FORUM

Field-Evolved Resistance: Assessing the Problem and Ways to Move Forward

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ABSTRACT "Field-evolved resistance" is defined as a "genetically based decrease in susceptibility of a population to a toxin caused by exposure to the toxin in the field." The key component of "field-evolved" resistance is that it does confer decreased susceptibility to an insecticide in the field. Another key component is that the decrease in susceptibility to the insecticide is because of previous exposure of the target insect to the toxin in the field. Several studies have reported field-evolved resistance to crops engineered to express proteins from the bacterium, *Bacillus thuringiensis* (Bt). However, there has not been a consistent standard in the application of the definition of field-evolved resistance for Bt crops. The inconsistency in applying the definition arises from differences in the methods used to detect resistance, the ecology of the interaction between the pest and the Bt crop, and the effective dose the pest encounters while feeding on the Bt crop. Using case studies of reported resistance to Bt crops, it is demonstrated resistance does not come in a single form, and that in most cases, resistance can still be managed.

KEY WORDS insect resistance management, field-evolved resistance, *Bacillus thuringiensis*, refuge

The National Research Council (NRC 1986) defined insecticide resistance as "genetically heritable changes in a population causing a reduction in susceptibility to a specific insecticide." However, the language in the NRC definition of resistance can be used to describe a range of situations from changes in susceptibility measured only in the laboratory to complete product failure in the field. Tabashnik et al. (2009) extended the NRC definition by introducing the term "field-evolved resistance" as a "genetically based decrease in susceptibility of a population to a toxin caused by exposure to the toxin in the field." Although it does not necessarily imply loss of economic efficacy in the field, the key component of field-evolved resistance is that it does confer decreased susceptibility to toxins (whether they be conventional insecticides or proteins from the bacterium, *Bacillus thuringiensis* [Bt proteins]) as encountered in the field. Another key component is that the decrease in susceptibility to the toxin is because of previous exposure of the target insect to the toxin in the field. In addition, inter-mating of resistant individuals is necessary to transmit resistance to future generations and cause the resistance to spread. Although these key components of field-evolved resistance are in agreement and extend the NRC (1986) definition, the application of the definition is difficult in practice, and may lead to ambiguous interpretations of the scope of the problem. The difficulty is because of demonstrating that changes in susceptibility are related to exposure of populations to the insecticide or Bt crop. Also problematic is the generic use of the term "population" in the resistance literature. Studies of resistance have used the term to describe individuals from the field collected for measurement in the laboratory (Mahon et al. 2007; Downes et al. 2010a,b; Zhang et al. 2011; Wan et al. 2012), a group of surviving individuals observed in the field (Van Rensburg 2007), or a group of interbreeding individuals (Storer et al. 2010). The latter use of population is what is implied by the field-evolved resistance definition, but in application of the definition, it has been the first use of population that has been most reported.

Before the availability of crops engineered to express Bt proteins, many insect pests had a history of evolving resistance to all of the major classes of conventional insecticides. Resistance evolution remains the major risk to the benefits provided by Bt crops (Gould 1998; Caprio and Sumerford 2007; Tabashnik et al. 2008, 2009). However, there are several key differences between Bt crops and conventional insecticides that directly affect the intensity of selection for resistance in an insect population. With conventional insecticides, resistance evolves via repeated applications of insecticides with increasing doses as efficacy declines because of less susceptibility in pest populations. Bt-expressing crops have an upper limit as to the dose that pests encounter, but the exposure is con-

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tinuous. Because of the continuous expression, the history of resistance evolution to conventional insecticides, and the benefits of Bt crops to the environment, the Environmental Protection Agency mandates insect resistance management (IRM) strategies for all Bt crops registered. Proactive IRM has resulted in the establishment of baseline susceptibilities to Bt proteins and conventional insecticides; one consequence of an established baseline is that resistance is more likely to be based on a laboratory-measured deviation from the baseline. Resistance to conventional insecticides is more easily confirmed based on poor efficacy in the field and increasing frequencies and doses of active ingredient necessary to control the target pest. Unlike the reactive resistance management for conventional pesticides in previous generations, current resistance management and monitoring practices make it possible to detect changes in susceptibilities before the complete loss of their efficacy. For Bt proteins, one major problem in doing so is defining resistance in a manner that results in responses to changes in susceptibility that are relevant to field performance.

