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# Seeds of Change: Corn Seed Mixtures for Resistance Management and Integrated Pest Management

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**ABSTRACT** The use of mixtures of transgenic insecticidal seed and nontransgenic seed to provide an in-field refuge for susceptible insects in insect-resistance-management (IRM) plans has been considered for at least two decades. However, the U.S. Environmental Protection Agency has only recently authorized the practice. This commentary explores issues that regulators, industry, and other stakeholders should consider as the use of biotechnology increases and seed mixtures are implemented as a major tactic for IRM. We discuss how block refuges and seed mixtures in transgenic insecticidal corn, *Zea mays* L., production will influence integrated pest management (IPM) and the evolution of pest resistance. We conclude that seed mixtures will make pest monitoring more difficult and that seed mixtures may make IRM riskier because of larval behavior and greater adoption of insecticidal corn. Conversely, block refuges present a different suite of risks because of adult pest behavior and the lower compliance with IRM rules expected from farmers. It is likely that secondary pests not targeted by the insecticidal corn as well as natural enemies will respond differently to block refuges and seed mixtures.

**KEY WORDS** insect resistance management, refuge, Bt corn, secondary pests, biological control

Grower adoption of transgenic insecticidal corn, *Zea mays* L., has expanded rapidly since its introduction in 1996. In 2010, 63% of corn planted in the United States was transgenic insecticidal hybrids (USDA–ERS 2010). The insecticides incorporated into transgenic

corn are derived from *Bacillus thuringiensis* Berliner (Bt) and are effective against two classes of pests: the Lepidoptera, including the European corn borer, *Ostrinia nubilalis* (Hübner) (Crambidae), and southwestern corn borer, *Diatraea grandiosella* Dyar; and the Coleoptera, such as *Diabrotica virgifera virgifera* LeConte (western corn rootworm) and *Diabrotica barberi* Smith & Lawrence (northern corn rootworm). In the first phase of this technology (1995–2008), the transgenic corn expressed one toxin for each order (Lepidoptera or Coleoptera). The primary strategy for insect resistance management (IRM) during this phase used a block of non-Bt plants to provide a refuge for producing susceptible or nonselected insects. During the second phase of transgenic-corn technology (2008–present), some seed companies are producing hybrids that contain multiple toxins (pyramided toxins or pyramids) for each group of pests. Furthermore, refuges implemented as seed mixtures are being considered. With seed mixtures (also called seed blends or refuge in a bag), growers purchase a bag of seed containing mostly insecticidal corn with a small proportion of non-Bt seed. Thus, the refuge is randomly planted within the same cornfield as the transgenic corn. The U.S. Environmental Protection Agency has approved a conditional registration of a

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seed mixture for corn rootworm IRM for a corn hybrid expressing only one toxin.

Corn hybrids can be stacked with herbicide-tolerance genes and multiple toxin-pyramids for controlling Lepidoptera and Coleoptera. Furthermore, most seed is sold with insecticidal coatings that are primarily targeted at secondary root pests. If priced competitively with nontransgenic or single toxin corn, pyramided, stacked corn could be expected to gain market share because of its increased efficacy and lower expected refuge requirements. Thus, it is likely that the total acreage planted to transgenic corn will increase. From a seed company perspective, these pyramided stacks present the opportunity to gain market share while reducing the cost and complexity of supplying transgenic seed. No longer will separate (yet similar) hybrids have to be maintained with activity against only Lepidoptera or only corn rootworms. One hybrid would have activity against both pest groups. Stacking also makes it possible to increase the value of seed to growers, so suppliers can command higher seed prices, with a negligible change in the cost of seed production (Duffy 2009, Schnitkey and Lattz 2009). From a grower's perspective, however, the move toward pyramided stacks restricts consumer choice and increases input costs, and may force them to deploy controls that are not necessary. Nevertheless, other incentives might tend to counterbalance a restricted marketplace. For example, growers can save money on crop insurance by using stacked varieties. The new Biotech Endorsement for federal crop insurance reduces insurance premiums for participating growers who plant 75% or more of their cornfields with approved stacked hybrids (USDA-RMA 2009). In 2009, this endorsement saved participating growers more than seven dollars per hectare on average (Edwards 2010), further increasing farmer incentives to fully adopt stacked transgenic corn hybrids.

