FORUM

Defining Terms for Proactive Management of Resistance to Bt Crops and Pesticides

BRUCE E. TABASHNIK,[1,2] DAVID MOTA-SANCHEZ,[3] MARK E. WHALON,[3] ROBERT M. HOLLINGWORTH,[3] AND YVES CARRIÈRE[1]

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ABSTRACT Evolution of pest resistance to pesticides is an urgent global problem with resistance recorded in at least 954 species of pests, including 546 arthropods, 218 weeds, and 190 plant pathogens. To facilitate understanding and management of resistance, we provide definitions of 50 key terms related to resistance. We confirm the broad, long-standing definition of resistance, which is a genetically based decrease in susceptibility to a pesticide, and the definition of "field-evolved resistance," which is a genetically based decrease in susceptibility to a pesticide in a population caused by exposure to the pesticide in the field. The impact of field-evolved resistance on pest control can vary from none to severe. We define "practical resistance" as field-evolved resistance that reduces pesticide efficacy and has practical consequences for pest control. Recognizing that resistance is not "all or none" and that intermediate levels of resistance can have a continuum of effects on pest control, we describe five categories of field-evolved resistance and use them to classify 13 cases of field-evolved resistance to five *Bacillus thuringiensis* (Bt) toxins in transgenic corn and cotton based on monitoring data from five continents for nine major pest species. We urge researchers to publish and analyze their resistance monitoring data in conjunction with data on management practices to accelerate progress in determining which actions will be most useful in response to specific data on the magnitude, distribution, and impact of resistance.

KEY WORDS evolution, field-evolved resistance, genetically engineered crop, *Bacillus thuringiensis*

Evolution of pest resistance to pesticides is an increasingly urgent problem that threatens human health and agriculture worldwide (Brent and Holloman 2007, Enayati and Hemingway 2010, Powles and Yu 2010, Heckel 2012, Wolstenholme and Kaplan 2012, Coetzee and Koekemoer 2013, Shalaby 2013, Sierotzki and Scalliet 2013), with resistance recorded in at least 546 species of arthropod pests (Fig. 1), 218 species of weeds, and 190 species of plant pathogens (Fungicide Resistance Action Committee 2013, Heap 2013, Whalon et al. 2013). Well-defined terms for detecting, analyzing, and categorizing resistance are needed to tackle this daunting challenge. However, the lack of a modern glossary for resistance was recently brought to our attention by an initiative of the U.S. Environmental Protection Agency (EPA) seeking input on definitions of terms about resistance (Whalon 2013).

Here, we provide a list of 50 key resistance terms and definitions aimed to facilitate understanding and management of resistance (Tables 1–4). This article emphasizes resistance to toxins from *Bacillus thuringiensis* (Bt) produced by transgenic plants, but our goal is to define the terms broadly so they can be applied to resistance to any pesticide. Although we consulted many references (Wilson and Bossert 1971, Hartl 1981, Li et al. 2007, Gassmann et al. 2009, and others cited below), the definitions here are not necessarily identical to those in the references. Whereas some definitions provided here might be accepted readily, others may be controversial. In controversial cases, the definitions proposed here can provide a point of reference for discussions, refinements, and revisions. In particular, we review the definitions of "resistance," "field-evolved resistance," and related terms to dispel confusion about these terms. We also illustrate various categories of field-evolved resistance using data obtained from monitoring pest resistance to Bt crops.

Resistance

We define resistance as a "genetically based decrease in susceptibility to a pesticide" (Table 1). The roots of this definition are in the book produced by the National Research Council of the National Academy of Sciences of the United States, in which Brent (1986) defines resistance as "any heritable decrease in sensitivity to a chemical within a pest population." Brent (1986) specifies that resistance can be "slight, marked, or complete" and "homogenous, patchy, or rare." In the same book, Dekker (1986) emphasizes that resis-

[1] Department of Entomology, University of Arizona, Tucson, AZ 85721.
[2] Corresponding author, e-mail: brucet@cals.arizona.edu.
[3] Department of Entomology, Michigan State University, E. Lansing, MI 48824.