Current use of the term field-evolved resistance has several drawbacks. The field-evolved resistance definition poorly addresses the magnitude of resistance (temporally or spatially), the extent of changes in Bt product efficacy, the containment of the resistance, and often relies on inferences based on laboratory measures; the inferences rarely include the pest ecology, especially, how the interaction between the pest and the Bt product shape the selection pressure that may or may not result in changes in susceptibilities. After >15 yr since Bt crops were commercially introduced, there have been few reported cases of sustained insect-control failures in the field. Cited cases of resistance to Bt crops result from two sources of evidence: 1) changes in susceptibility of pest collections from the fields measured by laboratory bioassays (Ali et al. 2006; Mahon et al. 2007; Downes et al. 2010a,b; Zhang et al. 2011; Wan et al. 2012); and 2) collections of individual insects surviving after having fed on the Bt crop in the field (Van Rensburg 2007, Gassmann et al. 2011, Kruger et al. 2011, Gassmann 2012), including loss of efficacy of the Bt product and withdrawal of the product in some locations (Storer et al. 2010, 2012). These two sources of evidence for resistance will be discussed in the context of the ecological and genetic factors that impact the intensity of selection pressure for Bt resistance. For each source, the challenges of applying the field-evolved resistance definition will also be discussed. Case studies of Bt resistance will be examined and discussed in light of the field-evolved resistance definition in its current use. The ultimate goal of this discussion is to move definitions of resistance toward more comprehensive assessments (laboratory and ecological) of all the factors impacting resistance evolution, without compromising the proactive need for detecting resistance in sufficient time for remediation. Of importance is the conclusion that detection of resistant individuals in the field or laboratory is not the equivalent to claiming field-evolved resistance; further analyses are necessary to demonstrate genetic changes in susceptibility directly because of the insecticide.

Sources of Observed Resistance

For documenting field-evolved resistance to be of value to IRM, it must occur in a timely manner, but also have relevance to true changes in field susceptibility caused by the Bt protein. The two forms of resistance most often reported are as follows: 1) changes in the performance, as measured in the laboratory, of field-collected insects; and 2) field collections of living individuals from a Bt crop, ideally, with subsequent confirmation through bioassays. These forms of "resistance" vary in how relevant they are with respect to the time required for their detection, whether they are truly capturing alleles with field significance, and the ability to confirm a causal link between field exposure to the Bt toxin and a genetically based decrease in susceptibility by a population to the toxin.

Changes in the Susceptibility of Insect Samples Measured in the Laboratory. Most of the reports of Bt resistance evolution are in the form of temporal changes in the susceptibility of insect samples measured by a laboratory bioassay (Ali et al. 2006, Tabashnik et al. 2008, Zhang et al. 2011, Wan et al. 2012). Rarely do these reports present follow-up studies to confirm that the laboratory-measured changes in susceptibilities have any relationship to the genetic ability of individuals to survive on Bt crops. We are not suggesting that observed changes should be ignored, but that further work is necessary to determine the field relevance of each reported case.

Laboratory measures of resistance have the potential to detect resistance early; their inability to directly correlate to performance of the Bt crop is a major limitation. Routine resistance monitoring programs are usually started before the commercial introduction of the Bt protein. The components of a routine monitoring program include a baseline of susceptibility during the several years before the Bt protein is introduced to the market, and a bioassay (dose–response or diagnostic dose) to quantify susceptibility within and across populations. This approach allows for the possibility of observing a shift in susceptibility from the baseline early. However, there may not be enough information at the onset to determine what the bioassay is actually measuring with respect to field relevance (Moar et al. 2008). The greatest challenge is how to relate bioassay results measuring tolerance (survival, larval development, or both) of the pest to the protein, with the protein expression the pest encounters under typical field conditions (see Moar et al. 2008 and Tabashnik et al. 2008 for a discussion of the technical issues associated with interpreting dose–response data and Caprio and Sumerford 2007 for technical discussion of diagnostic dose).

Field Collections of Individuals Surviving on the Bt Crop. On occasion, Bt crops have not controlled targeted insects as expected even though the product involved has continued to provide value (Van Rens-

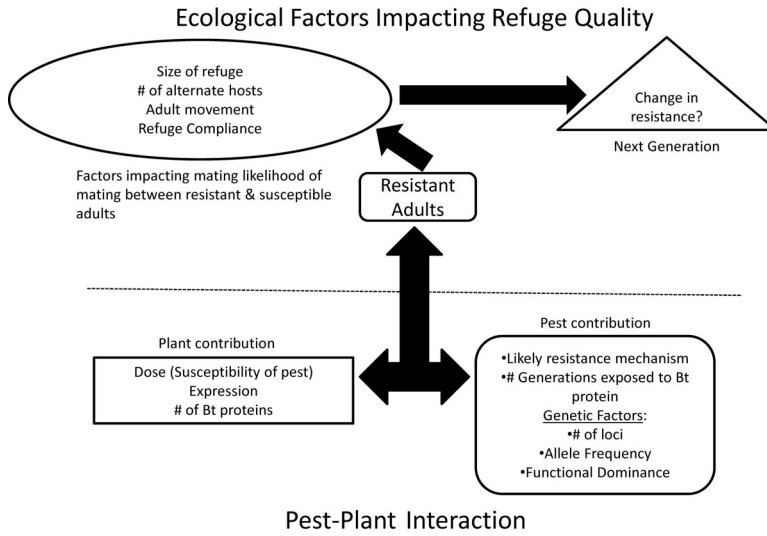

Fig. 1. Variables impacting the evolution of resistance to Bt crops. Variables below horizontal line affect the intensity of selection for surviving on the Bt crop. Above the horizontal line are the ecological variables that impact changes in allelic frequencies as mediated by mating patterns among resistant genotypes.