The purpose of this commentary is to explore issues that regulators, industry, and other stakeholders should consider as we continue to expand and alter our deployment of transgenic crops. Specifically, we focus upon the proposed use of seed mixtures as a major tactic in IRM. Stakeholders will benefit from a greater understanding of the short and long-term consequences of refuge choices not only for IRM but also for integrated pest management (IPM). We summarize many of the issues that this commentary addresses in Table 1. Monitoring, secondary pests and biological control are discussed in the first section of our commentary, which focuses on the effects of changes to IRM tactics upon IPM practices. With regard to control of secondary pests and biological control, we believe that the mixture of insecticidal and conventional corn plants will require new approaches that may include new challenges. We assume that traditional approaches will continue to be used in block refuges, and the use of insecticides and refuge quality are among the issues mentioned in the section on the corn landscape. The section on pest behavior develops important questions concerning larval and adult behavior. The final two subjects listed in Table 1 are covered

**Table 1.** Summary of IPM issues pertaining to choice of deployment of IRM refuges as seed mixtures or blocks

Issue	Seed mixture	Block
Pest monitoring	Difficult	Typical
Control of secondary pests	New approaches	Traditional
Biological control	New approaches	Traditional
Insecticide use	Less	More
Quality of refuge relative to Bt corn	Similar	Probably different
Effects on IRM of larval behavior	More risk	Less risk
Effects on IRM of adult behavior	Less risk	More risk
Adoption of PIP technology <sup>a</sup>	Higher	Lower
Compliance with IRM rules	Higher	Lower

<sup>a</sup> PIP, plant-incorporated pesticide.

in our section on human behavior. As Table 1 suggests, we present this document to encourage stakeholders to study the complex factors that influence decision making. For example, we recommend that the scientific community account for the impacts that transgenic corn and IRM strategies have on insects not targeted for control by seed companies. Scientists also should consider the many ways that the corn landscape and pest behavior affect natural selection of pest species, particularly how selection can occur throughout the life cycle of a pest. Finally, we recommend that the scientific community not forget the influence of human behavior when evaluating refuge options.

## IPM

With the expansion of Bt corn in the marketplace, host plant resistance (in the form of Bt corn) and prophylactic approaches (insecticidal seed treatments) to pest management have become the norm. These approaches control a large assemblage of above- and below-ground primary and secondary pests, some of which are not found in the area where the crop is planted. This risk-management approach to corn pest management has certainly provided tangible benefits to producers in corn-producing regions where target pests were once abundant, as in the case of Bt corn hybrids targeting European corn borers (Hutchison et al. 2010). However, this approach carries with it the tendency to ignore many aspects of IPM, such as monitoring pest levels and concentrating treatments when or where appropriate, because there is an assumption that most pests are controlled throughout the season, regardless of pressure levels. Although field corn has never been considered an IPM-intensive cropping system, there is less impetus than ever for growers or crop consultants to enter fields. And without objective assessments of pest population density and damage or the presence of non-Bt hybrids in the same field, emergence of novel pests, declines in pest populations, or the beginnings of resistance may not be identified until they have become widespread.

Scientists and most stakeholders usually focus their attention on the major targeted pest(s) listed on the label of the registered transgenic insecticidal corn.

Nontarget insects are abundant within corn agroecosystems, and they influence the productivity of cornfields, as well as providing ecosystem services to the surrounding landscapes. Nontarget insects include secondary pests, those species that feed on the corn plant and may or may not be targeted by the toxin. Beneficial species (e.g., natural enemies of crop pests and weeds, pollinators, detritivores) all provide free services to farmers, and their conservation within cropland is important (Toepfer et al. 2008, Lundgren et al. 2009a, Toepfer et al. 2009). Given their intimate association with cornfields, these nontarget arthropods may be affected by the refuge choice or actually determine the effectiveness of the refuge for IRM and IPM.

**Monitoring.** One indirect benefit of block refuges has been that growers and consultants have been aware that scouting is needed in these areas, often providing an early warning system of pest and disease problems that might otherwise go undiagnosed. Increased adoption of seed mixtures will probably contribute to a decline in pest monitoring; although plants in a block refuge can be easily located and monitored, a refuge plant in a seed mixture cannot be distinguished from other plants without the use of protein-specific test strips. Even though these test strips are relatively simple to use, their cost (US\$2.50–\$4.00 per protein per plant) makes their use unlikely over a large area. This kind of testing is also necessary to determine whether the mixture of refuge and Bt seed matches the label. We believe that it is unlikely that seed mixtures will be rigorously sampled for targeted pests by farmers, extension specialists, or crop consultants. At the same time, sampling in block refuges may decline as block refuge sizes decline and benefit:cost ratios for sampling small areas decline.

