Insect Resistance to Transgenic Bt Crops: Lessons from the Laboratory and Field

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ABSTRACT Transgenic crops that produce insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) grew on >62 million ha worldwide from 1996 to 2002. Despite expectations that pests would rapidly evolve resistance to such Bt crops, increases in the frequency of resistance caused by exposure to Bt crops in the field have not yet been documented. In laboratory and greenhouse tests, however, at least seven resistant laboratory strains of three pests (*Plutella xylostella* [L.], *Pectinophora gossypiella* [Saunders], and *Helicoverpa armigera* [Hübner]) have completed development on Bt crops. In contrast, several other laboratory strains with 70- to 10,100-fold resistance to Bt toxins in diet did not survive on Bt crops. Monitoring of field populations in regions with high adoption of Bt crops has not yet detected increases in resistance frequency. Resistance monitoring examples include *Ostrinia nubilalis* (Hübner) in the United States (6 yr), *P. gossypiella* in Arizona (5 yr), *H. armigera* in northern China (3 yr), and *Helicoverpa zea* (Boddie) in North Carolina (2 yr). Key factors delaying resistance to Bt crops are probably refuges of non-Bt host plants that enable survival of susceptible pests, low initial resistance allele frequencies, recessive inheritance of resistance to Bt crops, costs associated with resistance that reduce fitness of resistant individuals relative to susceptible individuals on non-Bt hosts ("fitness costs"), and disadvantages suffered by resistant strains on Bt hosts relative to their performance on non-Bt hosts ("incomplete resistance"). The relative importance of these factors varies among pest-Bt crop systems, and violations of key assumptions of the refuge strategy (low resistance allele frequency and recessive inheritance) may occur in some cases. The success of Bt crops exceeds expectations of many, but does not preclude resistance problems in the future.

KEY WORDS genetically modified crops, resistance, *Bacillus thuringiensis*, refuge, Bt crops

TRANSGENIC CROPS THAT PRODUCE toxins from the bacterium *Bacillus thuringiensis* (Bt) can control some key pests, thereby reducing reliance on insecticide applications (Shelton et al. 2002, Carrière et al. 2003). Ingested Bt toxins kill susceptible insects by binding to and disrupting their midgut membranes (Schnepf et al. 1998). Lepidopteran larvae are the primary targets of >99% of the acreage of Bt crops grown to date (James 2002). Large-scale planting of Bt crops began in 1996 and increased quickly, with >14 million ha grown worldwide in 2002 (James 2002). The cumulative area of Bt crops from 1996 to 2002 was 62 million ha (James 2001, 2002, and references therein), enough to cover the entire states of California and Iowa. Bt crops grown to date expose pest populations to Bt toxin throughout the growing season.

The widespread and prolonged exposure to Bt toxins represents one of the largest selections for resistance in insect populations the world has ever seen (Tabashnik 1994, Gould 1998, Shelton et al. 2002, Ferré and Van Rie 2002). Before Bt crops were grown commercially, many scientists expected that pests would rapidly evolve resistance. This view was supported by pervasive resistance to insecticides, laboratory-selected resistance to Bt toxins in many insects, and field-evolved resistance to sprays of Bt toxins in diamondback moth, *Plutella xylostella* (L.) (Georghiou and Lagunes-Tejeda 1991, Tabashnik 1994, Shelton et al. 2002, Ferré and Van Rie 2002, Heckel et al. 2003).

To counter the threat of resistance, the refuge strategy has been widely adopted. This strategy is based on theory developed in dozens of papers during the past 25 yr (e.g., Georghiou and Taylor 1977, Tabashnik and Croft 1982, Roush 1994, Gould 1998, Peck et al. 1999, Caprio 2001, Onstad et al. 2002) and limited experimental evidence from small-scale tests (Liu and Tabashnik 1997, Shelton et al. 2000, Tang et al. 2001). It entails planting refuges of non-Bt host plants along with Bt crops to promote survival of susceptible pests. Ideally, resistance is conferred by rare, recessive alleles and most resistant adults from Bt crops mate with

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Table 1. Survival of resistant laboratory strains on Bt crops

