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Insect resistance management in GM crops: past, present and future

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Transgenic plants expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt*) were first commercialized in 1996 amid concern from some scientists, regulators and environmentalists that the widespread use of *Bt* crops would inevitably lead to resistance and the loss of a 'public good,' specifically, the susceptibility of insect pests to *Bt* proteins. Eight years later, *Bt* corn and cotton have been grown on a cumulative area >80 million ha worldwide. Despite dire predictions to the contrary, resistance to a *Bt* crop has yet to be documented, suggesting that resistance management strategies have been effective thus far. However, current strategies to delay resistance remain far from ideal. Eight years without resistance provides a timely opportunity for researchers, regulators and industry to reassess the risk of resistance and the most effective strategies to preserve *Bt* and other novel insect-resistant crops in development.

Bt crops have already exceeded the length of time that typically passes in the field before resistance to most conventional neurotoxic pesticides is first documented[1], despite undergoing what has been hailed as one of the world's largest selections for resistance[2]. Does the lack of resistance indicate that insect resistance management (IRM)[3] strategies have been effective, or were fears about resistance perhaps unwarranted in the first place[2,4]? Although neither question can yet be answered with certainty, the increase in resistance to *Bt* sprays in the field[5] as well as the greenhouse[6], and laboratory selection of resistant strains of several major pests[2,7] (including at least three that can survive on *Bt* plants[2]) demonstrate that resistance to *Bt* crops most likely remains a question of not 'if' but 'when.'

The absence of field resistance to date is presumed to be due to one or more of the following factors[2]: first, large fitness costs or other disadvantages suffered by resistant individuals; second, an initial low frequency of resistant alleles; three, a dilution of resistant alleles with susceptible individuals from non-*Bt* plants or 'refuges'; and fourth, a high dose of toxin delivered by plants. In the laboratory, fitness costs have been observed with some resistant strains of Indianmeal moth (*Plodia interpunctella*)[8], pink bollworm (*Pectinophora gossypiella*)[9–11], diamondback moth (*Plutella xylostella*)[12], Colorado potato beetle (*Leptinotarsa decemlineata*)[13,14], cabbage looper (*Trichoplusia ni*)[6] and cotton bollworm (*Helicoverpa armigera*)[15], whereas other strains have not displayed such costs[16–18]. Lack of observable fitness costs and resistance stability[19] in some strains indicate that reduced competitiveness is not a firm barrier to resistance evolution. A more reliable strategy is the development, deployment and regulation of IRM strategies that are firmly rooted in theory and supported by experimentation, and that consider *Bt* crops as but one component of an overall integrated pest management (IPM) approach. In this article, we examine how current IRM tactics for *Bt* crops evolved and the continuing need for improved strategies. We then consider the role of second generation transgenic plants and IPM in developing more effective IRM tactics. Ultimately, the development of rigorous and responsive IRM strategies that are employed within an IPM framework will best preserve existing and future insect-resistant crops.

IRM in *Bt* crops—a brief history

IRM for *Bt* crops began largely as a theoretical exercise. In the decade leading up to the first commercial release of *Bt* plants, several deployment tactics designed to delay resistance were proposed. These strategies built on a considerable body of theoretical and empirical work on resistance to conventional insecticides, but most considered *Bt* crops as a 'special case'[20] where target pests are exposed to toxins over the entire season. These strategies included the following:

Moderate toxin dosage to ensure survival of fraction of susceptible insects. In this approach, expression of *Bt* toxins at a moderate level allows a proportion of susceptible insects to survive[21]. Models indicate that moderate expression provides some delay in resistance, but the delay is small compared to other tactics. Moreover, the efficacy of *Bt* at lower doses is variable and expression is vulnerable to environmental influences, as occurred in 1996 in Australia when the first commercialized *Bt* cotton plants performed less than ideally from a pest management perspective[21].

High toxin dosage to kill insects heterozygous for resistance. This strategy involves the expression of *Bt* toxins at a level high enough to ensure that individuals heterozygous for resistance are killed[21,22]. Because the initial occurrence of individuals homozygous for resistance is likely to be so rare that it can essentially be ignored, the rate of resistance evolution is driven primarily by the frequency and survival of heterozygotes[21,23]. Thus, from an IRM perspective, a dose that is high enough to cause mortality to heterozygotes is preferred. From an IPM perspective, a high dose will also ensure that crop damage is maintained below an economic threshold.

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Combination/stacking/pyramid of toxins. This strategy involves deployment of *Bt* varieties expressing different toxins simultaneously in a mosaic, in a rotation, sequentially (e.g., use one toxin until control failure occurs) or by incorporating both toxins in a single variety (that is, pyramided)[20,21]. Models and empirical data from our laboratory indicate that the use of two different toxins in a mosaic pattern is the most ineffective method of delaying resistance[24,25]. Thus, caution may be warranted if multiple varieties expressing different toxins are grown concurrently in the field, because this will result in a landscape-level mosaic[21].