burg 2007, Kruger et al. 2011). Observations like these are often thought to be an early warning before the occurrence of a complete product failure; however, complete loss of efficacy (deviation in the ability of a Bt crop to control a pest relative to its baseline percent control) may also be confirmed with field collections (Storer et al. 2010). Observations of changes in the efficacy of a moderate-dose product may arise without resistance evolution; unusual sizes of pest populations may cause observable damage to the crop without a genetic change in susceptibility. In situations involving loss of efficacy, it is usually unclear whether resistance alleles have increased in the pest populations. The nontrivial confirmation of a heritable basis for the observed survival of individuals is needed, as with the laboratory-measured resistance.

Confirmation of Field-Evolved Resistance

The obvious criteria for concluding that field-evolved resistance to a Bt plant is occurring include a demonstration of a genetic basis for lower susceptibility to the Bt protein as well as to the product that is expressing it. However, any inferences about resistance evolution must consider all of the variables that affect the increase and spread of resistance alleles (Fig. 1; Table 1). Figure 1 illustrates that it is not only the interaction between the pest and the Bt crop that will determine whether resistance becomes more prevalent in the field, but also the ecological variables that will influence how well the refuge delays resistance. For Bt plants, the expression of Bt proteins relative to the pest's susceptibility is the primary determinant of the likely path toward resistance evolution, and therefore directly impacts the inheritance of the resistance trait (Table 2; Heckel 1994; Tabashnik 1994; Gould 1998; Tabashnik et al. 2004, 2008, 2009; Tabashnik and Gould 2012). For example, pests that exhibit a greater susceptibility to the Bt toxin are likely to require a resistance allele with a major effect (Heckel 1994). Such alleles would not be expected to be detected in a gradual manner over time. In contrast, pests that are more tolerant of a Bt protein may exhibit gradual changes in susceptibility.

Table 1. Comparison of variables impacting resistance traits and laboratory bioassays for insect species exhibiting greater and lower susceptibility to Bt products

Impact variable	Greater susceptibility	Lower susceptibility
No. of resistance loci	One or few	More quantitative
Inheritance	Recessive	Mostly additive
Initial allelic frequency	Rare	Rare to moderate
Baseline LC_{50}	Lower, little variance among populations	Higher, with greater variability among and within populations
Variability	Small owing to rarity of resistance alleles; smaller impact of environmental factors on phenotype	Greater impact of environmental effects on phenotype; higher allelic frequencies
Change in dose–response	Easy to detect; may be abrupt with large effect	More gradual, likely with more ambiguous results
Diagnostic concentration	More easy to define	May need more than one
Greatest impediments to response to selection	Low allelic frequencies; recessive gene action, refuge	Ecological barriers, fitness costs associated with resistance alleles, refuge

Table 2. Case studies of Bt resistance, including important variables (source for measuring resistance, economic significance of resistance, survival on protein, survival on plant expressing Bt protein, and evidence of field damage to Bt product) for assessing field-evolved resistance (strength of evidence: weak [−] to very strong [++]; ± = inconclusive)

Insect, location	Bt product	Source	Economic significance	Field-evolved resistance criteria			
				Protein	Plant	Field	Relationship between exposure and decreased susceptibility
Spodoptera frugiperda[a], Puerto Rico	Cry1F/maize	Field change	+	+	++	++	++
Pectinophora gossypiella[b], India	Cry1Ac/cotton	Field change	−	+	++	++	++
Busseola fusca[c], South Africa	Cry1Ab/maize	Field change	+	+	−	−	+
Diabrotica virgifera virgifera[d], United States	Cry3Bb1/maize	Field change	+	−	+	+	+
Helicoverpa armigera[e], Australia	Cry1Ac and Cry2Ab/cotton	Laboratory	−	+	−	−	−
H. punctigera[f], Australia	Cry1Ac and Cry2Ab/cotton	Laboratory	−	+	−	−	−
H. zea[g], United States	Cry1Ac, cotton	Laboratory	−	±	−	−	−
P. gossypiella[h], China	Cry1Ac, cotton	Laboratory	−	±	−	−	−

[a] Storer et al. 2010, 2012.
[b] Dhurua and Gujar 2011.
[c] Van Rensburg 2007, Kruger et al. 2011.
[d] Gassmann et al. 2011, 2012.
[e] Downes et al. 2007, Mahon et al. 2007, Mahon and Olsen 2009.
[f] Downes et al. 2007, 2009, 2010a,b.
[g] Ali et al. 2006, Tabashnik et al. 2008.
[h] Wan et al. 2012.