**Secondary Pests.** Some of the most common secondary or nontargeted pests are noctuid moths: western bean cutworm, *Striacosta albicosta* (Smith); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); and corn earworm, *Helicoverpa zea* (Boddie). Western bean cutworm is susceptible to Cry1F, a Bt toxin, and the larvae tend to move more than European corn borer (Michel et al. 2010). Within hours of hatching, first instars will crawl into the tassel or down into silks; within a day of hatching, they can move one to two plants away from their natal plant. In 2 wk, virtually all larvae are in the ear zone feeding on kernels, husk, or silk (Michel et al. 2010). This movement in a seed mixture will expose them to different plant tissues as well as to refuge and Bt plants in and across the row of corn plants.

It is critical to note that although currently registered transgenic hybrids provide protection from a range of key pests, there are large assemblages of other potential pests that are unaffected, including slugs, mites, thrips, mirids, cicadellids, aphids, dipteran larvae, flea beetles, and many non-*Diabrotica* species of beetle larvae (J.G.L., unpublished data; Rose and Dively 2007). Although none of these groups are key pests (with the exception of spider mites on the Southern High Plains), these species occasionally cause spo-

radic outbreaks. Even in cases where insects are controlled, the control is not equal for all pests; for example, toxins with high efficacy against European corn borer do not perform as well against the western bean cutworm (Cantangui and Berg 2006). The purpose of highlighting these concerns is not to argue that control of any and all potential pests should be the benchmark for pest management strategies. Rather, we want to illustrate the point that our main warning system for novel pest problems—the eyes of IPM practitioners—will probably be far less prevalent in cornfields with seed mixtures.

**Natural Enemies.** Biological control by endemic and introduced natural enemies is a cornerstone of IPM, and seed mixtures may affect the ability of these natural enemies to persist in cropland and their contributions to pest management (Wilhoit 1991). Predators, parasitoids, and pathogens are a frequent and free source of biotic mortality for all pests in cropland (Brust et al. 1986; Lewis and Bing 1991; Andow 1992; Jackson and Hesler 1996; Cottrell and Yeagen 1998; Phoopholo et al. 2001; Pfannenstiel and Yeagen 2002; Toepfer et al. 2008; Lundgren et al. 2009a,b; Toepfer et al. 2009), and our farm management decisions should encourage their conservation and contributions to pest management whenever possible. Currently, there are no consistently documented toxicological effects of Cry toxins expressed in commercial Bt corn against predators or parasitoids (Marvier et al. 2007, Wolfenbarger et al. 2008, Duan et al. 2010), but Bt corn certainly changes the corn environment relative to non-Bt habitats, and these changes could have important implications for biological control (Dean and De Moraes 2006, Lundgren et al. 2009a).

Pest density and dispersion affect the foraging efficacy of natural enemies and the behavior and abundance of natural enemies that specialize on specific target and nontarget pests (Arpaia et al. 1997, Winder et al. 2001, Bohan et al. 2000, White and Andow 2005). Refuge areas with relatively dense patches of target pests allow local populations of specialist natural enemies to persist in cropland. These natural enemies may be an important additional source of mortality to resistant pests that emerge from adjacent Bt fields (Carrière and Tabashnik 2001, Gassmann et al. 2008, Lundgren et al. 2009a). Moreover, natural enemies commonly considered generalists actually vary substantially in their reliance on specific key corn pests (Kurtz et al. 2009, Lundgren et al. 2010). By integrating pests more evenly throughout a landscape, seed mixtures may facilitate the persistence of specialized natural enemies within cornfields better than the current refuge strategy, which localizes pests into one section of the landscape.

Whether seed mixtures are better or worse for natural enemies and biological control depends on how these biotic mortality sources respond to pest densities, which is largely unknown for natural enemies of key pests. Nevertheless, discussions of optimal IRM or IPM decisions are incomplete unless natural enemies are factored into the equation.



### Corn Landscape

Corn insects do not perceive the legal, regulatory, and management boundaries that humans create, and they are attuned to the heterogeneity across variable environmental conditions (Kennedy and Storer 2000). In this section, we treat the heterogeneous landscape as a hierarchical set of factors that causes the type of plant encountered by a pest to differ from one small area to another even when farmers attempt to plant a corn monoculture. This hierarchy consists of toxins, plants, and blocks or plant patches.

**Toxins and Plants.** Toxins are both intentionally and accidentally alternated across a field. Refuges without toxins are planted as part of an intentional strategy, whereas variations in plant growth, soil, fertilizer, and moisture may modify toxin expression in Bt cornfields (Krupke et al. 2009, Lundgren et al. 2009a). Corn hybrids vary across the landscape. In individual plants, toxins can be differentially expressed across tissues (Horner et al. 2003). Maturity of corn plants may influence toxin dose. These and other factors create a mosaic—the simultaneous planting of hybrids expressing different toxins in patches within a small area such as a farm or set of adjacent farms. Traditionally, scientists have urged stakeholders to avoid spatial mosaics of toxins across fields, because simultaneous selection for resistance to all toxins can occur in separate locations (Onstad 2008b).