At present, pyramiding two (or more) toxins into one variety appears to be the best way to delay resistance, but the toxins should have different binding sites to reduce the likelihood of cross-resistance[22]. Unlike resistance to a single toxin, it is not the mortality of heterozygous larvae that is the most influential factor, but the mortality of the susceptible homozygotes[21]. If both toxins are expressed at high levels, the frequency of individuals with complete resistance to two toxins will be far rarer than those resistant to one. Much longer delays in resistance are expected to occur with pyramided toxins, not just in comparison to single transgene plants, but other methods of deploying multiple toxins as well[21]. Pyramiding toxins is also favored over rotations because, in many cases, resistance to *Bt* toxins can remain stable or decrease only slowly in the absence of selection pressure[7,16,19,26]. For species with only moderate susceptibility to a given *Bt* toxin, such as *Helicoverpa* spp., pyramided plants can also provide superior control[27].

Temporal or tissue-specific toxin expression. In this approach, expression of *Bt* toxins in plants occurs only at certain times or in certain parts through the use of temporal, tissue-specific or chemically inducible promoters[21]. The use of selective expression through nonconstitutive promoters was recognized early on as a potentially effective way to reduce selection for resistance to *Bt* crops[28], but has been limited by the available technology. In the case of tissue-specific promoters, however, larvae that move easily between toxic and nontoxic plant structures may negate the benefits of selective *Bt* expression[23,29].

Provision of nontoxic plants. This strategy provides non-toxin-bearing plants to maintain susceptible insects within the field in seed mixtures or external to the transgenic crop in refuges[20,21]. Seed mixtures once seemed attractive because they required little effort on the part of growers to implement resistance management. They were also expected to favor random mating between susceptible and resistant insects[21]. Subsequent studies have shown, however, that for some pests, interplant movement by larvae would render this strategy less effective[21–23,29,30]. Larvae may actively avoid feeding on *Bt* plants[28,29], or larvae developing on non-*Bt* plants may move to toxic plants and die, thus reducing the effective size of the refuge[23,30]. In field tests using the diamondback moth and transgenic broccoli, our group found that unsprayed refuges external to the *Bt* crop were more effective for conserving susceptible alleles and reducing the overall number of resistant insects on transgenic plants compared to mixed or sprayed refuges[31]. However, care must be taken to ensure the insects in the refuge are managed from both an economic and IRM standpoint. Managing insects in the refuge, while conserving *Bt* susceptible alleles, continues to be a challenge.

Evaluating the potential effectiveness of IRM strategies is made especially difficult by the limited ability of researchers to run empirical tests. Most resistant strains are unable to survive on *Bt* hosts, and field tests using resistant insects raise concerns about creating a wider resistance problem. One exception to this rule is the use of *Bt* broccoli and resistant diamondback moth strains in regions where the insect does not overwinter. This system has been used extensively by our laboratory to evaluate IRM concepts and strategies, although it is limited by the inability to conduct landscape-sized, long-term trials. For the most part, the development of current IRM strategies has relied largely on computer simulations. When *Bt* crops were first commercialized in 1996, most experts agreed that the best strategy available at the time was a combination of high expression and a refuge to conserve susceptible alleles. Until transgenic cotton containing two pyramided transgenes received regulatory approval in Australia and the US in 2002, the high-dose of a single toxin, in conjunction with a refuge, was the only IRM strategy commercially available. Because of the current premium associated with two-transgene plants, plants expressing a single transgene may continue to dominate the market, unless their use is limited by regulatory agencies, or companies are provided with incentives to substitute two-transgene plants.

Box 1 Monitoring for resistance

As part of their IRM requirements, companies selling *Bt* plants are mandated by the EPA to implement an annual resistance monitoring program, the goal of which is to detect changes in resistance levels in pest populations. However, considerable debate centers on which monitoring technique(s) to use. Currently, the most widely used method is a diagnostic or discriminating dose incorporated into an artificial diet. Such a dose, when carefully selected, will allow only resistant individuals to survive. This relatively inexpensive method allows many individuals to be tested and will detect both polygenic resistance and multiple resistance mechanisms. However, it is less useful for detecting resistance alleles that are highly recessive or that occur at low frequencies[65], which may limit the window of opportunity to adjust IRM guidelines before resistance becomes widespread.

The F_2 screen[66] was proposed as a means to detect rare recessive alleles, but this method has limitations including the inability to detect polygenic resistance[67]. The 'Holy Grail' of monitoring techniques may be the development of appropriate molecular methods. Mutations affecting a Cry1A-binding midgut cadherin protein[68,69] have been shown to be tightly linked with laboratory-selected resistance in *H. virescens*[70] and *P. gossypiella*[71] and thus the cadherin gene has been considered the prime target for DNA-based screening for resistance[71]. However, recent work has shown that field-evolved resistance in *P. xylostella* has a different genetic basis (S.W. Baxter, University of Melbourne, Australia, personal communication), suggesting that a single molecular test will not be suitable for detecting Cry1A-resistance-allele frequency in all insect species, or perhaps even populations within a species. A similar case may exist for other *Bt* proteins and other non-*Bt* insecticidal traits. Thus, monitoring for resistance alleles may require complementary molecular tests and diagnostic assays, as well as increased field scouting for any surviving larvae.