Dosing: High Versus Less-Than-High Dose and Pest Susceptibility. Heckel (1994) and Bourguet et al. (2000) discussed the relationship between the dose expressed by the Bt crop and the most likely resistance mechanism that will evolve in the insect. When a high dose is present, resistance is more likely to be a consequence of a reduction in binding affinities for larval-midgut receptors and more likely to be inherited in a monogenic recessive manner. In contrast, proteolytic mechanisms of resistance, exhibiting a more additive and quantitative inheritance, are more likely when the Bt expression of the crop creates a less-than-high dose scenario. Behavioral mechanisms of resistance are also possible when the dose of Bt is more moderate. As a consequence, a greater range of possible outcomes for resistance are expected in a lower-dose situation (Caprio and Sumerford 2007).

The frequency of resistance alleles are expected to be rare when Bt doses are high and likely to exhibit recessive inheritance. Although resistance alleles may be rare at moderate doses, there may be a greater likelihood of nonrecessive inheritance (Heckel 1994). When insects encounter high doses, the scarcity of resistance alleles and recessive inheritance makes their early detection extremely difficult because of the number of field-collected individuals necessary to sample the resistance alleles (Roush and Miller 1986). However, if it is detected, it may appear more abruptly and may be of greater significance and field relevance. Many baseline studies of susceptibility for pests that are not highly susceptible to individual Bt proteins report great variation among populations in LC_{50} values (Sims et al. 1986, Luttrell et al. 1999, Ali et al. 2006). Because of the greater variation in susceptibility, laboratory measures in a lower-dose system are often ambiguous. Temporal variation may be because of changes in laboratory methods (intended, otherwise, or both) (Moar et al. 2008), sampling variance within and among populations (Sumerford et al. 2010), or poor correspondence between survival during the short duration of a laboratory bioassay and completion of the larval stage on Bt crops under field conditions.

Implications for Application of Field-Evolved Resistance to Bt Crops. Interpreting measures of susceptibility should be dependent on the nature of the Bt product. Observing surviving individuals on Bt crops or detecting shifts in laboratory measures of susceptibility is not sufficient to document resistance. Resistance evolution will occur only if individuals complete their life cycles after exposure to Bt proteins and transmit alleles conferring greater fitness on the Bt crop to their progeny. For generational increases of resistant individuals to be confirmed, it is therefore necessary to correlate observed changes in measures of susceptibility (laboratory and field efficacy) with the ability of individuals to complete their life cycle on the Bt product, mate with other genetically resistant individuals, and therefore transmit resistance alleles to their offspring. The short duration of laboratory measures (protein and plant) of resistance do not directly lend themselves to the assessment of this type of response. In addition, when insect species are less susceptible to a Bt protein, one would expect to observe some short-term survival on diets containing Bt proteins in the laboratory, as well as on plants expressing the Bt protein in the field and the laboratory. At some point, it is necessary to demonstrate that individuals become reproductive adults after exposure to the protein.

If the goal is to not only detect resistance but more importantly show a causal relationship between field exposure to the toxin and a decrease in susceptibility

by a population, the likelihood that resistance evolution produced the resistant phenotypes must be measured to determine whether that is a reasonable conclusion based on the variables in Table 1 and Fig. 1. The greatest impediments for high-dose products are recessive inheritance and the rare frequency of resistance alleles, thus allowing the refuge to be more effective at a given size relative to a moderate dose. As a consequence, we would not expect to observe gradual shifts in observations of resistance measured with products presenting a high dose to the target pest. In contrast, moderate-dose situations allow for the possibility of evolution to occur in small shifts, but there is also a greater role for ecological impediments to slow the increase of resistance alleles (Gould 1998). Fitness costs may also play an important role in delaying resistance evolution (Gassmann et al. 2009). Another important variable is the expression in the Bt plant of one or more additional Bt proteins. For a well-designed product with Bt protein pyramids, a single protein will be as effective against resistant genotypes for the other proteins expressed in that Bt product as they are for susceptible genotypes.

The Economic Perspective for Resistance Detection. The economic relationship between the insect pest and the Bt crop is a variable not usually considered in the interpretation of resistance detection and its consequences. Claims of resistance may trigger unnecessary resistance remediation and regulatory decisions if not discussed in an appropriate context. For example, the present Bt crops were developed to target major pests of corn and cotton in different geographies. However, additional benefits from the Bt proteins were found in the form of control of many secondary pests of lesser economic importance. Altering resistance management strategies or removing products from the market because of resistance in pests of lesser economic importance would impact the yield protection that Bt products offer to growers, and may also remove the environmental benefits of Bt products as a consequence of the need for more sprays of conventional insecticides.

