COMMENTARY

Delaying insect resistance to transgenic crops

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In her seminal work, *Silent Spring*, Rachel Carson writes: "If Darwin were alive today the insect world would delight and astound him with its impressive verification of his theories of survival of the fittest. Under the stress of intensive chemical spraying the weaker members of the insect populations are being weeded out." (1)

Evolution of insecticide resistance in >400 species of insects not only confirms Darwin's theories, it threatens agriculture and human health worldwide (www.pesticideresistance.com/; ref. 2). To reduce reliance on insecticide sprays, corn and cotton have been genetically engineered to produce insecticidal crystal (Cry) proteins derived from the bacterium *Bacillus thuringiensis* (Bt). Transgenic Bt corn and Bt cotton grew on 42 million ha during 2007, with a cumulative total of >200 million ha planted worldwide since their commercialization in 1996 (3). However, the history of insecticide resistance informs us that adaptation by insects could diminish the long-term efficacy of Bt crops and the associated economic, health, and environmental benefits (4–6). To date, field-evolved resistance to Bt crops has been documented in only 3 insect species (Fig. 1) (7–10). Along with other evidence, the report by Meihls *et al.* (11) in this issue of PNAS suggests that refuges of plants that do not produce Bt toxins may be useful for delaying insect resistance to Bt crops.

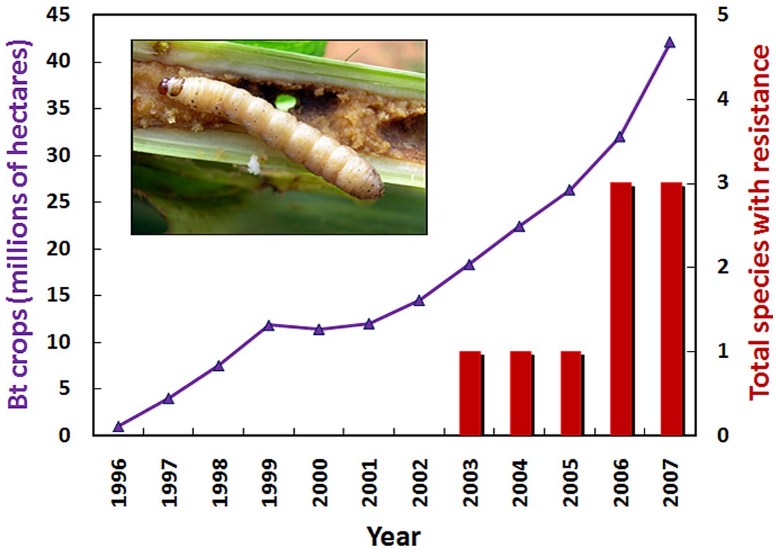

Fig. 1. Global adoption of Bt crops and evolution of insect resistance. The cumulative total planting of Bt crops worldwide was >200 million ha from 1996 to 2007 (3), but so far, resistance in the field has been detected in only 3 lepidopteran species: *Helicoverpa zea* (bollworm), to Bt cotton producing Cry1Ac in the southeastern United States in 2003 (7), *Spodoptera frugiperda* (fall armyworm) to Bt corn producing Cry1F in Puerto Rico in 2006 (9), and *Busseola fusca* (stem borer) to Bt corn producing Cry1Ab in South Africa in 2006 (8), depicted in the photo (Courtesy: Prof. Johnnie van den Berg, North-West University, Potchefstroom, South Africa).

The refuge strategy, which is mandated in the United States and elsewhere, is based on the idea that most of the rare resistant pests surviving on Bt crops will mate with abundant susceptible pests from nearby refuges of host plants without Bt toxins (12, 13; www.epa.gov/EPA-PEST/1998/January/Day-14/paper.pdf). If inheritance of resistance is recessive, the hybrid progeny from such matings will die on Bt crops, substantially slowing the evolution of resistance. This approach is sometimes called the "high-dose refuge strategy" because it works best if the dose of toxin ingested by insects on Bt plants is high enough to kill all or nearly all of the aforementioned hybrid progeny (12, 13). In principle, if a high dose is not achieved, resistance can be delayed by increasing refuge abundance, which lowers the proportion of the population selected for resistance to compensate for survival of hybrid progeny on Bt plants (12, 13).