Bt crop	Bt toxin[a]	Insect	Strain	RR[b]	Survival on Bt crop (%)[c]	Reference
			Commercially grown Bt crops			
Corn	Cry1Ab or Cry1Ac	*O. nubilalis*	KS-SC	70	0	Huang et al. (2002)
Cotton	Cry1Ac	*H. armigera*	Cry1Ac-sel	13	25	Fan et al. (2000)
			BX	57	58	Akhurst et al. (2003)
		H. virescens	YHD2	10,100	0	Gould et al. (1995), F. Gould (personal communication)
		P. gossypiella	AZP-R	3,100	45[d]	Tabashnik et al. (2000a, 2002a, b), Liu et al. (2001a), Morin et al. (2003)
			APHIS-98R	>100	37	Liu et al. (1999, 2001b)
Potato	Cry3A	*L. decemlineata*	Bt-R	>400	0	Wierenga et al. (1996)
			Experimental Bt crops			
Broccoli	Cry1Ac	*P. xylostella*	Loxahatchee	300	90	Roush (1994), Metz et al. (1995)
	Cry1C		Cry1C-Sel	12,400	91	Zhao et al. (2000)
Canola	Cry1Ac	*P. xylostella*	NO-QA	>6,800	100	Tabashnik et al. (1993), Ramachandran et al. (1998)

[a] For each insect strain, the same Bt toxin was produced in transgenic plants and tested in laboratory bioassays, except for *O. nubilalis* and the Loxahatchee strain of *P. xylostella.* In the two exceptional cases, the Bt plants produced the toxins listed and the bioassays were done with formulations (Dipel and Javelin, respectively) containing Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, spores, and other components.

[b] RR (resistance ratio) = LC_{50} of resistant strain ÷ LC_{50} of susceptible strain based on results from bioassays with Bt toxin or formulation.

[c] (Survival on Bt crop ÷ survival on non-Bt variety of the same crop) × 100%; for the YHD2 strain of *H. virescens*, survival was 0% on Bt and non-Bt cotton.

[d] Median from four experiments, range = 39–100%.

susceptible adults from refuges. If so, the theory predicts that resistance will be delayed substantially. Although large-scale tests of the refuge strategy have not been reported, experiments show that violations of some key assumptions (low resistance allele frequency and recessive inheritance of resistance to Bt crops) may occur in several pest-Bt crop systems examined to date (Tabashnik et al. 2000a, Burd et al. 2003, Akhurst et al. 2003). Thus, given the widespread use of Bt crops against various pests, resistance might evolve quickly in some situations despite the presence of refuges.

Contrary to this expectation, increases in the frequency of resistance to Bt toxins in field populations caused by exposure to commercially grown Bt crops have not been documented yet. To better understand this outcome, we review in this study the current status of pest resistance to Bt crops, including responses of resistant laboratory strains to Bt plants and frequencies of resistance in field populations targeted by Bt crops. Because Bt corn that produces Cry1Ab or Cry1Ac and Bt cotton that produces Cry1Ac have accounted for nearly all of the Bt crop acreage grown to date, we emphasize the key lepidopteran pests targeted by these crops. We also consider responses of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), to Bt potato, which was grown commercially on a relatively small scale (<100,000 ha from 1996 to 2001), and of diamondback moth to Bt crucifers that were developed primarily for resistance research and have not been grown commercially. We focus on studies published or in press in refereed journals, using references to other sources primarily to supplement information about strains and populations described in refereed journal articles.

Survival of Resistant Laboratory Strains on Bt Plants

We review here responses to Bt plants of 10 Bt-resistant strains of six species of pests. An important finding is that resistance to Bt toxins or formulations in diet or leaf dip bioassays does not necessarily confer the ability to survive on Bt plants (Table 1). Strains that have been selected with Bt toxins are considered resistant if they show genetically based reduction in susceptibility compared with unselected conspecific strains (Tabashnik 1994). However, this reduced susceptibility to Bt toxins or formulations is not always sufficient to enable completion of larval development on Bt plants (Roush 1994, Tabashnik et al. 2000b). Difficulty surviving on Bt plants despite resistance to Bt toxins or formulations in laboratory bioassays could be caused by longer exposure to toxins in tests with Bt plants, higher toxin concentrations in Bt plants, interactions between plant chemistry and Bt toxins, production of the active form of the toxin in Bt plants rather than the protoxin form sometimes tested in laboratory bioassays, or differences in the sets of toxins produced by Bt plants and those tested in laboratory bioassays.