Case Studies of Reported Sources of Resistant Populations

Several cases are reported in the literature as examples of field-evolved resistance to Bt (Table 1). In the following paragraphs, the cases are evaluated to determine whether they meet the following standards to be designated as "field-evolved" resistance: 1) Is there a change in susceptibility to the Bt protein?; 2) Does it correlate with the selection pressure exerted by the Bt product?; 3) Will individuals survive and complete their life cycle on the plant?; and 4) Is there an effect on the efficacy of a Bt crop?

Fall Armyworm—Puerto Rico: Strong Evidence of Field-Evolved Resistance. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), feeding on Cry1 F-expressing corn in Puerto Rico is an example of an undisputed case of field-evolved resistance (Storer et al. 2010, 2012). Not only was significant economic damage observed, resistance was further documented in laboratory dose–response studies (Storer et al. 2010, 2012). Inheritance studies documented that resistant populations collected from Cry1-F-expressing maize produced resistant and mostly susceptible offspring when mated with resistant and completely susceptible populations, respectively. Four years after the initial collections of resistant populations from the field, Storer et al. (2012) confirmed that resistance was still prevalent in Puerto Rico. There are several factors that favored resistance evolution in Puerto Rico. First, there is the opportunity for multiple generations of selection per crop cycle in Puerto Rico (Storer et al. 2012). Corn is planted year-round in Puerto Rico; Storer et al. (2012) estimate that ≈30–40 generations of fall armyworm occurred before resistance was observed, and that it is very likely that all of these generations were exposed to selection pressure for Cry1 F resistance. Second, being on an island, it is likely that fewer susceptible migrants were available to mate with Cry1 F survivors. The commercial response was to remove the product from the local (Puerto Rico) marketplace as soon as resistance was confirmed.

Pink Bollworm—India: Strong Evidence of Field-Evolved Resistance. Although *Pectinophora gossypiella* (Saunders) is highly susceptible to Cry1Ac, field-evolved resistance to cotton expressing only Cry1Ac has been documented in the state of Gujarat, India (Dhurua and Gujar 2011). Greater than expected damage to bolls of Bollgard cotton was reported during 2008 (one field with 90% of bolls infested) and 2009 (53 fields tested, 44 with ≥fourth instars). Laboratory bioassays of 2009 populations found a median of 70% survival at a diagnostic concentration ≈500× the LC_{50} of susceptible populations. During 2010, resistance also was detected in populations collected from non-Bt cotton. The reasons for resistance evolution likely were the monophagous nature of pink bollworm (it only feeds on plants of Malvaceae, and almost exclusively on cotton in most locations), insufficient compliance with refuge requirements, and significant plantings of unapproved Bt cotton grown in India before and after official approval (unapproved hybrids may have had lower protein expression levels with no refuge seed provided).

Resistance to Cry1Ac in populations of pink bollworm has not led to the removal of Bt cotton from the Indian marketplace because Bollgard II cotton remains effective in the control of pink bollworms because of the lack of cross-resistance between Cry1Ac and Cry2Ab and even Bollgard cotton remains effective against *Helicoverpa armigera* (Hübner), its primary target. Nevertheless, adjustments to the resistance management strategy for Bt cotton in India are being considered. Replacement of Bollgard cotton with Bollgard II cotton remains the best solution for the control of Cry1Ac-resistant pink bollworms, and resistance has remained localized since 2010. However, in areas where Cry1Ac resistance has been documented, Bollgard II will be a single-gene product for pink bollworm. Stewardship efforts to better educate growers as to the importance of refuge have been

implemented to improve compliance. Refuge seed mixed in the bag with Bollgard II seed is also an attractive resistance management plan to ensure grower compliance. Pyramids involving new Bt proteins, especially those with independent modes of action, will also aid in the management of Cry1Ac-resistant pink bollworms.