Another concern identified by Chilcutt and Tabashnik (2004) is the potential contamination of plants due to cross-pollination. The refuge plants could be sprinkled with possibly toxic pollen that could harm targeted and nontarget arthropods. Or each type of corn could be cross-pollinated resulting in kernels that may be more toxic in refuges and possibly less toxic in Bt corn patches. Chilcutt and Tabashnik (2004) studied block refuges, but the issue may be a greater concern in seed mixtures because of the proximity of the two kinds of plants.

The heterogeneity of toxin dose creates a mosaic, but whether the same or different problems for IRM will occur with seed mixtures remains to be determined. Scientists need to be more specific about the potential occurrence of low-dose tissues (pollen and ear tissues). Will these tissues be encountered by pests while feeding? In the future, toxin-expression levels should be documented precisely enough to contribute to scientific IRM. We should not expect toxin dose to be consistent over time and space in patches of Bt corn.

In continuous corn systems, seed left in the field over the winter from the previous years' harvest may germinate, grow, and mix with the crop. Not only are there no mechanisms for controlling these volunteer plants in cornfields (assuming they contain similar genes for herbicide tolerance as the crop), but an unpredictable and varying level of Bt toxin expression in corn plants may affect damage, insect movement and potentially even violate the assumptions of the IRM plan that is the foundation for seed mixtures (Krupke et al. 2009).

**Blocks or Plant Patches.** Since the first introduction of Bt corn, entomologists have been concerned about refuges and blocks of Bt corn differing in quality. The nutritional and environmental conditions encountered by the insects may differ because of the planting of refuge corn in lower quality soils or the planting of different maturity classes in different blocks. Soils and soil moisture vary among patches. Furthermore, the planting of the same corn types at different times, could also produce phenological differences. When soil or foliar insecticides are applied only to refuges, the quality of blocks will differ even more. The quality of refuge is more likely to be the same as that of the Bt corn when the same type of non-Bt seed is included in a seed mixture.

### Pest Behavior

Understanding pest behavior is critical to scientific IRM and IPM (Onstad 2008a). We need to know how adults and larvae move from block to block and plant to plant. Larval feeding and movement essentially occurs within and between plants. However, adult behaviors may be more complex. We need to know the sequence of movement, mating, feeding and egg laying as well as the locations of each. Ultimately, we want to use our knowledge to predict the selection pressure on the population and the production of susceptible insects on the various types of plants.

Traditionally, block refuges have the IRM advantage of minimizing differential selection due to larval movement from toxic to non-Bt plants, but possibly have disadvantages relative to mating of susceptible individuals from refuges with heterozygotes from Bt corn blocks. Seed mixtures are generally thought to permit very good mixing of genotypes as adults but are risky with regard to differential selection during the larval stage (Onstad and Gould 1998). However, we should stress that the strengths or weaknesses for either type of refuge have not been rigorously explored or defined. Furthermore, each species must be considered independently from any historical work on other pests. We should not expect a one-size-fits-all IRM plan to be ideally suited to all pest species. For example, R.L.H.'s recent data (unpublished) suggest that no differential selection between susceptibles and heterozygotes occurs when European corn borer neonates on Bt plants move to non-Bt plants. This observation negates the standard assumption in early, conservative models by Onstad and Gould (1998) and Davis and Onstad (2000), which assumed that homozygous susceptibles and heterozygous individuals survived differently. Likewise, Binning et al. (2010) collected data indicating that differential selection due to movement by neonate western corn rootworms is negligible. With regard to mating, the models of Caprio (2001), Sisterson et al. (2005), and Onstad and Meinke (2010) found that intermediate levels of adult dispersal delayed resistance more than other levels. Thus, pure random mating may not be preferred in all cases.

**Adult Behavior.** Seed mixtures promise to mitigate some of the postulated adult movement problems associated with structured refuge, especially as refuge size shrinks for pyramided hybrids. However, as more observations are made in cornfields, entomologists are realizing that the adult behaviors and their quantitative measurement are much more complex than we ever appreciated (Spencer et al. 2005). This ignorance is epitomized by work on corn rootworm species.