Limitations of the high-dose/refuge strategy

Although the high-dose/refuge appears to have contributed at least in part to the success of IRM in *Bt* corn and cotton, it suffers from several disadvantages that make it far from ideal for long-term preservation of *Bt* crops. Efficacy of the strategy has three underlying assumptions: first, the initial allele frequency for resistance is low (e.g., $<10^{-3}$); second, inheritance is recessive; and third, resistant and susceptible insects will mate more or less randomly. However, studies have shown that one or more of these assumptions may be violated with some pest species. For example, a disparity in development time between resistant and

susceptible pink bollworms suggested that assortative mating could occur[10]. Incomplete or nonrecessive inheritance to various *Bt* products or toxins has also been documented in strains of European corn borer (*Ostrinia nubilalis*), tobacco budworm (*Heliothis virescens*), *H. armigera*, *Helicoverpa zea*, *P. xylostella* and *L. decemlineata*[7,15,32], although insect resistance to *Bt* in high-expressing transgenic plants has thus far proven functionally recessive[2,33].

In addition to potential violations of key assumptions, the high-dose refuge strategy is also limited by a number of practical considerations. Delivery of a high-dose and separation of toxic and nontoxic refuge plants in space may be compromised by the expected contamination of *Bt* seed lots with nonexpressing 'off-types' that may comprise up to 3% of the crop[33] and create, in effect, a seed mixture. Models indicate that even a low frequency of nontoxic plants in *Bt* seed could result in a marked acceleration of resistance development[22]. In addition, recent data suggest that pollen flow from *Bt* plants to nontoxic plants may result in exposure of pests to low levels of *Bt* in developing seed tissue in at least some areas of the refuge[34]. Another disadvantage of the high-dose refuge strategy is that multiple pests with differing degrees of susceptibility to *Bt* may be targeted by the toxin. The success of the high-dose/refuge strategy is based on the assumption that heterozygote mortality is high, but a *Bt* plant may deliver a dose that is highly toxic to one species but only moderately toxic to another[21,35]. Reduced mortality of less susceptible heterozygous insects may be compensated for by increasing the size of the refuge, but this may have economic trade-offs[21,22]. Thus, the high-dose/refuge strategy may not be appropriate as a one-size fits all IRM tool for *Bt* crops with multiple pests.

Debate over the appropriate size, placement and management of refuges is arguably the most contentious issue surrounding the high-dose/refuge tactic[36,37]. Size recommendations for *Bt* corn and cotton refuges in the US range from 4% to 50% of the crop, depending on whether or not it is sprayed with insecticide, with US Environmental Protection Agency (EPA, Washington, DC, USA) refuge size requirements generally falling well below those recommended by some organizations and researchers[37]. Refuges are required to be within a specified distance of the *Bt* crop to permit random mating between insects from the transgenic crop and the refuge (e.g., 0.5 miles of the *Bt* crop for corn) but for many key pests targeted by *Bt* crops, insufficient data regarding dispersal ability and mating behavior (e.g., pre-or post-dispersal)[33] are available to guide refuge placement requirements[38] (although recent models indicate that the importance of random mating may have been overemphasized[39,40]). Nor do current refuge recommendations necessarily take into account other aspects of the pest's biology that may influence the ability of the refuge to dilute resistant alleles, such as oviposition preference and the availability of alternate hosts that can serve as a refuge[38]. Stable isotope analysis has provided valuable information regarding patterns of alternate host use by *H. zea* in some areas of the United States[41]; landscape-level studies of alternate host use by other pests targeted by *Bt* and their role in maintaining susceptibility to *Bt* would greatly aid development of appropriate and effective refuge guidelines.

Adding to the concern over inadequate refuge size and placement is the issue of grower compliance. Data from the US Department of Agriculture (Washington, DC, USA) indicated that 21% of farms growing *Bt* corn in 10 states in 2002 were, to varying degrees, in violation of the refuge requirements mandated by the EPA[42]. Given the low margin of error that some scientists believe is associated with the current recommendations, noncompliance could pose a threat to the high-dose/refuge strategy, particularly for insects with few wild hosts to act as an alternative refuge. However, social and economic factors that result in less than 100% adoption of *Bt* crops may result in regional 'unstructured' refuges that may compensate in part for some violations of refuge

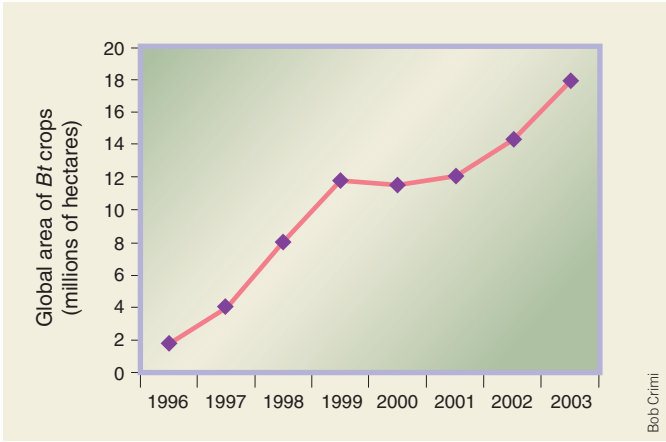

Figure 1 Global area planted to *Bt* crops (adapted from ref. 42).

requirements[43]. Risk of economic damage to refuges may be one reason for the lack of compliance among farmers growing *Bt* crops, and remains a major drawback of the high-dose/refuge strategy.

China, which grew 2.8 million ha of *Bt* cotton in 2003 (ref. 44) has no formal refuge requirements[45,46]. Small, resource-poor farms in China and other developing countries represent a particular challenge for IRM in *Bt* crops. In small farm situations where refuges are difficult to implement, the use of seed mixtures for crop pests that generally do not move between plants as larvae may be a more practical alternative to a separate refuge strategy[47].