For example, larvae from the resistant (KS-SC) strain of European corn borer, *Ostrinia nubilalis* (Hübner), did not survive on transgenic corn that produces Cry1Ab or Cry1Ac (Huang et al. 2002). In laboratory bioassays with artificial diet, this strain had up to 70-fold resistance to Dipel (Huang et al. 1999), a formulation of Bt subsp. *kurstaki* containing Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, and other components (Abbott Laboratories 1992).

In a similar case, larvae of Colorado potato beetle from a resistant strain did not survive on Bt potato

plants that produce Cry3A despite >400-fold resistance of neonates to Cry3A in diet tests (Wierenga et al. 1996). Because the resistant strains of European corn borer and Colorado potato beetle survived on nontransgenic corn and potato (Huang et al. 2002, Wierenga et al. 1996), respectively, their inability to survive on Bt plants can be attributed to the Bt toxins in the plants or interactions between the toxins and other plant traits.

In contrast, the YHD2 strain of *Heliothis virescens* (F.) with 10,100-fold resistance to Cry1Ac (Gould et al. 1995) did not survive to pupation on Bt cotton or non-Bt cotton (F. Gould, personal communication). However, hybrid progeny produced by crossing adults from YHD2 and a susceptible strain survived on non-Bt cotton. Although YHD2 larvae are probably not killed by high concentrations of Cry1Ac in Bt cotton, they may die in the fifth instar on cotton plants with or without Cry1Ac because of inbreeding depression or fitness costs directly associated with resistance (F. Gould, personal communication).

Unlike the examples reviewed above, survival on transgenic plants producing high concentrations of Bt toxin has been documented for at least five highly resistant strains of diamondback moth and pink bollworm, *Pectinophora gossypiella* (Saunders). Whereas unselected strains of these pests are highly susceptible to the toxins in Bt plants and cannot survive on Bt plants, the resistant strains develop successfully on them. The origins of these strains, their performance on Bt plants, and their inheritance of resistance to Bt plants are summarized below.

We emphasize that, as far as we know, no pest population has evolved resistance in the field to a Bt crop. Nonetheless, intensive exposure to Bt sprays in the field has caused moderate to high levels of resistance to Bt toxins in many diamondback moth populations (Ferré and Van Rie 2002, Heckel et al. 2003). Laboratory selection of progeny derived from some resistant field populations has produced strains that thrive on experimental Bt plants (Roush 1994; Metz et al. 1995; Tang et al. 1997, 1999; Ramachandran et al. 1998; Zhao et al. 2000, 2002).

At least two resistant strains of diamondback moth survived on Cry1Ac-producing Bt crucifers developed primarily for research. The NO-QA strain derived in 1989 from a watercress field in Hawaii (Tabashnik et al. 1990) survived on Bt canola (Ramachandran et al. 1998), and the Loxa A strain established in 1992 from a kohlrabi field in Loxahatchee, Florida, survived on Bt broccoli (Metz et al. 1995, Tang et al. 1997). Another diamondback moth strain established in 1994 from Chinese cabbage in Loxahatchee (Perez et al. 1997) has been reared on Cry1Ac-producing transgenic broccoli for >75 generations (Zhao et al. 2002). This strain has been referred to as Loxahatchee (Perez et al. 1997), Cry1A^R (Cao et al. 1999), and Cry1Ac-R (Zhao et al. 2002). The Cry1C-Sel strain of diamondback moth, which was started in 1997 from a collard field in South Carolina, survived on Cry1C-producing transgenic broccoli (Zhao et al. 2000).

Each of the strains of diamondback moth mentioned above completed larval development on Bt crucifers. Conservatively, lumping the strains from Loxahatchee, survival on Bt crucifers is well documented for at least three independent strains of diamondback moth: NO-QA from Hawaii, Loxahatchee from Florida, and Cry1C-Sel from South Carolina. The NO-QA and Cry1C-Sel strains were selected repeatedly in the laboratory before they were tested on Bt plants, but the Loxahatchee strain started in 1992 had <50% mortality on Bt broccoli before laboratory selection was peformed, and only 10% mortality on Bt broccoli after a single selection (Roush 1994).