Sorting out the demographics of western corn rootworm populations emerging from refuge and transgenic corn may provide one of the greatest hurdles to IRM. Kang and Krupke (2009a) conducted laboratory studies on western corn rootworm that demonstrated that males averaged less than three matings in the 3 wk after they first mate (far fewer than the 8.2 times reported by Branson et al. 1977), and the majority were no longer competent for mate-seeking beyond those 3 wk. Given the multiweek delay in adult emergence from refuge versus transgenic hybrids in block plantings, these data suggest the realized mating activity between susceptible refuge males and potentially resistant transgenic-field female may be quite low. In a companion study, Kang and Krupke (2009b) found that mating among WCR was not random—female size exhibited a significant positive relationship with male courtship and mating. Murphy et al. (2010) observed more synchrony in beetle emergence from seed mixtures.

J.L.S. (unpublished) has observed pre-mating movement by western corn rootworm females along with low levels of male mating activity. Using different Bt proteins as unique ingestible markers for refuge and insecticidal hybrids (Spencer et al. 2003), J.L.S. (unpublished) observed nonteneral females from refuge mating in transgenic corn. These surprising observations suggest that block-refuge males may be 1) too sparsely distributed to rapidly mate with receptive females as they emerge, 2) refractory to mating (i.e., they have not replenished or acquired sufficient accessory gland materials to deposit a (another) spermatophore in a mating female), or 3) too old to successfully court or mate with females (Kang and Krupke 2009a). Because seed mixtures eliminate spatial isolation, a greater mixing of genotypes and both sexes is inevitable, making it more likely that males from refuge plants encounter receptive females on Bt corn in time to mate.

**Larval Behavior.** Goldstein et al. (2010) observed neonate movement and dispersal behavior of the European corn borer under controlled conditions on Bt and non-Bt corn. With continuous airflow, neonates on a Bt corn plant for 24 h abandoned the plants more frequently than neonates on a non-Bt corn plant. The proportion abandoning Bt corn ranged from 55 to 76%, whereas the range for non-Bt corn was 35 to 42%. Goldstein et al. (2010) concluded that neonates are unable to detect Bt in the corn within 10 min but that they can detect it within the first hour.

Predicting how larval behavior and survival influence IRM is difficult because of the interactions between processes (Onstad 2006). To demonstrate how

plant toxicity, larval movement and larval survival can influence the evolution of resistance with block refuges and seed mixtures, a simulation model of bivoltine European corn borer population dynamics was evaluated. We added the following equations for calculating neonate survival from Onstad and Gould (1998; also used by Davis and Onstad 2000). Let  $T$  be the proportion of Bt corn in a seed mixture and  $E(g, i)$  and  $N(g, i)$  equal the densities of eggs and neonate larvae of genotype  $g$  on plant type  $i$  ( $i = r$  refuge,  $i = b$  Bt corn).

$$\begin{aligned} N(g, r) &= E(g, r) \times (1 - Z) \\ &+ E(g, r) \times Z \times (1 - T) \\ &+ E(g, b) \times V \times (1 - T) \times \text{pdts}(g) \\ N(g, b) &= E(g, b) \times (1 - V) \\ &+ E(g, r) \times Z \times T \\ &+ E(g, b) \times V \times T \end{aligned}$$

Where  $Z$  is the probability of a neonate moving from a refuge plant,  $V$  is the probability of a neonate moving from a toxic plant, and  $\text{pdts}$  is the survival rate due to predispersal tasting of the toxin (Onstad and Gould 1998). We assume that no larvae die during movement and that toxin mortality occurs after neonates settle on each type of corn. Parameter values can be found in Table 1 of Onstad et al. (2002). The single toxin is expressed at a practical-high dose (SS survival of 0.001, RR survival of 1, and RS survival of 0.01). The resistance allele has an initial frequency of 0.001. Predispersal tasting survival ( $\text{pdts}$ ) was varied from 0.5 to 1.0 for homozygous susceptibles (note labels below bars in Fig. 1);  $\text{pdts} = 1$  for all other genotypes. Thus, the scenarios defined in Fig. 1 are the  $\text{pdts}$  values for susceptible larvae and the movement rates ( $Z, V$ ) for all genotypes. Mean dominance of the resistance allele is 0.009 in block refuges and for scenarios A and B with seed mixtures. A dominance value of 0 means resistance is recessive. For the other simulated scenarios, the dominance changes as the parameters change. As usual, the threshold for declaring resistance is 50% resistance allele frequency.

Figure 1 clearly shows that predictions are sensitive to model parameters concerning larval behavior and survival. When there is no larval movement or movement occurs equally among all plants and no differential survival due to tasting occurs among the genotypes (scenario A) 5% and 20% refuges delay resistance 10 and 36 yr, respectively (Fig. 1a and b). Block refuge results are the same (data not shown). When  $\text{pdts} = 1$  and larvae are repelled from Bt corn ( $V = 0.5$ ) but none leave refuge corn ( $Z = 0$ ), resistance evolves in 13 yr for a 5% refuge and 40 yr for a 20% seed mixture (scenario B, Fig. 1a and b). These results show the value of extra larvae being able to find the refuge in a seed mixture, at least when there is no differential selection due to tasting and moving.