Despite its limitations, the high-dose/refuge is still considered by most experts to be the most effective strategy currently available for delaying resistance. Clearly, however, there is a continuing need for improved IRM strategies that are sound yet practical. Rigorous IRM strategies will help preserve not only *Bt* crops that are already marketed, but other insect-resistant transgenic crops in development as well.

Second generation insect-resistant transgenic plants and IRM

Several new technologies in various stages of development are ushering in the next era of IRM for *Bt* crops. These take the form of new transgenic strains that mix and match existing toxins, the discovery and deployment of nontraditional insecticidal toxins, and the application of more traditional integrated pest management strategies, including cultural and biological controls.

Pyramided transgenic strains. We believe the recent release of transgenic cotton (Bollgard II, Monsanto, St. Louis, MO, USA) expressing two pyramided *Bt* genes (*cry2Ab* and *cry1Ac*) will provide superior resistance management options for cotton growers in the United States and Australia. In addition to increasing the life expectancy of *Bt* varieties, pyramided transgenes have two key advantages over the conventional high-dose/refuge strategy. First, in crops with multiple pests, two-transgene plants may provide better control of the entire pest complex, because species that are less susceptible to one toxin may still obtain a high-dose from the other. Second, plants with pyramided *Bt* toxins require a smaller refuge. Models have indicated that a 5–10% refuge with plants expressing two transgenes can provide a delay in resistance equivalent to a 30–40% refuge when two transgenes are deployed sequentially[21]. The maximum benefits of pyramided *Bt* genes will be realized if there is no cross-resistance between the two toxins[21].

Using broccoli expressing two *Bt* genes (*cry1Ac* and *cry1C*) and a synthetic population of diamondback moth in greenhouse tests, our group has demonstrated empirically that pyramided toxins delayed resistance

to both toxins compared to deployment of the same toxins in a mosaic pattern, and to at least Cry1Ac when the latter was deployed first in a sequence[25]. Subsequent tests using the same model system have also shown that the concurrent use of both two-transgene plants and plants expressing either transgene singly will select for resistance more rapidly than using two-transgene plants alone. Although these greenhouse tests do not necessarily reflect all the possible dynamics of resistance development on a landscape scale, we believe these findings have important implications for regulators and seed companies. In addition to Cry2Ab, Bollgard II cotton produces the same Cry1Ac toxin as its single transgene predecessor, Bollgard. Release of Bollgard II in areas where single-transgene cotton continues to be grown may shorten the lifespan of both toxins. WideStrike, a two-transgene cotton developed by Dow AgroSciences (Indianapolis, IN, USA) which also employs Cry1Ac in conjunction with Cry1F, will be available in the United States in 2005[48].

From an IRM perspective, the most judicious deployment of two-transgene technology may be to avoid using it concurrently with one-transgene plants, or to develop plants with *Bt* transgenes dissimilar to those already marketed. Australia, which permitted the use of both one- and two-transgene cotton in 2003, has now limited the use of *Bt* cotton to two-transgene varieties only. Economic considerations may render this an unlikely option for other countries, whereas practical considerations will limit the development of two-transgene plants with novel *Bt* genes, at least in the near future.

The most effective way to delay resistance to any insecticide remains the avoidance of unnecessary exposure to the toxin[21]. Inducible promoters could potentially allow *Bt* expression to occur only where or when needed (e.g. when the pest density exceeds an economic threshold, or vulnerable reproductive plant structures are formed) by the application of an otherwise benign compound. Creation of large temporal refuges requires less stringent assumptions regarding insect dispersal and mating[28]. We have shown that *Bt* production can be rapidly induced at high levels using transgenic broccoli expressing *cry1Ab* under the control of a chemically inducible promoter[49]; however, the effectiveness of this plant-promoter system as a resistance management tool remains to be established.

Novel toxins. Pyramided transgenes and inducible promoters offer new deployment options, but the use of novel Cry toxins with dissimilar target sites and alternative, non-Cry toxins with different modes of action and no cross-resistance to current *Bt* toxins also has tremendous value as an IRM tool that may be more rapidly and easily implemented. In addition to the widely studied crystalline endotoxins that are generally produced during sporulation, *B. thuringiensis* produces several toxins during vegetative growth as well[50]. These vegetative insecticidal proteins (Vips) are structurally and functionally dissimilar to their crystalline counterparts and show insecticidal activity against a wide range of lepidopteran and coleopteran pests[51,52]. Transgenic cotton producing a Vip toxin is currently being evaluated in field tests by Syngenta (Greensboro, NC, USA) and commercial release is anticipated in 2006 in the United States.

Another non-Cry toxin with potential for IRM is the insecticidal protein 'toxin A' isolated from *Photorhabdus luminescens*, the symbiotic bacteria associated with certain entomopathogenic nematodes that causes host mortality during nematode infection. *Arabidopsis thaliana* was recently transformed to express the toxin A gene at extremely high levels, and transformed plants had good insecticidal activity against at least one lepidopteran pest and moderate activity against a coleopteran pest[53]. In addition to toxin A, the genome of *P. luminescens* is believed to contain several other toxin-encoding genes with insecticidal activity[54].