In addition to survival of resistant diamondback moth on Bt crucifers developed for research, survival on commercial cultivars of Cry1Ac-producing Bt cotton has been demonstrated for two laboratory-selected strains of pink bollworm from Arizona (Liu et al. 1999, 2001a; Tabashnik et al. 2000a, 2002b; Morin et al. 2003). The AZP-R strain was created by pooling survivors of bioassays with Cry1Ac in wheat germ diet from 10 strains derived in 1997 from Arizona cotton fields (Patin et al. 1999, Tabashnik et al. 2000a). The APHIS-98R strain was started by selecting a long-term laboratory susceptible strain, first with leaf powder from Bt cotton in diet and subsequently with Cry1Ac in diet (Liu et al. 2001b). While APHIS-98R was selected in the laboratory for many generations before it was tested on Bt cotton (Liu et al. 1999, 2001a), AZP-R survived on Bt cotton after only three laboratory selections with Cry1Ac in diet (Tabashnik et al. 2000a).

Although the five aforementioned resistant strains completed development on Bt plants that killed virtually all individuals tested from susceptible strains, detailed life history studies reveal variation in the extent to which performance of these resistant strains was impaired by Bt plants. For the NO-QA strain of diamondback moth, differences were not detected between individuals reared on Bt and non-Bt canola in extent of defoliation, larval survival, and head capsule width at 5 d, percentage pupation, pupal weight, or percentage adult emergence (Ramachandran et al. 1998). In parallel, differences between Bt and non-Bt broccoli were not detected in larval survival or weight gain for a Loxahatchee strain of diamondback moth (Tang et al. 1999). Furthermore, during four generations of rearing on non-Bt broccoli, resistance to Bt broccoli did not decline in two hybrid strains derived by crossing a Loxahatchee strain with a susceptible strain (Tang et al. 1997).

In contrast, adverse effects of Bt plants were detected for the Cry1C-Sel strain of diamondback moth (Zhao et al. 2000) and both resistant pink bollworm strains (Liu et al. 1999, 2001a; Tabashnik et al. 2000a). After Cry1C-Sel had been reared for 13 consecutive generations on Cry1C-producing broccoli, it still had a cumulative disadvantage on Bt broccoli relative to non-Bt broccoli (Zhao et al. 2000). Only survival at 3 d differed significantly between Bt and non-Bt broccoli, yet the trend of poorer performance on Bt broccoli

Table 2. Field surveys for resistance in pests targeted by Bt crops; none show increases in resistance

Bt crop	Insect	Region	Bioassay approach	Years	Estimated frequency[a]	
					Initial	Final
Corn	*O. nubilalis*[b]	United States	Isofemale lines (F_2) vs Bt corn	1996–2001	0	0
		France	Isofemale lines (F_2) vs Bt corn	2000–2001	0	0
Cotton	*H. armigera*[c]	N. China	Field-derived strains vs Cry1Ac	1998–2000	0.0095	0.0022
	H. zea[d]	North Carolina	Isofemale lines (F_1) vs Cry1Ac	2000–2001	0.00043	0
	P. gossypiella[e]	Arizona	Field-derived strains vs Cry1Ac	1997–2001	0.16	0.075

[a] Values are estimates of resistance allele frequency except for *H. armigera*, which are survival to third instar at 1 μg Cry1Ac per ml diet. Initial refers to estimate from first year (e.g., 1996 for *O. nubilalis*); final refers to estimate from last year (e.g., 2001 for *O. nubilalis*).
[b] Andow et al. 1998, 2000; Bourguet et al. 2003.
[c] Wu et al. 2002.
[d] Jackson et al. 2002, Burd et al. 2003.
[e] Tabashnik et al. 2000a, Sims et al. 2002, and unpublished data.

was evident in all of the life history parameters examined (survival, pupal weight, fecundity, and percentage of eggs hatched). In four of five independent greenhouse tests conducted from 1998 to 2002, survival of resistant pink bollworm strains was lower on Bt cotton than on non-Bt cotton (Liu et al. 1999, 2001a; Tabashnik et al. 2000a, 2002a; Morin et al. 2003). Relative to performance on non-Bt cotton, Bt cotton adversely affected developmental rate, pupal weight, and fecundity, but not percentage of eggs hatched (Liu et al. 1999, 2001a). This type of impaired performance in resistant strains that complete development on Bt plants is called "incomplete resistance" (Carrière and Tabashnik 2001).