In most cases, larval movement hastens resistance evolution when the extra differential selection exists

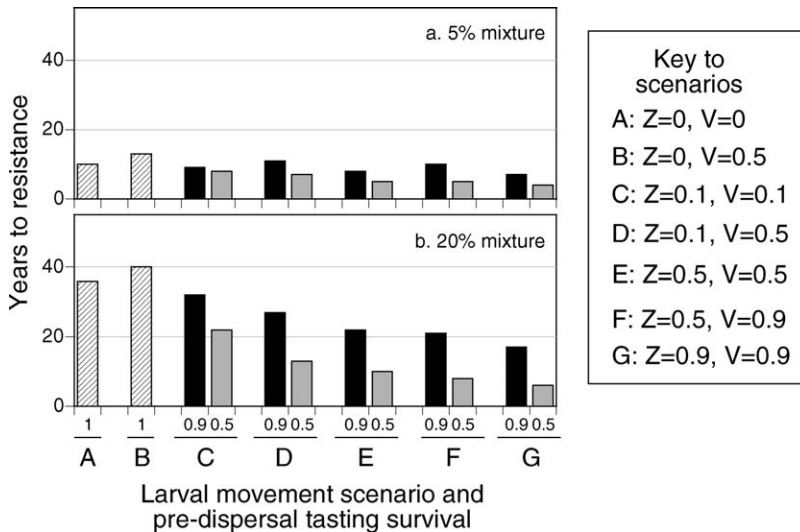


Fig. 1. Influence of three larval movement and survival parameters on evolution of resistance (years to 50% resistance allele frequency) to single-toxin Bt corn with 5% refuge (a) and 20% refuge (b) seed mixtures. Parameters Z and V are probabilities of a neonate moving away from conventional corn plant and Bt corn plant, respectively.

( $pdts < 1$ ) and the heterozygotes have a selective advantage over the homozygous susceptibles. Scenario C with  $pdts = 0.9$  produced results lower than but similar to the benchmark values in scenario A (Fig. 1). Almost all other cases in Fig. 1 have predictions smaller than those for block refuges or seed mixtures without movement (scenario A). The exceptions are scenarios D and F with a 5% seed mixture and with  $pdts = 0.9$  (Fig. 1a); evolution takes  $>10$  yr because the repellency from Bt corn provides the benefit of escape to refuge plants and little differential selection due to the high  $pdts$  value.

Note that when movement from Bt corn plants (V) is held constant, evolution is faster when movement away from non-Bt corn (Z) increases. This can be seen by comparing scenarios D and E or F and G (Fig. 1). This is due to larvae leaving refuge plants in the seed mixture. Thus, in these scenarios, the seed mixture is less effective in producing susceptible insects than is a block refuge because movement of larvae in a block refuge can produce higher numbers of adults. L.L.B. (unpublished) observed only 17 and 20% survival (first and second generation) in the seed mixture compared with the block refuge in his field studies of European corn borer.

As with the pollen contamination study performed by Chilcutt and Tabashnik (2004), W.D.H. et al. (unpublished) conducted a pollen-dispersal trial in Minnesota in 2009 and found that non-Bt ears in a refuge block, as far as 12 m from the Bt field edge were readily compromised with Bt pollen. These studies illustrate the potential for individual non-Bt plants within a Bt field, via seed mixture, to have a high probability of pollen contamination, and variable Bt expression in kernel tissue. Neonate European corn borer, corn earworm, and western bean cutworm are all able to feed initially on non-Bt leaf, silk tissue, or both before

entering the ear. As a second, third or late instar feeding on kernel tissue with variable Bt expression, survival will be much higher than on Bt plants with maximum Bt expression in husk, silk, and kernel tissue. This concern for European corn borer was confirmed in a previous replicated field trial in Minnesota, by using Cry1Ab corn. European corn borer mortality was measured on non-Bt ears that had been pollinated by nearby Bt plants shedding pollen. Naturally occurring infestations of European corn borer larvae on these ears averaged only 50% mortality, compared with 100% mortality on Bt ears fertilized with Bt pollen. Thus, the late instars surviving on these ears are exposed to low-dose concentrations of Bt toxin that could select for heterozygous survivors.