Novel compounds with insecticidal activity that can be incorporated in crop plants represent valuable alternatives to the *Bt* toxins now expressed in transgenic crops. A key advantage of toxins such as Vip and toxin A is that, unlike *Bt* Cry toxins, there is potential to control unrelated pests from multiple orders. Unlike the Cry toxins, however, the safety of some of these alternative toxins to nontarget organisms has yet to be fully investigated. Regardless of the toxin, the same caution must be observed to avoid pest resistance. No single IRM strategy can delay resistance indefinitely, but combining multiple tactics (e.g., pyramided transgenes) and inducible promoters with *Bt* alone (or with other novel insecticidal toxins) could be the best defense against the evolution of resistance to insect-resistant transgenic crops.

Integrated pest management. At the same time, molecular breeding should not be the only focus of improved IRM strategies for transgenic crops. Many traditional aspects of integrated pest management, including cultural and biological controls, can and should play a valuable role in IRM. In Australia, for example, cultural control of *H. armigera* remains an important component of managing this pest and its resistance to *Bt* cotton. Suppression of overwintering pupae by soil disturbance, narrow planting windows that reduce the number of generations under selection and a mandated cap on *Bt* acreage have played an important role in reducing the selection pressure for resistance[21,55]. The use of 'trap' crops highly attractive for oviposition, such as pigeon pea for *H. armigera*, can provide susceptible insects and/or help concentrate late season adults and their progeny for elimination[56]. Management of insects in the refuge—in many ways the Achilles heel of the high-dose/

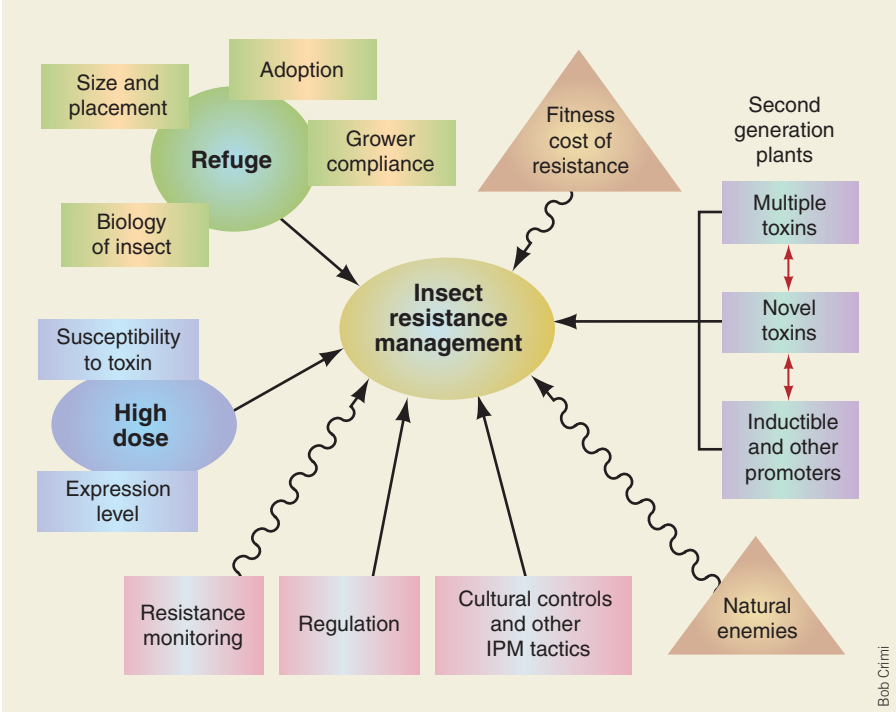

Figure 2 Factors affecting the efficacy of IRM strategies for insect-resistant transgenic crops.

refuge strategy—can also be approached with an integrated pest management philosophy that strives to maintain susceptible alleles in the refuge with minimal economic damage. Insect-tolerant varieties derived from classical breeding may have sufficient herbivore resistance to serve as suitable refuge plants and could provide a refuge that avoids excessive economic losses[57], while providing IRM and IPM benefits in the main crop. Alternatively, a refuge consisting of plants less preferred for oviposition than the main crop could also potentially reduce insect density in the refuge while still delaying resistance substantially[58].

Natural enemies deserve special note. There is a growing body of literature concerning the potential nontarget effects of *Bt* crops on predators and parasites, but relatively little emphasis has been placed on their influence on resistance evolution. Models have indicated that predators and parasites may slow the rate of resistance development, if mortality from natural enemies is higher for resistant insects feeding on toxic hosts than susceptible insects in the refuge[59]. On the other hand, the high concentration of prey on refuge plants may result in a disproportionately higher mortality of susceptible insects by natural enemies, which in turn would accelerate resistance. However, at least one field study has shown an inverse relationship between host egg density and predation[60]. The only way to properly quantify the effects on IRM is to conduct the appropriate field tests. In those situations where a higher density of insects in the refuge concentrates natural enemies, the anticipated cost of higher mortality among refuge insects must be weighed against other refuge management options (e.g., spraying) that would result in an even greater mortality differential.