The most direct way to test the high-dose hypothesis is to let resistant and susceptible adults mate in the laboratory and measure survival of their hybrid progeny on Bt plants. Because suitable resistant strains for direct tests usually are not available, indirect tests are used. One such method relies on the reasonable assumption that if Bt plants do not kill virtually 100% of susceptible individuals, they probably will not kill nearly all hybrid individuals. Thus, the U.S. Environmental Protection Agency guidelines for a high dose specify that Bt plants should kill at least 99.99% of susceptible insects in the field (www.epa.gov/scipoly/sap/meetings/1998/0298_mtg.htm).

Meihls *et al.* (11) studied a case in which the high-dose standard is not satisfied: resistance of western corn rootworm, *Diabrotica vergifera vergifera*, to transgenic corn producing Bt toxin Cry3Bb. This devastating beetle pest and closely related species cost U.S. farmers approximately $1 billion annually (14). Important advances incorporated by Meihls *et al.* (11) include use of Bt corn plants in the greenhouse to select rootworm colonies for resistance and estimation of larval survival in the field on Bt corn plants relative to nearly identical ("isoline") non-Bt corn plants. The failure to achieve a high dose was indicated first by the survival of susceptible rootworm larvae on this type of Bt corn. Meihls *et al.* confirmed this conclusion by showing that survival in the greenhouse on Bt corn relative to non-Bt corn was 48–73% for hybrid progeny of resistant and susceptible adults.

Supporting predictions from the refuge theory, Meihls *et al.* (11) report that resistance evolved quickly without refuges and slower or not at all with refuges. They exposed rootworm colonies to Bt corn in the greenhouse under 4 selection regimes: constant exposure, neonate exposure, late exposure, and no exposure (control). The constant-exposure colony was reared on Bt corn throughout the larval development period. Larvae in the neonate-exposure colony were placed on Bt corn as neonates, then shifted to non-Bt corn to complete development. Larvae in

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the late-exposure colony ate non-Bt corn as neonates and completed development on Bt corn. After 3 generations of selection, the constant exposure colony was highly resistant; its survival on Bt corn plants was similar to survival of the control colony on non-Bt corn plants. In marked contrast, after 3 generations of selection, the neonate- and late-exposure colonies remained as susceptible to Bt corn as the control colony. After 6 generations of selection, survival on Bt corn increased significantly in the late-exposure colony, but not in the neonate-exposure colony. Meihls *et al.* (11) suggest that the 2 partial-exposure regimes might emulate situations in the field where refuges are provided by non-Bt corn or grassy weed host plants interspersed with Bt corn.

The results of Meihls *et al.* (11) parallel those of Lefko *et al.* (15) showing that in greenhouse experiments without refuges, rootworm rapidly evolved resistance to transgenic corn producing Bt toxins Cry34Ab and Cry35Ab, which also does not meet the high-dose standard (11). It remains to be seen what will happen in the field where refuges of at least 20% non-Bt corn are required (www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_006484.htm). Bt corn producing Cry3Bb for rootworm control was registered in 2003 and first exceeded 1 million ha planted in the United States in 2005 (www.monsanto.com/pdf/investors/2008/2008_biotech_acres.pdf). The more extensive field experience since 1996 with Bt corn and cotton producing Cry1 toxins for caterpillar control is helpful for putting the rootworm results in context.

Analysis of more than a decade of resistance monitoring data for 6 major caterpillar pests targeted by Bt corn and cotton suggests that the principles of the refuge strategy may apply in the field (10). Resistance to Bt cotton producing Cry1Ac was detected after 7–8 years in some field populations of *Helicoverpa zea* in the southeastern United States, but field-evolved resistance to Bt crops was not found in the 5 other pests examined: *Helicoverpa armigera*, *Heliothis virescens*, *Ostrinia nubilalis*, *Pectinophora gossypiella*, and *Sesamia nonagriodes* (10). As with rootworm and Bt corn, tests with *H. zea* showed that Bt cotton producing Cry1Ac does not meet the high-dose standard, based on both survival of susceptible larvae on the transgenic plants and dominant inheritance of resistance (10). In contrast, Cry1Ac-producing cotton and Cry1Ab-producing corn meet the high-dose criterion for the 3 other pests from the continental United States (*H. virescens*, *O. nubilalis*, and *P. gossypiella*) that have remained susceptible to Bt crops for >10 years. This comparison suggests that the lack of a high dose for *H. zea* may have favored its faster evolution of resistance.

Plants that do not produce Bt toxins may be useful for delaying insect resistance to Bt crops.