It is clear that we need to learn more about larval behavior. We are targeting this life stage with biotechnology, and yet we know very little about larval behavioral ecology. Can larvae perceive non-Bt and Bt plants or plant tissues before ingestion of tissues? Can they change their behavior to find nontoxic or less-toxic tissues? How is larval behavior affected by larval density? Do isolated refuge plants support more or fewer susceptible larvae than a plant in a block refuge? We also should be aware that there could be selection against larval movement away from non-Bt plants. It is not clear how fast that selection could proceed. This evolution could actually act to lengthen the time to resistance.

#### Human Behavior

Human behavior motivated by human values plays a critical role in pest management and IRM (Mitchell and Onstad 2005, 2008; Hurley and Mitchell 2008). Technology adoption and IRM compliance are particularly relevant when considering how the expan-

sion of current IRM requirements to include seed mixtures will impact the efficacy of IRM for Bt corn. The impact of these two factors will differ regionally, with variation in the frequency and severity of pest infestations and crop productivity and in market forces such as prices for corn and other crops.

A surprising observation is that many farmers do not fully adopt Bt corn in their operations; that is, they planted less than the maximum amount of Bt corn permitted by IRM requirements (Goldberger et al. 2005, Frisvold 2006). This trend has been favorable for IRM because it has meant fewer hectares of Bt corn and hence reduced selection pressure. Besides an increase in hectares planted with Bt hybrids, seeding densities are also increasing, more so for Bt hybrids than for conventional hybrids (Mitchell et al. 2009). This increased seeding density is economical because the increased yield more than compensates for the increased seed costs even with higher seed prices (Stanger and Lauer 2006, Lauer and Stanger 2006). Future research should determine how differential planting densities for conventional and Bt seed affects the risk of resistance evolution.

Expanding IRM requirements to include seed mixtures will encourage even more growers to adopt Bt corn and also encourage growers who have adopted Bt corn to increase their percentage of Bt corn hectares. Research has shown that farmers value convenient and time-saving technologies (Carpenter and Gianessi 2001; Marra et al. 2004; Fernandez-Cornejo et al. 2002, 2005). Because block refuge requirements reduce the convenience of planting Bt corn and increase planning and planting time (e.g., time spent planning how IRM requirements will be met), they discourage adoption of Bt corn. Greater planting of Bt corn promoted by the use of seed mixtures will increase selection pressure and the risk of insect resistance.

Refuges can effectively reduce the risk of insect resistance only if growers comply with them. Initial estimates of grower compliance in 2002 were high (ABSTC 2005; Goldberger et al. 2005) and increased through 2006 (Jaffe 2009). Compliance with IRM requirements, including planting of a structured refuge, has declined remarkably since 2006 and is now up to 9% lower for Bt corn than in 2002 (Jaffe 2009). Based on a 2009 grower survey on compliance in Canada, compliance levels are down to 61% of farmers, where 16% are not even planting a refuge (Canadian Corn Pest Coalition 2009). The reasons for this decline are unknown, but there are logistical and financial considerations associated with a structured refuge. Through the 2010 growing season, registered single-toxin corn planted in the Corn Belt was required to have a 20% structured refuge (until recently, the minimum refuge requirement in cotton growing areas of the United States was 50%). In addition to the inconvenience of planting refuge as blocks or row-strips, many growers perceive that the best agronomics and yield potential are only being included in transgenic corn hybrids, so that "refuge corn" is considered inferior to the transgenic corn in terms of yield potential. Furthermore, traits have the potential to dramatically

increase yield under certain circumstances on their own (Mitchell et al. 2004).

With the U.S. Environmental Protection Agency's recent approval of a new set of IRM requirements for several new stacked corn hybrids, IRM requirements are no longer unified and have become more complex. Given the increasing complexity of IRM requirements, current trends of diminishing compliance rates will probably continue.

Seed mixtures may or may not reduce the general confusion concerning IRM, but they will make compliance more convenient. Although this increased convenience will make it more likely for growers to comply with IRM, a reduction in the risk of resistance is not guaranteed if the trend toward more varied and complex IRM requirements continues. Growers may start to question the appropriateness and fairness of requirements because they do not understand the rationale underlying the differences. If growers raise such questions, they may be less likely to comply (Hurley and Mitchell 2008). For example, to reduce seed costs, growers may create their own seed mixtures using unapproved hybrids (e.g., mixing stacked Bt hybrids and hybrids with a single Bt trait or conventional hybrids and hybrids with a single Bt trait), a practice 29% of South Dakota growers surveyed by Wang and Van der Sluis (2005) reported using. An even more serious possible outcome is that growers may simply choose to ignore IRM requirements for Bt corn that is not approved for seed mixtures.