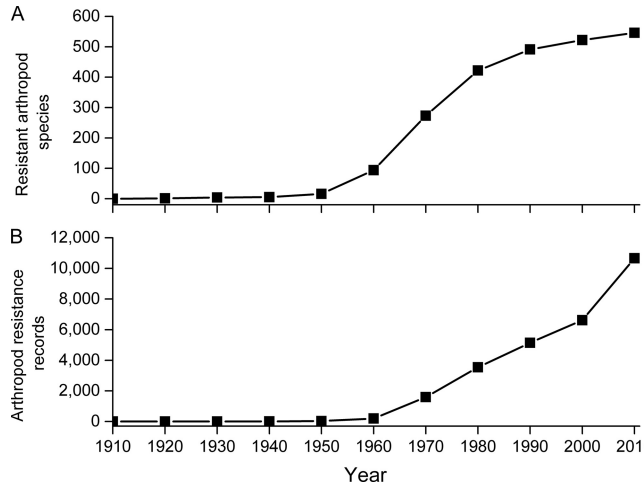

Fig. 1. A century of arthropod resistance to pesticides. A) Number of arthropod pest species with resistance to one or more pesticides. B) Records of arthropod pest resistance to pesticides. Each record consists of a published report of resistance in one pest species to one pesticide in a particular geographic region during a particular time period (Mota-Sanchez et al. 2008). Less than 4% of the records reflect laboratory-selected resistance. Unlike some previous summaries, this figure excludes resistance recorded in 40 species of nonpest arthropods, such as natural enemies and pollinators. As of 16 October 2013, totals were 546 arthropod pest species with resistance and 11,254 resistance records. From 2000 to 2010, the number of resistance records increased by 61% (from 6,617 to 10,661), while the number of species with resistance increased by only 4.6% (from 522 to 546) because resistance to at least one pesticide was already recorded in nearly all major arthropod pest species by 2000. The data were obtained from Whalon et al. (2013).

tance is a heritable decrease in sensitivity, citing an expert panel of the Food and Agriculture Organization of the United Nations (FAO 1979). The book's glossary gives a similar definition: "the inherited ability in a strain of a pest to tolerate doses of toxicant that would prove lethal to a majority of individuals in a normal population," and adds: "Laboratory documentation of resistance, however, does not necessarily indicate a current or impending loss of economic efficacy in the field."

The definition stated here captures the essence of the three definitions from the National Research Council (1986), which all emphasize any heritable changes that reduce susceptibility of pests relative to conspecifics and do not include economic impact as a criterion for resistance. The definition offered here includes resistance in organisms that are not pests (Tabashnik and Johnson 1999, Pedra et al. 2004) and thus is broader than the earlier definitions. With this general definition of resistance as the base, we define three more specific terms about resistance: field-evolved resistance, laboratory-selected resistance, and practical resistance (Table 1; Fig. 2).

Table 1. General terms: pesticides and resistance

Efficacy: the extent to which a pesticide controls a pest population
Evolution of resistance: the process by which a genetically based decrease in susceptibility to a pesticide occurs in a population
Field-evolved resistance (= field-selected resistance): genetically based decrease in susceptibility to a pesticide in a population caused by exposure to the pesticide in the field
Incipient resistance: field-evolved resistance in which a statistically significant, genetically based decrease in susceptibility has occurred, but the percentage of resistant individuals is <1%
Laboratory-selected resistance: genetically based decrease in susceptibility to a pesticide in a population caused by exposure of the population to the pesticide in the laboratory
Mode of action: how a pesticide works
Pesticide: a synthetic or natural substance that kills or harms pests (e.g., insecticides such as permethrin and Bt toxins; as well as fungicides, herbicides, miticides, and nematicides)
Practical resistance (= field resistance): field-evolved resistance that reduces pesticide efficacy and has practical consequences for pest control
Resistance: genetically based decrease in susceptibility to a pesticide
Resistant individual: an individual with a genetically based decrease in susceptibility to a pesticide relative to other individuals of the same species
Susceptibility (= sensitivity): the tendency to be killed or harmed by a pesticide
Tolerance: We discourage use of this term because it has several definitions, which fosters confusion. If the term is used, we recommend using and citing the definition of Finney (1971): the highest concentration of a particular pesticide that an individual can withstand without being killed. We urge the use of "inherent susceptibility" to signify the baseline susceptibility to a pesticide of a species before it is exposed to the pesticide. We favor "low level of resistance" or "small decrease in susceptibility" to indicate a low level of resistance
Toxin: a poison produced by an organism (e.g., Bt toxin)

