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Review

Can Pyramids and Seed Mixtures Delay Resistance to Bt Crops?

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The primary strategy for delaying the evolution of pest resistance to transgenic crops that produce insecticidal proteins from *Bacillus thuringiensis* (Bt) entails refuges of plants that do not produce Bt toxins and thus allow survival of susceptible pests. Recent advances include using refuges together with Bt crop 'pyramids' that make two or more Bt toxins effective against the same pest, and planting seed mixtures yielding random distributions of pyramided Bt and non-Bt corn plants within fields. We conclude that conditions often deviate from those favoring the success of pyramids and seed mixtures, particularly against pests with low inherent susceptibility to Bt toxins. For these problematic pests, promising approaches include using larger refuges and integrating Bt crops with other pest management tactics.

Evolution of Pest Resistance Threatens the Benefits of Transgenic Bt Crops

The world population is expected to grow from 7.2 billion now to at least 9.6 billion by 2100, greatly increasing demand for agricultural output [1–3]. Crops genetically engineered to produce insecticidal proteins from the bacterium Bt can help meet this demand by suppressing pest populations locally and regionally [4–7], increasing or stabilizing yield [8–10], reducing reliance on conventional insecticides [10–12], and enhancing favorable effects of beneficial arthropods [13–16]. From 1996 to 2014, farmers planted **Bt crops** (see Glossary) on a cumulative total of 648 million ha worldwide, consisting almost entirely of Bt corn and Bt cotton [17]. Bt soybean was planted in Brazil on a cumulative total of 7.4 million ha in 2013 and 2014, and Bt eggplant was planted commercially in 2014 on a small scale in Bangladesh [17]. Yield gains and insecticide reductions with Bt crops are often sufficient to increase farmer profits, which is the primary reason that farmers use these crops in the USA [10,12]. In the USA in 2015, Bt corn accounted for 81% of all corn and Bt cotton for 84% of all cotton [18].

As Bt crops have become more widely adopted, some of their economic and environmental benefits have been lost because of rapid evolution of resistance by pests, particularly to the earliest commercialized Bt crops that produced only one Bt toxin [19] (Box 1 and Table 1). Since Bt crops were first commercialized 20 years ago, the **refuge** strategy has been the primary approach used to delay pest resistance [19,20]. In this strategy, refuges of non-Bt host plants allow the survival of susceptible pests that can mate with resistant pests emerging from Bt plants (Figure 1). Laboratory and greenhouse experiments, large-scale studies, and retrospective comparisons of patterns of **field-evolved resistance** show that refuges can delay resistance [19,21–23]. This review focuses on two recent developments in managing resistance to Bt crops, both of which are refinements of the refuge strategy: using refuges in conjunction with Bt crop **pyramids** that have two or more toxins effective against the same pest, and planting random mixtures of Bt and non-Bt seeds.

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Conditions in the field often deviate substantially from those promoting success of the refuge strategy for delaying insect pest resistance to pyramided Bt crops, particularly in pests with low inherent susceptibility to Bt toxins.

Phasing out plants that produce only one toxin effective against target pests could increase the durability of Bt crop pyramids.

Evolution of pest resistance to Bt crops could be slowed by using combinations of toxins that are structurally distinct, such as Cry and Vip toxins, or Cry toxins with low amino acid sequence similarity in domain II.

Gene flow between Bt and non-Bt corn plants in seed mixtures produces a mosaic of Bt and non-Bt kernels in ears of non-Bt corn plants, which could accelerate the evolution of resistance in pests feeding on ears.

In some regions of the USA, where western corn rootworm has evolved resistance to Cry3Bb and mCry3Aa, all pyramided Bt corn hybrids targeting this pest are effectively single-toxin crops.

Many conditions favoring success of the refuge strategy deviate from the ideal for western corn rootworm, implying that the risk of resistance in this pest is high for all currently available Bt corn hybrids in the USA.