The widely adopted refuge strategy is expected to delay resistance most effectively when inheritance of resistance is functionally recessive (Tabashnik and Croft 1982, Gould 1998). In other words, the greatest benefits of refuges are expected when Bt plants kill heterozygous hybrid progeny of homozygous resistant and homozygous susceptible adults. Although resistance to Bt toxins is not recessive in some insect strains (Tabashnik 1994, Ferré and Van Rie 2002), inheritance of resistance to transgenic plants with high concentrations of Bt toxins is recessive in all cases examined to date involving pests that have high inherent susceptibility to the toxins produced: diamondback moth strains Loxa A (Metz et al. 1995, Tang et al. 1997) and Cry1C-Sel (Zhao et al. 2000), and pink bollworm strains AZP-R (Tabashnik et al. 2000a, Liu et al. 2001a, Morin et al. 2003) and APHIS-98R (Liu et al. 1999).

The Loxa A and NO-QA strains of diamondback moth and both resistant strains of pink bollworm have mode 1 resistance, the most common type of lepidopteran resistance to Cry1A toxins (Tabashnik et al. 1998). Along with high resistance to at least one Cry1A toxin, mode 1 resistance entails recessive inheritance, reduced binding of at least one Cry1A toxin, and little or no cross-resistance to Cry1C. In pink bollworm, resistance to Bt cotton is associated with mutations in a cadherin gene encoding a Cry1Ac-binding protein (Morin et al. 2003). A closely related gene is disrupted in the Cry1Ac-resistant YHD2 strain of *H. virescens* (Gahan et al. 2001), which also shows mode 1 resistance.

In the examples reviewed above, unselected strains of the pests are extremely susceptible to the Bt toxins produced by the transgenic plants, and their mortality on Bt plants is essentially 100%. However, this is not true for all pests targeted by Bt crops, as illustrated by *Helicoverpa armigera* (Hübner) and Cry1Ac-producing Bt cotton. In artificial diet bioassays with unselected susceptible strains, the LC_{50} of Cry1Ac was 100–1,000 times higher for *H. armigera* (Akhurst et al. 2003) than for *H. virescens* (Gould et al. 1995). Thus, control of *H. armigera* by Bt cotton is marginal and can be overcome by much lower levels of resistance than those required for survival on Bt cotton by some other pests. Indeed, some unselected *H. armigera* complete larval development on Bt cotton in the field when Cry1Ac concentration declines at the end of the season in Australia (Fitt and Wilson 2000). Nonetheless, in experiments in which survival on Bt cotton was 0% for unselected strains, two resistant strains of *H. armigera* survived on Bt cotton (Table 1). The Cry1Ac-sel strain from China had 13-fold resistance to Cry1Ac in diet and 25% survival on Bt cotton relative to non-Bt cotton (Fan et al. 2000). The BX strain from Australia had 57-fold resistance to Cry1Ac in diet and 58% survival on Bt cotton relative to non-Bt cotton (Akhurst et al. 2003).

In summary, at least seven resistant strains of three pests survive on Bt crops. Three resistant strains of diamondback moth survive on Bt crucifers produced for research purposes. Two resistant strains of pink bollworm survive on commercially grown varieties of Bt cotton that produce Cry1Ac. Two resistant strains of *H. armigera*, which has inherently low susceptibility to Cry1Ac, also survive on Bt cotton.

Frequency of Resistance to Bt Toxins in Field Populations

As far as we know, no reports published in refereed journals demonstrate that exposure of field populations to Bt crops has increased the frequency of alleles conferring resistance to Bt toxins. Indeed, as summarized below, such increases have not been detected in 2- to 6-yr studies of four major pests (Table 2).

Table 3. Pink bollworm Bt resistance (r) allele frequency estimated from bioassays

Year	Sites tested		Larvae tested		Mean r allele frequency (95% confidence limits)[a]
	Total	With ≥1 survivor	Total	Survivors	
1997	10	5	500	16	0.16 (0.05–0.26)
1998	10	1	1,100	1	0.007 (0–0.017)
1999	13	0	1,179	0	0
2000	14	0	4,840	0	0
2001	17	10	2,950	31	0.075 (0.03–0.12)

[a] Genetic analyses revealed that larvae surviving 21 days of exposure to 10 μg Cry1Ac per ml diet were homozygous for resistance at a major locus. Thus, resistance allele frequency in each field-derived strain was estimated as the square root of the frequency of survivors (after adjustment for control mortality) of this diagnostic test. Confidence limits were estimated by bootstrapping with 10,000 repetitions (Tabashnik et al. 2000a, Sims et al. 2002, and unpublished data).

