabashnik et al. 1991).

For the colony initiated from the 18 May collection from SO, the LC_{50}s (all expressed in mg AI/l) were 48 for the F_1, 18 for the F_2, and 23 for the F_3. The LC_{50} for the 15 September collection from SO declined from 32 in the first generation to 17 in the second generation. None of the differences among laboratory-reared generations of SO colonies were significant.

Between 30 June and 15 September the SO field population was not treated with *Bt*. However, the LC_{50} for 15 September (24 mg AI/l) was not lower than the LC_{50} for 30 June (11 mg AI/l).

Discussion

Microbial pesticides, particularly *Bt*, are likely to become increasingly important as pest resistance and environmental concerns reduce the usefulness of conventional insecticides. Although laboratory selection has increased resistance to *Bt* in several species of insects (McGaughey 1985; McGaughey and Beeman 1988; Stone et al. 1989; Miller et al. 1990), the lack of documented cases of resistance to *Bt* in open field populations led to the assumption that such resistance was unlikely (Briese 1981; Wilcox et al. 1986; Wilding 1986; de Barjac 1987).

Our results show, however, that at least three field populations of DBM in Hawaii have evolved resistance to *Bt*. Each of these populations has been treated repeatedly with *Bt*. Indeed, the frequency of use of *Bt* to control these populations may have been comparable to excessive use of conventional pesticides. Thus, our results support Sun's prediction that if *Bt* is used like a conventional insecticide, then development of resistance to *Bt* can be expected (p. 398 in Talekar and Griggs 1986).

One field population (NO) with a history of frequent *Bt* treatments was highly resistant to *Bt* compared with other populations. We also documented substantial and statistically significant increases in resistance to *Bt* in two other field populations (SO and KH) in response to numerous treatments with *Bt* that were made between 1986-87 and 1989-90. Our data show that nearby field populations that were not treated repeatedly with *Bt* and two untreated laboratory colonies showed no comparable increase in resistance to *Bt* during the same period. In conjunction with growers' reports of reduced efficacy, our results provide strong evidence that field populations of DBM have developed resistance to *Bt* that is sufficiently high to thwart control.

Our finding of resistance to *Bt* in DBM populations occurring on two different islands (Oahu and Hawaii) and two different crops (watercress and cabbage) strongly suggests that resistance to *Bt* evolved independently at least twice in Hawaii. If so, we would anticipate that genes for resistance to *Bt* are also present in DBM populations outside of Hawaii. Thus, we would expect resistance to *Bt* to develop in other field populations of DBM that are treated repeatedly with *Bt*. Indeed, we are aware of suspected resistance to *Bt* in DBM from Asia and North America.

The rapid response to laboratory selection in our experiments showed that the resistant NO population contained genetic variation for resistance to *Bt*. We hypothesize that susceptible laboratory populations did not evolve resistance to *Bt* in previous selection experiments (Devriendt and Martouret 1976; Krieg and Langenbruch 1981) because they were relatively small and genetically homogeneous.

In our selection experiments, five generations of selection caused 5-7-fold increases in LC_{50}s. After nine generations of selection, LC_{50}s were 40-70 times greater than the recommended field rate of *Bt*. These results show that DBM has the potential to attain much higher levels of resistance than those previously found in the field.

In contrast to our laboratory results, we found that five treatments with *Bt* in the field did not cause a detectable increase in resistance to *Bt*. We hypothesize that the selection intensity imposed by five field treatments was weaker than that imposed by five generations of laboratory selection because more larvae escaped treatment in the field and concentrations of *Bt* used in the laboratory were higher than field rates. Other potential differences that might have affected the results include immigration and emigration in the field, and differences in initial genetic variation between populations.

In conclusion, resistance of DBM to *Bt* provides warnings about intensive use of microbial insecticides. First, DBM can evolve resistance to mixtures of *Bt* toxins. Field populations of

DBM developed resistance to Dipel, which contains a mixture of *Bt* toxins (Höfte and Whiteley 1989). We do not know if DBM resistance to Dipel is primarily or entirely due to resistance to one of the toxins in Dipel. Nonetheless, the ability of DBM to resist Dipel shows that mixtures of *Bt* toxins are not impervious to resistance development. Experiments are needed to determine if resistance to single toxins evolves more slowly than resistance to mixtures of toxins. Limited experimental evidence from conventional insecticides suggests that mixtures do not always retard resistance development (Tabashnik 1989; Immaraju et al. 1990).

Second, rotations or other management strategies that assume rapid restoration of susceptibility when *Bt* treatments are stopped may not be particularly useful. Our results show that field-selected resistance declined slowly and inconsistently when exposure to *Bt* was discontinued. Our data suggest that leaving several generations untreated may not always reduce resistance. Approximately 1 year of rearing the NO-U subcolony without exposure to insecticide caused a significant and substantial decline in its LC_{50} to *Bt*. Survival at the field rate of *Bt* decreased from 66 to 43%, but this was still much higher than the 0-10% survival of susceptible populations treated with the field rate. This decline in resistance would be too slow to markedly enhance the success of rotations.

Third, intensive use of *Bt* against resistant populations can cause rapid development of extremely high levels of resistance. The NO population, which had reached approximately 30-fold resistance in the field, quickly attained greater than 400-fold resistance when selected with high concentrations of *Bt* in the laboratory. These results suggest that attempts to overwhelm moderately resistant populations with high concentrations of *Bt* are likely to backfire. After resistance to *Bt* has increased to detectable levels, alleles for resistance to *Bt* are likely to be common enough to give the 'high dose' strategy little chance of suppressing resistance (Tabashnik and Croft 1982).

In summary, as in most cases, the best opportunity to manage resistance to *Bt* in DBM is to take action before resistance occurs. *Bt* should be used judiciously to conserve its efficacy against DBM. Management programs that emphasize biological and cultural controls can integrate *Bt* and other insecticides sparingly, thereby prolonging their usefulness.

We do not know if results from DBM can be extrapolated to provide insight into the potential for resistance to *Bt* in other pests. The most detailed knowledge of resistance to *Bt* currently available is derived from laboratory-selected strains of other insects, particularly *Plodia interpunctella* (Hübner), the Indian meal moth (McGaughey 1985; Johnson et al. 1990; van Rie et al. 1990). Because field- and laboratory-selected resistances can differ markedly, studies of field-selected resistance in DBM may be particularly valuable for understanding and managing resistance to *Bt*.

The remarkable success of insects in overcoming virtually every type of insecticide suggests that we would be wise to assume that the threat of resistance to *Bt* is imminent (Gould 1988a, 1988b; Raffa 1989). Underestimating our enemy could be costly; proceeding with caution may help to conserve the effectiveness of an extraordinarily specific and environmentally safe insecticide.

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