The future of *Bt* crops and IRM

On a global scale, selection for resistance to *Bt* is still in its infancy. In 2003, *Bt* varieties accounted for only 9% and 17% of the world's corn and cotton acreage, respectively; however, adoption rates are rising rapidly, with the global area grown to *Bt* crops increasing 24% between 2002 and 2003 (refs. 44,61; **Fig. 1**). *Bt* crops are currently grown in 17 countries and adoption is predicted to continue its steady growth, influenced by an increasing confidence in the benefits of *Bt* crops and the availability of new *Bt* varieties that confer protection against additional crop pests (e.g., Monsanto's MON863 corn for corn rootworm control)[44]. In addition, several other *Bt* crops, including potato, rice, canola, soybean, tobacco, tomato, apple, peanuts and broccoli, are in various stages of development[62]. As the use of *Bt* crops continues to grow, so too will the selection pressure on various pests from these crops.

The lack of resistance observed in the field to current *Bt* crops attests to the potential sustainability of this technology, but caution continues to be warranted because at least one insect species, the diamondback moth, has evolved resistance in the field to foliar *Bt* sprays, and the selection pressure for resistance in other species to *Bt* crops will only increase as adoption levels rise. The development of second-generation insecticidal transgenic crops should not be seen as a panacea for the problems of pest management and *Bt* resistance, but as a call for improved IRM strategies that keep pace with the new technology. The alternative is to risk repeating the pesticide treadmill that has dominated pest management for the last five decades, where products are used until resistance causes them to fail, with the assumption that replacements will always be available. Although *Bt* may provide a suite of useful insecticidal proteins, each will have a limited period of effectiveness if improperly managed, and alternative products sharing the same level of efficacy, selectivity and consumer safety are not likely to be found quickly or easily.

To help preserve both existing and future insect-resistant transgenic crops, IRM strategies should be both rigorous and responsive. The most effective strategy for a given crop will be based on the best available models and data, and will not remain static in the face of our rapidly changing knowledge regarding the ecology and genetics of resistance to *Bt* (**Fig. 2**). Monitoring for changes in resistant-allele frequencies in field populations (**Box 1**) will play an increasingly critical role in future IRM. Sensitive monitoring techniques may provide sufficient lead time to implement tactics designed to contain the spread of resistant individuals[38,63] and/or adjust deployment strategies of subsequent crops accordingly.

IRM strategies can do much to delay resistance to *Bt* plants. However, the most effective use of *Bt* crops will be as a component of overall IPM programs. For example, the use of *Bt* crops in conjunction with cultural or biological methods that have limited efficacy on their own may help increase the feasibility of large refuges (where only moderate insect control is desired) or help suppress local pest populations in the near or long term[23]. Indeed, the use of *Bt* cotton has already suppressed some regional populations of pink bollworm in Arizona[64]. Although treating *Bt* crops as a silver bullet for pest management will almost certainly hasten the evolution of resistance, incorporation of transgenic crops with traditional, integrated approaches to pest management should help ensure their long-term sustainability and maximize their environmental and human health benefits.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. McCaffrey, A.R. Resistance to insecticides in heliothine Lepidoptera: a global view. *Phil. Trans. R. Soc. Lond. B* **353**, 1735–1750 (1998).
2. Tabashnik, B.E. *et al.* Insect resistance to transgenic *Bt* crops: lessons from the laboratory and the field. *J. Econ. Entomol.* **96**, 1031–1038 (2003).
3. US Environmental Protection Agency. *Biopesticides registration action document -Bacillus thuringiensis plant-incorporated protectants* (EPA, Washington, DC, USA 2001). http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm
4. Fox, J.L. Resistance to *Bt* toxin surprisingly absent from pests. *Nat. Biotechnol.* **21**, 958–959 (2003).
5. Tabashnik, B.E., Cushing, N.L., Finson, N. & Johnson, M.W. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* **83**, 1671–1676 (1990).
6. Janmaat, A.F. & Meyers, J. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. *Proc. R. Soc. Lond. B Biol.Sci.* **270**, 2263–2270 (2003).
7. Frutos, R., Rang, C. & Royer, M. Managing resistance to plants producing *Bacillus thuringiensis* toxins. *Crit. Rev. Biotechnol.* **19**, 227–276 (1999).
8. Oppert, B., Hammel, R., Throne, J.E. & Kramer, K.J. Fitness costs of resistance to *Bacillus thuringiensis* in the Indianmeal moth, *Plodia interpunctella*. *Entomol. Exp. Appl.* **96**, 281–287 (2000).
9. Carrière, Y. *et al.* Fitness costs and maternal effects associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **94**, 1571–1576 (2001).
10. Liu, Y.B. *et al.* Effects of *Bt* cotton and Cry1Ac toxin on survival and development of pink bollworm (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **94**, 1237–1242 (2001).
11. Carrière, Y. *et al.* Overwintering cost associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera: Gelechiidae*). J. Econ. Entomol.* **94**, 935–941 (2001).
12. Groeters, F.R., Tabashnik, B.E., Finson, N. & Johnson, M.W. Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). *Evolution* **48,** 197–201 (1994).
13. Trisyono, A. & Whalon, M.E. Fitness costs of resistance to *Bacillus thuringiensis* in Colorado potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **90**, 267–271 (1997).
14. Alyokhin, A. & Ferro, D.N. Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. *J. Econ. Entomol.* **92**, 510–515 (1999).
15. Akhurst, R.J., James, W., Bird, L.J. & Beard, C. Resistance to the Cry1Ac δ-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **96**, 1290–1299 (2003).
16. Tang, J.D., Gilboa, S., Roush, R.T. & Shelton, A.M. Inheritance, stability, and lack of fitness costs of field-selected resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae) from Florida. *J. Econ. Entomol.* **90**, 732–741 (1997).
17. Ramachandran, S. *et al.* Survival, development, and oviposition of resistant diamondback moth (Lepidoptera: Plutellidae) on transgenic canola producing a *Bacillus*