The first estimates of Bt resistance (r) allele frequencies in pest populations were obtained indirectly (Tabashnik 1994). These are lower limits based on the assumption that at least one r allele occurred among the field-collected founders of strains that responded to laboratory selection for resistance. Applying this approach, Gould et al. (1995) estimated that the initial r allele frequency in the YHD2 strain of *H. virescens* was at least 0.008 (one allele in 63 diploid individuals) or that resistance arose by mutation in the laboratory. Based on tests in which wild males were mated with resistant YHD2 females, Gould et al. (1997) estimated that the r allele frequency in field populations of *H. virescens* from four states was 0.0015 before commercialization of Bt cotton. We are not aware of subsequent studies with this pest checking for increases in the r allele frequency caused by Bt cotton.

The frequency of pink bollworm resistance to Cry1Ac did not increase from 1997 to 2001 in Arizona, even though Cry1Ac-producing Bt cotton accounted for more than half of the state's cotton during this period (Table 3; Tabashnik et al. 2000a, Carrière et al. 2001a, Sims et al. 2002). In this case, r allele frequency was estimated by collecting 300–2,000 cotton bolls from each of 10–17 cotton fields across Arizona annually during August to December from 1997 to 2001. The progeny of pink bollworm obtained from these bolls initiated strains that were reared on wheat germ diet. Larvae from these field-derived strains were tested in diet bioassays with a concentration of Cry1Ac that kills close to 100% of homozygous susceptibles and heterozygotes, but essentially no resistant homozygotes (Tabashnik et al. 2000a, 2002a; Sims et al. 2002). Although r allele frequency estimates were based on assumed Hardy-Weinberg equilibrium and the observed recessive inheritance of resistance, the conclusion that the frequency of resistant individuals did not increase from 1997 to 2001 does not depend on assumptions or inferences about the dominance of resistance, the number of loci, or Hardy-Weinberg equilibrium. Independent field data also show that Bt cotton remained extremely effective against pink bollworm throughout this period (Tabashnik et al. 2000a, Sims et al. 2002, Carrière et al. 2003), which spans ≈20 generations of the pest.

Analysis of models suggests that refuges, fitness costs, and incomplete resistance could contribute to the observed delay in pink bollworm resistance to Bt cotton (Carrière and Tabashnik 2001). Experiments show that when Bt toxin is not present, large fitness costs occur in resistant individuals compared with susceptibles, including reduced survival on non-Bt cotton and reduced overwintering survival (Carrière et al. 2001b, c). Experiments also reveal incomplete resistance, which entails disadvantages suffered by resistant pink bollworm on Bt cotton compared with their performance on non-Bt cotton, including lower survival and fecundity (Liu et al. 2001a).

Similar to results with pink bollworm in Arizona, widespread use of Bt cotton did not cause detectable increases in resistance of *H. armigera* to Cry1Ac in northern China from 1998 to 2000 (Wu et al. 2002). In this region, Bt cotton use increased rapidly from 10,000 ha in 1997 to 1 million ha (≈90% of cotton in the region) in 2000 (Wu et al. 2002). Using methods similar to those applied to pink bollworm, Wu et al. (2002) collected at least 100 larvae per site from 13 to 15 sites in each of 3 yr. They established laboratory strains on artificial diet and tested them with diet bioassays. The percentage of larvae surviving 1 μg Cry1Ac per ml diet was 0.95% in 1998, but only 0.22% in 2000. Also, survivors at this concentration occurred in four populations in 1998 compared with only one in 2000.

In related work, the frequency of *Helicoverpa zea* (Boddie) resistance to Cry1Ac did not increase in North Carolina from 2000 to 2001, even though Bt cotton accounted for a high proportion of the state's cotton (Jackson et al. 2002, Burd et al. 2003). In bioassays of progeny of wild females, Burd et al. (2003) found that 1 female of the 583 screened in 2000 carried a major nonrecessive allele for resistance to Cry1Ac. Assuming four alleles per family (two from the wild female and two from the wild male parent), the estimated r allele frequency is 0.00043 (1/2,332) for 2000. For 2001, none of the 561 females screened had a major nonrecessive allele for Cry1Ac resistance (Jackson et al. 2002). Large refuges of untransformed alternate crops may help to delay resistance in *H. armigera* and *H. zea*, which are polyphagous (Gould et al. 2002).