***Busseola fusca*—South Africa: Evidence of Field-Evolved Resistance.** The African stem borer, *Busseola fusca* (Fuller) is a major pest of maize in South Africa. Cry1Ab-expressing maize is a less-than-high dose for *B. fusca.* Van Rensburg (2007) reported severe damage caused by the African stem borer to Bt corn hybrids containing the event MON 810 that produce the Cry1Ab protein. The survival of progeny of diapausing larvae collected from both a Bt and non-Bt planting were compared when feeding on various Bt and non-Bt hybrids. Using field and greenhouse grown plants artificially infested with neonate larvae, Van Rensburg (2007) observed most larvae from the non-Bt-derived population surviving to at most the eighth day versus substantial numbers of larvae of the Bt-derived population surviving over the entire trial period (23 d). However, in subsequent research (Kruger et al. 2011), median times to mortality (LT_{50}) for the progenies from refuge- and Bt-maize collected in the Vaalarts area were not significantly different in a greenhouse study (3.41 vs 3.27 d for refuge- and Bt-collected, respectively). In a laboratory study, there was a slight increase in the LT_{50} for the progeny of the Bt-collected colony versus the progeny of the refuge-collected colony (8.96 vs. 6.84 d, respectively); the resistance to Cry1Ab therefore appears to be heritable, but weak, and consistent with expectations of resistance evolution in a nonhigh dose scenario. The observed change in susceptibility of *B. fusca* to Cry1Ab was because of poor management of the refuge corn. *B. fusca* larvae are vulnerable to low humidity; growers are reluctant to irrigate refuges, and rely on rain-fed plantings near irrigated Bt corn to maintain humidity in their refuge. During periods of low rainfall, the production *B. fusca* populations are negatively impacted, resulting in too few adults.

MON 810 remains on the market in South Africa. The studies described above indicate the Cry1Ab resistance in *B. fusca* is relatively weak; MON 810 still provides economic value to South African growers. Pyramided corn, MON 89034, expressing Cry1A.105 and Cry2Ab2, is replacing products expressing only Cry1Ab. Populations of resistant *B. fusca* are also managed by pesticide oversprays. Stewardship efforts have improved refuge management and compliance. The feasibility of seed mixes is also being measured. Cry1Ab resistance in *B. fusca* can therefore be managed.

Western corn rootworm—Iowa, United States: Evidence of Field-Evolved Resistance. Western corn rootworm is the major pest of maize in the mid-western United States (Gray et al. 2009). Corn hybrids expressing single Bt proteins do not exhibit a high-dose scenario for western corn rootworm and Cry3Bb1-expressing corn, MON 863 and subsequently MON 88017, has been grown since 2003. Gassmann et al. (2011) reported findings from four fields in Iowa identified by farmers as having severe rootworm feeding injury to Cry3Bb1-expressing maize. Laboratory bioassays revealed that progeny from these collections displayed significantly higher survival on Cry3Bb1 maize in laboratory bioassays than did the progeny of western corn rootworm from fields exhibiting little feeding damage (survival from problem fields was three times greater than for western corn rootworm from fields not associated with feeding injury to Cry3Bb1 maize) and that the inheritance of survival was incomplete (Gassmann et al. 2011). The four fields where putative resistant populations were collected also had a history of several years of continuous use of Cry3Bb1-expressing maize, suggesting a relationship between exposure to the Bt product and a change in the susceptibility of these western corn rootworm populations. What is currently unknown is whether the change in susceptibility is widespread and to what extent inadequacy of current refuge recommendations (Tabashnik and Gould 2012) and poor compliance with refuge recommendations are causes. Surveys of locations reporting field-performance concerns with MON 88017 indicate that continuous corn-on-corn production has created an opportunity for localized selection with a single-protein event that does not produce a high dose.

Products containing pyramided Bt proteins (i.e., SmartStax, Cry3Bb1, and Cry34/35) effectively control the problem populations Gassmann et al. isolated from Iowa. The registration of Cry3Bb1-containing products, including SmartStax, with seed-mix refuge will substantially improve refuge compliance. Educating growers of the value of improved cultural practices such as crop rotation also will likely mitigate resistance evolution.

***Helicoverpa spp.*—Australia: Poor Evidence of Field-Evolved Resistance.** *Helicoverpa spp.* are common agricultural pests around the world. They generally exhibit relatively low susceptibility to Bt proteins. Australia has a very aggressive and proactive resistance monitoring program for the Bt proteins expressed in Bollgard II (Downes et al. 2007, 2009, 2010a, 2010b; Mahon et al. 2007, Mahon and Olsen 2009) cotton varieties. The species monitored are *H. armigera* and *Helicoverpa punctigera.* F_1 and F_2 screens revealed unexpectedly high frequencies of alleles conferring resistance to Cry2Ab2 (Mahon et al. 2007; Downes et al. 2009, 2010a) before the registration of Bollgard II cotton. However, there was no within-season increase in the frequency of these resistance alleles in subsequent years nor has there been any observed change in field performance of Bollgard II across years. Characterization of the inheritance of resistance as measured by the laboratory bioassay was determined to be monogenic and very close to completely recessive for both species (Mahon et al. 2007, Downes et al. 2010a,b). Mahon and Olsen (2009) also reported limited survival of Cry2Ab2-resistant, laboratory-selected strain of *H. armigera*-fed Bollgard II cotton.