thuringiensis toxin. *J. Econ. Entomol.* **91**, 1239–1244 (1998).
18. Sayyed, A.H. & Wright, D.J. Fitness costs and stability of resistance to *Bacillus thuringiensis* in a field population of the diamondback moth, *Plutella xylostella*. *Ecol. Entomol.* **26**, 502–508 (2001).
19. Tabashnik, B.E. Groeters, F.R., Finson, N. & Johnson, M.W. Instability of resistance to *Bacillus thuringiensis*. *Biocont. Sci. Technol.* **4,** 419–426 (1994).
20. Tabashnik, B.E. Evolution of resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* **39**, 47–79 (1994).
21. Roush, R.T. *Bt*-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pestic. Sci.* **51**, 328–334 (1997).
22. Roush, R.T. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? *Phil. Trans. R. Soc. Lond. B* **353**, 1777–1786 (1998).
23. Roush, R.T. Managing resistance to transgenic crops. in *Advances in Insect Control* (eds. Carozzi, N. & Koziel, M.) 271–294 (Taylor and Francis, London, UK, 1997).
24. Roush, R.T. Designing resistance management programs: how can you choose? *Pestic. Sci.* **26**, 423–441 (1989).
25. Zhao, J-Z. *et al.* Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nat. Biotechnol.* **21**, 1493–1497 (2003).
26. Huang, F., Higgins, R.A. & Buschman, L.L. Heritability and stability of resistance to *Bacillus thuringiensis* in *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* **89**, 449–454 (1999).
27. Jackson, R.E., Bradley, J.R. & Van Duyn, J.W. Performance of feral and Cry1Ac-selected *Helicoverpa zea* (Lepidoptera: Noctuidae) strains on transgenic cottons expressing one or two *Bacillus thuringiensis* ssp. *kurstaki* proteins under greenhouse conditions. *J. Entomol. Sci.* **39**, 46–55 (2004).
28. Tabashnik, B.E. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. *Proc. R. Soc. Lond. B* **255**, 7–12 (1994).
29. Mallet, J. & Porter, P. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proc. R. Soc. Lond. B.* **250,** 165–169.
30. Tang, J.D. *et al.* Greenhouse tests on resistance management of *Bt* transgenic plants using refuge strategies. *J. Econ. Entomol.* **94**, 240–247 (2001).
31. Shelton, A.M., Tang, J.D., Roush, R.T., Metz, T.D. & Earle, E.D. Field tests on managing resistance to *Bt*-engineered plants. *Nat. Biotechnol.* **18**, 339–342 (2000).
32. Burd, A.D., Gould, F., Bradly, J.R., Van Duyn, J.W. & Moar, W.J. Estimated frequency of nonrecessive *Bt* resistance genes in bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in eastern North Carolina. *J. Econ. Entomol.* **96**, 137–142 (2003).
33. Gould, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.* **43**, 701–726 (1998).
34. Chilcutt, C.F. & Tabashnik, B.E. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. *Proc. Natl. Acad. Sci. USA* **101**, 7526–7529 (2004).
35. Gould, F. Potential and problems with high-dose strategies for pesticide engineered crops. *Biocont. Sci. Technol* **4**, 451–461 (1994).
36. Hargrove, T.R. Wrangling over refuge. *Am. Sci.* **87**, 24–25 (1999).
37. Dove, A. *Bt* resistance plan appraised. *Nat. Biotechnol.* **17**, 531–532 (1999).
38. Glaser, J.A. & Matten, S.R. Sustainability of insect resistance management strategies for transgenic *Bt* corn. *Biotechnol. Adv.* **22**, 45–69 (2003).
39. Caprio, M.A. Source-sink dynamics between transgenic and non-transgenic habitats and their role in the evolution of resistance. *J. Econ. Entomol.* **94**, 698–705 (2001).
40. Ives, A.R. & Andow, D.A. Evolution of resistance to *Bt*-crops: directional selection in structured environments. *Ecol. Lett.* **5**, 792–801 (2002).
41. Gould, F. *et al. Bacillus thuringiensis*-toxin resistance management: stable isotope assessment of alternate host use by *Helicoverpa zea*. *Proc. Natl. Acad. Sci. USA* **99,** 16581–16586.
42. Jaffe, G. *Planting Trouble Update.* (Center for Science in the Public Interest, Washington, DC, USA, 2003). http://www.cspinet.org/new/pdf/planting_trouble_update1.pdf
43. Hurley, T.M. & Secchi, S., Babcok, B.A. & Hellmich, R.L. Managing the risk of European corn borer resistance to *Bt* corn. *Environ. Resour. Econ.* **22**, 537–558 (2002).
44. James, C. Global status of commercialized transgenic crops: 2003. ISAAA Brief No. 30. (International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, USA, 2003).
45. Zhao, J.-Z., Zhao, K., Lu, M., Fan, X. & Guo, S. Interactions between *Helicoverpa armigera* and transgenic cotton in North China. *Sci. Agr. Sinica* **31**, 1–6 (1998).