It is easy to view the expansion of IRM requirements to include seed mixtures as a practical solution to the decreasing trend in IRM compliance. However, it is important to remember that adding seed mixtures to IRM requirements will affect what types of Bt corn are grown, how much Bt corn is grown, and how it is grown, in addition to affecting compliance. For example, if seed mixtures increase both compliance and the total acres of Bt corn planted, in terms of the evolution of resistance, how does this situation compare with planting fewer acres of Bt corn, but with higher levels of noncompliance? The intertwined psychological, sociological, and economic factors driving the seed corn industry and corn grower behavior are complex, dynamic, and warrant further research before their impacts on the evolution of insect resistance can be more fully understood.

## Conclusions

One of the central tenets of IPM is the judicious use of pesticides; that is, pesticides are applied only when warranted. Thus, robust IRM strategies are complemented by good IPM, and both should be considered together (Onstad 2008a). The challenge before entomologists is to not only anticipate the ramifications of refuge implementation for scouting and chemical insecticide use (usually in block refuges) but also attempt to adjust IRM strategies to complement the overall IPM paradigm for targeted pests, nontargeted pests, and diseases. The summary in Table 1 indicates that neither blocks nor mixtures are clearly superior.



For proper decision making, quantitative assessments of important ecological processes are needed to properly weigh their influence relative to the influence of other issues concerning refuge configuration and size. For example, the general trend in Fig. 1 is that increased larval movement and greater differential selection due to tasting by neonates both hasten the evolution of resistance in seed mixtures compared with block refuges. However, note that minimal selection due to tasting or minimal larval movement is not likely to influence IRM.

Furthermore, if entomologists and stakeholders truly want to emphasize IPM and believe that it encompasses and incorporates IRM, then the economics of the system cannot be ignored. In fact, the Food Quality Protection Act of 1996 requires the United States Department of Agriculture and the United States Environmental Protection Agency to promote IPM as part of their mandate. IPM is based on making rational and efficient pest management decisions (e.g., the concept of the economic threshold). We can ensure that these are being made only when we quantify the costs and benefits of IRM strategies over the long-term. This is true whether the focus is on farmer profit in a given growing season or on societal interests over the long-term. To account for economics, pest densities and pest damage will need to be considered, as they typically are in almost all discussions of IPM.

We can learn from previous studies how to efficiently and effectively investigate pests in the current and expected corn landscapes, even if we cannot directly apply conclusions drawn from abstract studies or studies of other species in other landscapes to our efforts to improve IRM and IPM for corn pests. For example, we should not directly apply conclusions from the abstract modeling of Mallet and Porter (1992), as they focused on scenarios in which larvae feed extensively on tissues before moving to another plant. In corn, neonates of many of the primary pest species move from plant to plant before significant feeding has occurred.

However, even when we focus on a single corn pest, reliance on classic assumptions and data sets may not always be ideal. Western corn rootworm in the Corn Belt provides an object lesson. Over recent decades, western corn rootworm populations, management strategies, and the agricultural landscape itself have changed. For example, the eastern Corn Belt is populated with rotation-resistant western corn rootworm that fly amid cornfields where European corn borers are rare, 80% of all corn carries at least one type of transgene, and most seeds are herbicide resistant and protected by one of a very few neonicotinoid seed treatments. The agroecosystem where these organisms live and are under selection has changed dramatically in the last two decades. The problem that regulators face is that the science of protecting transgenic crop technology via IRM is mostly based on western corn rootworm ecology and behavior in a system that has changed substantially.

Finally, stakeholders must face two other problems associated with the production of transgenic insecticidal

corn. First, trends indicate that the pool of nontransgenic hybrid available to growers will shrink in the future. We expect that growers will have fewer choices in what hybrids they grow in their fields. Therefore, from the perspective of overall IPM, we are concerned that seed companies will provide fewer options for regional needs, secondary pests, disease control, and refuge plantings. For example, will adequate supplies of non-Bt corn seed be available for areas of low pest density or if many farmers must replant their block refuges due to flooding or where corn is double cropped for silage production? Will the available non-Bt hybrids be appropriate for the region and be qualitatively similar to the Bt corn block after replanting? Secondly, we question whether pyramided toxins will actually increase mortality in targeted pests. Without this increase in mortality through independent activity of each toxin, the pyramid has much less value for IRM (Onstad and Meinke 2010). The U.S. Environmental Protection Agency recently acknowledged that a corn hybrid pyramided with two toxins active against corn rootworms does not significantly increase larval mortality (USEPA 2009). Both of these issues directly challenge our assumptions about the supposed simplicity and ease of implementation of IRM plans.

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