Field experience with caterpillar pests also suggests that if the high-dose standard is not met, increasing the abundance of refuges relative to Bt crops can delay resistance. For *H. zea*, higher refuge abundance was associated with slower resistance evolution in North Carolina compared with Arkansas and Mississippi (10). Analogously, relatively high abundance of non-Bt host plant refuges in Australia and China may have helped to slow *H. armigera* resistance to Bt cotton producing Cry1Ac (10).

Recently, field-evolved resistance to Bt corn has been reported for 2 additional caterpillar pests: *Busseola fusca* resistance to Cry1Ab-producing corn in South Africa (8) and *Spodoptera frugiperda* resistance to Cry1F-producing corn in Puerto Rico (9). Although published data are limited for these instances, failure to achieve the high-dose standard and to implement adequate refuges may have hastened resistance (8, 9). To maximize knowledge gained from these and other cases, it is important for scientists in academia, industry, and government to make publicly available the relevant information on the efficacy of transgenic crops and refuge abundance.

The first decade of transgenic crops producing Bt toxins for insect control has been successful, despite the few examples of resistance noted above. The second and subsequent decades of insecticidal transgenic crops will entail an increasing diversity of toxins, greater use of plants producing 2 or more distinct toxins, and other, novel approaches for countering insect resistance (16–18). The new data reported by Meihls *et al.* (11) can be used to refine models for predicting evolution of resistance to Bt corn by western corn rootworm. This pernicious pest has but 1 generation per year, which might slow its resistance evolution relative to caterpillar pests with multiple generations. It will be intriguing to determine whether, as observed so far with caterpillars, the field outcomes with beetles confirm the concepts of the refuge strategy. If so, this would bolster management of insect resistance to transgenic crops as a compelling illustration of applying evolutionary principles to benefit humankind.

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1. Carson R (1962) *Silent Spring* (Houghton Mifflin, New York).
2. Onstad DW (2008) *Insect Resistance Management: Biology, Economics and Prediction* (Academic, London).
3. James C (2007) *Global Status of Commercialized Biotech/GM Crops: 2007.* ISAAA Briefs No. 37 (Ithaca, NY, International Service for the Acquisition of Agribiotech Applications).
4. Tabashnik BE (1994) Evolution of resistance to *Bacillus thuringiensis. Annu Rev Entomol* 39:47–79.
5. Cattaneo M, *et al.* (2006) Farm-scale evaluation of transgenic cotton impacts on biodiversity, pesticide use, and yield. *Proc Natl Acad Sci USA* 103:7571–7576.
6. Romeis J, Shelton AM, Kennedy GG, eds (2008) *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs* (Springer, New York).
7. Luttrell RG, *et al.* (2004) Resistance to Bt in Arkansas populations of cotton bollworm. *Proceedings of the 2004 Beltwide Cotton Conferences*, San Antonio, TX, January 5–9, 2004, ed Richter DA (National Cotton Council of America, Memphis, TN), pp 1373–1383.
8. Van Rensburg JBJ (2007) First report of field resistance by stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize *S African J Plant Soil* 24:147–151.
9. Matten SR, Head GP, Quemada, HD (2008) How governmental regulation can help or hinder the integration of *Bt* crops into IPM programs. *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*, eds Romeis J, Shelton AM, Kennedy GG (Springer, New York), pp 27–39.
10. Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y (2008) Insect resistance to Bt crops: Evidence versus theory. *Nat Biotech* 26:199–202.
11. Meihls LN, *et al.* (2008) Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proc Natl Acad Sci USA* 105:19177–19182.
12. Gould F (1998) Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annu Rev Entomol* 43:701–726.
13. Tabashnik BE, Gould F, Carrière Y (2004) Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. *J Evol Biol* 17:904–912.
14. Rice ME (2003) Transgenic rootworm corn: Assessing potential agronomic, economic, and environmental benefits. *Plant Health Progress*, 10.1094/PHP-2004-0301-01-RV.
15. Lefko SA, *et al.* (2008) Characterizing laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122–7. *J Appl Entomol* 132:189–204.
16. Baum JA, *et al.* (2007) Control of coleopteran insect pests through RNA interference. *Nat Biotech* 25:1322–1326.
17. Soberón M, *et al.* (2007) Engineering modified Bt toxins to counter insect resistance. *Science* 318:1640–1642.
18. Bravo A, Soberón M (2008) How to cope with insect resistance to Bt toxins? *Trends Biotech* 26:573–579.