46. Zhao, J.-Z. & Rui, C. Insect resistance management (IRM) for transgenic *Bt* cotton. In *Transgenic Cotton*, (eds. Jia, S.R. *et. al.*), 165–180, (China Science Press, Beijing, 2001).
47. Gould, F. Testing *Bt* refuge strategies in the field. *Nat. Biotechnol.* **18**, 266–276 (2000).
48. http://www.dowagro.com/usag/resource/20030423a.htm
49. Cao, J., Shelton, A.M. & Earle, E.D. Gene expression and insect resistance in transgenic broccoli containing a *Bacillus thuringiensis cry1Ab* gene with the chemically inducible PR-1a promoter. *Mol. Breed.* **8**, 207–216 (2001).
50. Schnepf, E. *et al. Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **62**, 775–806 (1998).
51. Lee, M.K., Walters, F.S., Hart, H., Palekar, N. & Chen, J.-S. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. *Appl. Environ. Microbiol.* **69**, 4648–4657 (2003).
52. Warren, G.W. Vegetative insecticidal proteins: novel proteins for control of corn pests. in *Advances in Insect Control* (eds. Carozzi, N. & Koziel, M.) 109–121 (Taylor and Francis, London, UK, 1997).
53. Liu, D. *et al.* Insect resistance conferred by 283-kDa *Photorhabdus luminescens* protein TcdA in *Arabidopsis thaliana*. *Nat. Biotechnol.* **21**, 1222–1228 (2003).
54. Williamson, V.M. & Kaya, H.K. Sequence of a symbiont. *Nat. Biotechnol.* **21**, 1294–1295 (2003).
55. Fitt, G.P. An Australian approach to IPM in cotton: integrating new technologies to minimize insecticide dependence. *Crop Prot.* **19**, 793–800 (2000).
56. Sequeira, R.V. & Playford, C.L. Abundance of *Helicoverpa* (Lepidoptera: Noctuidae) pupae under cotton and other crops in central Queensland: implications for resistance management. *Aust. J. Entomol.* **40**, 264–269 (2001).
57. Smith, M. Public sector plant breeding and pest resistance management. in *Online Proceedings of CAST Pest Resistance Management Symposium, Indianapolis, IN, USA, April 10–11, 2003.* (The Council for Agricultural Science and Technology, Washington, DC, USA, 2003). http://www.pestmanagement.info/rmworkshop/
58. Alstad, D.N. & Andow, D.A. Managing the evolution of insect resistance to transgenic plants. *Science* **268**, 1894–1896 (1995).
59. Gould, F. Potential and problems with high-dose strategies for pesticide engineered crops. *Biocont. Sci. Technol* **4**, 451–461 (1994).
60. Arpaia, S., Gould, F. & Kennedy, G. Potential impact of *Coleomegilla maculata* predation on adaptation of *Leptinotarsa decemlineata* to *Bt*-transgenic potatoes. *Entomol. Exp. Appl.* **82**, 91–100 (1997).
61. James, C. Global status of commercialized transgenic crops: 2002. ISAA Brief NO. 27 (International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY, USA, 2002).
62. Shelton, A.M., Zhao, J-Z. & Roush, R.T. Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annu. Rev. Entomol.* **47**, 845–881 (2002).
63. Andow, D.A. & Ives, R.A. Monitoring and adaptive resistance management. *Ecol. Appl.* **12**, 1378–1390 (2002).
64. Carrière, Y. *et al.* Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proc. Natl. Acad. Sci. USA* **100**, 1519–1523 (2003).
65. Hawthorne, D., Sigfried, B., Shelton, A.M. & Hellmich, R. Monitoring for Resistance Alleles: a Report from an Advisory Panel on Insect Resistance Monitoring Methods for Bt corn. Agricultural Biotechnology Stewardship Committee Report (Agricultural Biotechnology Stewardship Technical Committee, Washington, DC, 2002).
66. Andow, D.A. & Alstad, D.N. F2 screen for rare resistance alleles. *J. Econ. Entomol.* **91**, 572–578 (1998).
67. Zhao, J-Z., Li, Y., Collins, H.L. & Shelton, A.M. Examination of the F2 screen for rare resistance alleles to *Bacillus thuringiensis* toxins in the diamondback moth. *J. Econ. Entomol.* **95**, 14–21 (2002).
68. Nagamatsu, Y., Koike, T., Sasaki, K., Yoshimoto, A. & Furukawa, Y. The cadherin-like protein is essential to specificity determination and cytotoxic action of the *Bacillus thuringiensis* insecticidal CryIAa toxin. *FEBS Lett.* **460**, 385–390 (1999).
69. Vadlamudi, R.K., Weber, E., Ji, I., Ji, T.H. & Bulla Lee, A. Jr. Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. *J. Biol. Chem.* **270**, 5490–5494 (1995).
70. Gahan, L.J., Gould, F. & Heckel, D.G. Identification of a gene associated with *Bt* resistance in *Heliothis virescens*. *Science* **293**, 857–860 (2001).
71. Morin, S. *et al.* Three cadherin alleles associated with resistance to *Bacillus thuringiensis* in pink bollworm. *Proc. Natl. Acad. Sci. USA* **100**, 5004–5009 (2003).