It is likely that for *Helicoverpa* in Australia, a combination of pyramided proteins, cotton refuges (structured and natural), and other ecological variables and fitness costs has prevented resistant phenotypes from occurring at damaging frequencies in the field. Tabashnik et al. (2008) correctly argue that Australian populations of *Helicoverpa* do not meet the criteria to demonstrate field-evolved resistance. Although there were resistance alleles present before exposure of populations to these Bt proteins, there is no evidence that current allelic frequencies in populations of these species has increased because of selection by Bollgard cotton.

***Helicoverpa zea*—United States: Poor Evidence of Field-Evolved Resistance.** Early resistance monitoring of *Helicocverpa zea* (Boddie) revealed great variation among populations in their susceptibility to Cry1Ac and Cry2Ab (Luttrell et al. 1999, Luttrell and Jackson 2012). The expression of individual proteins in Bt cottons targeting *H. zea* does not represent a "high dose" scenario. A retrospective by Tabashnik et al. (2008), using LC_{50} data from Ali et al. (2006), claimed field-evolved resistance to Cry1Ac, and cited some strikingly elevated LC_{50} values, mostly from populations collected from non-Bt crops. However, industry monitoring during 2008–2011 failed to detect strains with susceptibility outside of the expected range (100% of collections produced no surviving individuals at the diagnostic dose of 30 $\mu g/cm^2$ Cry1Ac, $N =$ 100 populations). Ali et al. (2006) reported that relationships between bioassays of 2008 field strains and survival on plant tissue assays "were limited and unclear." However, to date there have been no consistent reports of greater-than-expected damage in cotton for consecutive years, either across the U.S. cotton belt or at specific locations.

In a summary of the history of resistance monitoring and management of *H. zea* infesting U.S. cotton, Luttrell and Jackson (2012) argue that "conclusions about resistance evolution based solely on bioassay responses of field-collected bollworms does not appear to be sufficient to explain all influences on field activity of Bt cottons." Several factors argue against a change in susceptibility of *H. zea* to Cry1Ac-expressing cotton. Moar et al. (2008) argue that the resistance ratios assessed by Tabashnik et al. (2008) were elevated because of the poor quality of the susceptible, comparator population that had been maintained in the laboratory for several years. Collections from Bt crops are also biased samples. LC_{50} estimates from these collections are not representative of the range of susceptibility to Cry1Ac, and also do not measure the impact of the refuge. Head et al. (2010) have demonstrated a sufficient natural refuge to delay resistance evolution in U.S. cotton. If larvae surviving on Bt cotton emerge as adults, matings with adults from the refuge will likely delay resistance.

Pink Bollworm—China: Poor Evidence of Field-Evolved Resistance. Although no problem populations of pink bollworm have been reported, Wan et al. (2012) report a less than twofold increase in the median LC_{50} values (micrograms per milliliter) for Cry1Ac measured in populations of pink bollworm in China. It is argued that because the percentage of Cry1Ac-expressing cotton has increased during the years of the study, the decrease in susceptibility is correlated with exposure to the Bt cotton. Survivors from diet bioassay work were used to initiate laboratory selection for Cry1Ac resistance. During the F_{11} generation, the selected colony exhibited only 2.1% survival on Bt cotton bolls after 21 d of exposure in the laboratory (vs. 56.2% survival on non-Bt bolls). It is not likely that any adults would have emerged under typical field conditions. Resistance evolution for a high-dose scenario is expected to cause a major shift in susceptibility to the Bt protein (e.g., the Cry1Ac resistance in pink bollworm from India). Considering that Cry1Ac-resistant pink bollworm from India can complete larval development on Cry1Ac-expressing cotton, and the lack of required refuges in China, a twofold increase in LC_{50} values is not a likely response for this high-dose scenario.

Discussion

Although the need for a timely detection of evolving resistance is critical, there is a scientific obligation to interpret data in the context of the pest–crop ecology, as it may influence the evolutionary response to Bt in the field. What is argued for in this manuscript is greater rigor when examining measures of resistance that are based on biological and operational details of the pest–Bt product interaction. The necessary minimum criteria to confirm resistance in the field include the following: 1) proving a heritable basis for the shift in susceptibility; and 2) the ability of individuals possessing the alleles causing the shift in susceptibility to complete their development on plants expressing the toxin to transmit resistance alleles to their offspring. The purpose of presenting the case studies is to emphasize the difficulty in relating laboratory quantifications of resistance to evidence of field-induced changes in susceptibility that improves the ability of a pest to survive on the Bt product in the field. In particular, laboratory assays that show a shift are not sufficient evidence of "field-evolved" resistance but rather should be a warning of the potential for resistance evolving in the field. For example, if a statistically significant increase in resistance allele frequency is documented in lab assays, but no survivors are obtained on the Bt plant tissue and product performance has not changed in the field, it is not reasonable or useful to conclude there is field-evolved resistance.