To date, extensive screening of European corn borer, the major pest targeted by Bt corn, has not identified any individuals with alleles conferring resistance to Bt corn (Andow et al. 1998, 2000; Bourguet

et al. 2003). The primary tool used to estimate r allele frequency in this pest has been the F_2 screen, in which second generation progeny of field-collected females are tested with bioassays. This approach detected no alleles for resistance to Bt corn in 697 isofemale lines (2,788 alleles) derived from United States corn belt populations during 1996–2001 (Andow et al. 1998, 2000; Bourguet et al. 2003). Likewise, none of 721 isofemale lines (2,884 alleles) derived in 2000 and 2001 from French corn belt populations produced progeny that survived on Bt corn (Bourguet et al. 2003). Collectively, no alleles for resistance to Bt corn have been found in 1,418 isofemale lines (5,672 alleles) derived from French and United States corn belts from 1996 to 2001. Thus, it appears that alleles for resistance to Bt corn were rare initially and remained below detection level in this key pest.

Although tests with the F_2 screen detected no alleles for resistance to Bt corn in European corn borer, alleles conferring resistance to Cry1Ab in diet bioassays were more common (Andow et al. 1998, 2000; Bourguet et al. 2003). Also, as noted above, selection of European corn borer with Dipel in the laboratory decreased susceptibility to Dipel, but did not enable survival on Bt corn (Huang et al. 2002). Because resistance to Bt toxins in diet does not necessarily confer resistance to Bt crops, the estimates of r allele frequency summarized above for *H. virescens*, *H. armigera*, *H. zea*, and pink bollworm represent upper limits as they are based on diet bioassays.

Conclusions

In summary, the ability to thrive on Bt plants is well documented for at least seven resistant laboratory strains of three major pests: diamondback moth, pink bollworm, and *H. armigera.* However, after 7 yr of large-scale planting of Bt crops, resistance of pests to Bt crops in the field has not been reported. In particular, monitoring of pink bollworm in Arizona for 5 yr and *H. armigera* in northern China for 3 yr did not reveal increases in the frequency of resistance despite widespread adoption of Bt cotton.

The two aforementioned examples are important because they appear to violate key assumptions of the refuge strategy. In pink bollworm, the estimated frequency of a major resistance allele was 0.16 in 1997 (Tabashnik et al. 2000a), which is not considered rare. Given that Bt cotton is not extremely toxic to susceptible *H. armigera* (Fitt and Wilson 2000, Akhurst et al. 2003), resistance is probably not recessive (i.e., heterozygotes are likely to survive on Bt cotton). Perhaps these two pests will evolve resistance soon. However, some theoretical work suggests that while refuges are crucial for delaying resistance, violation of one or more key assumptions can be overcome by especially favorable assumptions about other factors (Carrière and Tabashnik 2001, Carrière et al. 2002). Thus, for pink bollworm, recessive inheritance of resistance, fitness costs, and incomplete resistance (Liu et al. 1999, Tabashnik et al. 2000a, Carrière et al. 2001b, c) may delay resistance considerably, even though resistance was not rare initially. For others, such as *H. armigera* and *H. zea*, in which nonrecessive inheritance of resistance occurs (Burd et al. 2003), large refuges of alternate host crops that do not produce Bt toxins may be crucial (Carrière et al. 2002, Gould et al. 2002). Therefore, refuges may be useful even when one or more key assumptions of the refuge strategy are violated.

One lesson learned from 7 yr of Bt crops is humility, particularly recognition that current ability to predict rates of resistance evolution in the field is limited. Second generation Bt cotton, which produces Cry2Ab as well as Cry1Ac, kills Cry1Ac-resistant pests, and thus has the potential to prolong efficacy (Tabashnik et al. 2002b). The success of Bt crops to date exceeds the expectations of many, but does not preclude resistance problems in the future. When will the first documented case of field-evolved resistance to a Bt crop occur? It could happen this field season, or maybe not for another 7 yr. Only time will tell.

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