The refuge strategy has been successful for delaying resistance to Bt crops in pests with high inherent susceptibility to Bt toxins, but larger refuges are needed and Bt crops must be integrated with

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Box 1. Categories and Patterns of Field-Evolved Resistance to Bt Crops

Recognizing that resistance is not 'all or none', and that various levels of resistance can have a continuum of effects on pest control, five categories of field-evolved resistance to Bt crops have been described [28,29]. All five categories entail a statistically-significant and genetically based decrease in susceptibility in field populations of pests, but only one category (practical resistance) indicates resistance is severe enough to generate reports of reduced pest control in the field: (i) incipient resistance, <1% resistant individuals; (ii) early warning of resistance, 1–6% resistant individuals; (iii) >6% to 50% resistant individuals; (iv) >50% resistant individuals and reduced efficacy expected but not reported; and (v) practical resistance, >50% resistant individuals and reduced efficacy reported. In a recent analysis, 12 of 27 cases examined (44%) showed no significant increase in resistance after 2–15 years (median, 8 years) of exposure to Bt crops [29]. Of the remaining 15 cases, three were characterized as incipient resistance, four were early warning of resistance, one was >50% resistant individuals with reduced efficacy expected but not reported, and seven demonstrated practical resistance. All seven cases of practical resistance involved resistance to single-toxin crops (see Table 1 in main article). Field-evolved resistance to Cry2Ab, which has been used only in combination with one or more other Bt toxins, has been documented in populations of two closely related species (*Helicoverpa punctigera* and *Helicoverpa zea*) that were exposed extensively to a Bt cotton pyramid of Cry1Ac and Cry2Ab, but neither of these cases has been categorized as practical resistance [19,55,82].

Bt Crop Pyramids

Each of the original Bt crops commercialized in 1996 was engineered to make a single crystalline (Cry) toxin to kill larvae of some key lepidopteran pests [24]. To delay resistance, improve efficacy against some pests, and broaden the spectrum of pests controlled, most newer Bt crops produce two or more Bt toxins [20]. Current multi-toxin crops produce two or more Bt toxins that belong to either the Cry protein family or to the vegetative insecticidal protein (Vip) family (Table 2). Pyramided Bt crops are a special kind of multi-toxin crop designed to delay the evolution of resistance by producing two or more distinct toxins that kill the same pest [20,25]. First commercialized in 2003, such pyramids have become increasingly prevalent in recent years in the USA and other countries [19,26]. For example in 2014, a pyramid producing Bt toxins Cry1Ac and Cry2Ab accounted for 96% of the 12 million ha of Bt cotton in India [27].

Conditions Promoting the Durability of Bt Crop Pyramids

Five conditions that promote the durability of both single-toxin and pyramided crops are: (i) refuges are sufficiently abundant, (ii) alleles conferring resistance are rare, (iii) resistance is recessive, (iv) **fitness costs** are associated with resistance, and (v) **resistance is incomplete** [19,20]. Retrospective analyses show that all cases of field-evolved **practical resistance** to single-toxin crops involve substantial deviations from one or more of the first three conditions [19,28,29]. Conversely, previous reviews have concluded that fitness costs associated with resistance and incomplete resistance can increase the durability of Bt crops [30–32]. Here we synthesize theory and evidence about three conditions that are especially important for the

Table 1. Seven Cases of Field-Evolved Practical Resistance to Single-Toxin Bt Crops[a]

Insect	Bt Crop	Toxin	Country	Durability (Years)[b]	Initial Detection[c]
Helicoverpa zea	Cotton	Cry1Ac	USA	6	2002
Busseola fusca	Corn	Cry1Ab	South Africa	6	2004
Spodoptera frugiperda	Corn	Cry1Fa	USA	3	2006
Pectinophora gossypiella	Cotton	Cry1Ac	India	6	2008
Diabrotica v. virgifera	Corn	Cry3Bb	USA	6	2009
Diabrotica v. virgifera	Corn	mCry3Aa	USA	4	2011
Spodoptera frugiperda	Corn	Cry1Fa	Brazil	2	2011

[a] Data from [19,29].

[b] Years elapsed in the region studied between the first year of commercial use and the first year of field observations or field sampling that yielded evidence of practical resistance

[c] First year of field observations or field sampling that provided evidence of practical resistance; publication of this evidence often occurred several years later. For example, evidence of *S. frugiperda* resistance to Cry1Fa in Brazil was published first in 2014 based on bioassay data from progeny of insects sampled from the field in 2011 [83].

other pest management tactics to sustain their efficacy against pests with low inherent susceptibility to Bt toxins

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