The four case studies detailed in Table 2 (*S. frugiperda*, *P. gossypiella* in India, *B. fusca*, and *D. virgifera virgifera*) where it was concluded that available data support the conclusion of field-evolved resistance have the following in common: reasonable evidence of increased adult production when larvae fed on the Bt plant, evidence of increased feeding damage in an agronomic situation, and a lack of detection by routine resistance monitoring before evidence of damaged fields. It can also be argued that in each of these cases, there was potential for geographic isolation. Although

fall armyworm adults have great mobility, the reported case of resistance occurred on an island with minimal refuge to produce susceptible adults. In contrast, pink bollworm and western corn rootworm adults exhibit limited mobility, thereby mimicking, to some extent, the isolating "island effect." The lack of compliance with refuge recommendations generated a perfect storm for the evolution of resistance. Laboratory studies after the collections from problem fields also confirmed the resistance. The substantiated reports of field-evolved resistance indicate that the resistance can be managed. The strategies to manage the resistance include the replacement of single-gene products, with crops expressing multiple Bt proteins, improvement in refuge strategies, and changes in management practices that slow the spread of resistance.

In contrast, studies in Table 2 that did not meet the proposed standards for field-evolved resistance (*H. armigera*, *H. punctigera*, *H. zea*, and *P. gossypiella* in China) as defined in this article all have the same limitation: there is little evidence of increased adult production and sustained increasing feeding damage per larva. Several of the articles imply an understanding of the need to provide evidence of adults to support the idea that field populations responded to selection with the Bt crop. Downes et al. (2009) and Wan et al. (2012) use market penetration of the Bt crop as a proxy for the intensity of selection, in an attempt to correlate selection pressure with changes in laboratory measures of susceptibility. Without evidence of completion of the pest's life cycle on the Bt crop, these types of analyses are only correlative and potentially misleading. As mentioned, there are alternative explanations to changing measures of susceptibility, especially in the case of moderate doses of the toxin (Moar et al. 2008).

Laboratory bioassays are problematic as the only evidence of field-evolved resistance. The case studies in Table 2 not providing strong evidence for field-evolved resistance are all moderately susceptible to the Bt proteins to which they are putatively resistant. Genetic mechanisms that allow an individual to survive the short duration of a laboratory bioassay are expected to be relatively common. The aggressive resistance monitoring in Australia for *Helicoverpa spp.* has isolated several families that exhibit tolerance of Cry2Ab. However, there has been no significant increase in the frequencies of resistance alleles within populations of *Helicoverpa spp.* In tests using field-grown cotton, all larvae from a resistant colony of *H. armigera* were dead by the end of the second week of exposure to BGII plants (Mahon et al. 2009).

The largest missing piece in the definition of field-evolved resistance is: "Where do we go from here?" As commonly applied, the term field-evolved resistance implies an endpoint. Several follow-up issues are usually not considered: geographic scope of the problem and is it likely to disperse to other populations, the potential for remediation to delay further resistance evolution, and whether susceptibility remains in populations from regions where field-evolved resistance has been reported. The latter point will be especially important for pests feeding on a nonhigh dose Bt crop, as it will dictate whether further evolution is possible for these populations. For example, if laboratory-selected colonies are used as a yardstick, Bt-adapted western corn rootworm stopped responding to selection when exposed to Cry3Bb1 (Meihls et al. 2008, 2011) and Cry34/35Ab1-expressing maize (Lefko et al. 2008). The incomplete nature of the inheritance of these resistance traits did not allow for 100% survival, and created a selection plateau for these western corn rootworm populations.

Brent (1986) discusses the detection and monitoring of pesticide resistance in the NRC (1986) publication mentioned at the beginning of this manuscript. During his discussion, Brent (1986) argues that "resistance" and "resistant" have many "different shades of meaning," and it is therefore necessary that a particular usage must be "specified as the correct one or resistance must be defined clearly whenever it is used." Brent concluded that the second was more feasible. The case studies indicate the lack of specificity in the application of field-evolved resistance. As discussed here, field-evolved resistance has been reported as a small change in a laboratory bioassay (Wan et al. 2012) to complete loss of efficacy (Storer et al. 2010). The second use of resistance described by Brent (1986), defining resistance clearly when used, provides a better context for actions that may result from resistance claims. Without more descriptive information in the application of "field-evolved resistance," there is no real added value when using this definition relative to other definitions of resistance. Several variables important for Bt crops necessary for inclusion in the assessment of field-evolved resistance are as follows: 1) the geographic or spatial scale of the reported resistance; 2) consideration of whether there are strategies to manage the resistance that is reported (or is a change necessary); 3) a quantification of the resistance within the population reported as resistant; 4) consideration of the effectiveness of IRM strategies introduced to delay resistance, especially compliance; 5) determine whether losses in the field efficacy of a Bt crop are associated with resistance rather than another factor; and 6) is there a heritable basis for resistance to the Bt crop that allows the production of reproductive adults.

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