Table 2. Genetic, evolutionary, and ecological terms relevant to resistance

Additive resistance: in reference to a single resistance gene, inheritance in which the phenotype for heterozygotes is intermediate between the phenotypes of susceptible and resistant homozygotes; or in reference to two or more resistance genes, inheritance in which the effect of resistance genes on the phenotype is additive across the genes
Allele: any one particular form of the several forms of a gene
Dominant resistance: inheritance of resistance in which the phenotype is resistant for individuals with either one or two resistance alleles at a genetic locus that determines susceptibility
Evolution: changes in allele frequency in a population
Fitness: the ability to survive and produce offspring relative to other individuals of the same species
Fitness cost: a trade-off in which alleles conferring resistance to a pesticide reduce fitness in environments lacking the pesticide
Genotype: the genetic makeup of an organism
Incomplete resistance: resistance in which fitness is lower for resistant individuals exposed to a pesticide relative to resistant individuals not exposed to the pesticide
Monogenic resistance: resistance conferred primarily or entirely by a single gene
Polygenic resistance: resistance conferred by two or more genes
Phenotype: an observable trait or set of traits of an organism
Population: a group of individuals of the same species that live in a particular geographic area
Quantitative trait loci: genes that contribute to a quantitative trait, such as polygenic resistance
Recessive resistance: inheritance of resistance in which individuals have a resistant phenotype only if they have two resistance alleles at a genetic locus that determines susceptibility
Resurgence: rapid increase in numbers of a pest population that was previously suppressed by a pesticide, natural enemy, or other factors

Field-Evolved Resistance, Laboratory-Selected Resistance, and Practical Resistance

Field-evolved (or field-selected) resistance is defined here as a genetically based decrease in susceptibility of a population to a pesticide caused by exposure to the pesticide in the field. Because this definition uses the term "pesticide," it is more general than the definition of Tabashnik et al. (2009) that focuses on Bt crops and uses "toxin," which specifies a poison produced by an organism (e.g., a Bt toxin). One can document field-evolved resistance directly by showing decreases in susceptibility through time for a population, or indirectly by showing that a population with a history of relatively high exposure to a pesticide is less susceptible than conspecific populations that have had less exposure (Tabashnik 1994).

We use the term "field" in the broad sense to mean any environment in which the pesticide is used to control a pest, such as fields of crops, greenhouses (Janmaat and Myers 2003), or inside organisms that host parasites (Wolstenholme and Kaplan 2012). Whereas field-evolved resistance results from exposure to a pesticide in the field, laboratory-selected resistance results from exposure to a pesticide in the laboratory (Table 1; Fig. 2). This distinction is important because control of pests in the field can be reduced by field-evolved resistance, but not by resistance that is confined to the laboratory. Further, the genetic basis, mechanism, and magnitude of resistance are not necessarily the same in laboratory-selected and field-evolved resistance (Zhang et al. 2012).

We define "practical resistance" as field-evolved resistance that reduces the efficacy of a pesticide and has practical consequences for pest control (Table 1; Fig. 2). The efficacy of a pesticide can be evaluated as the percentage reduction in pest density caused by the pesticide, which is calculated as the density of the pest in an untreated control minus its density after exposure to the pesticide, divided by its density in the untreated control (Tabashnik et al. 2000, Burkness et al. 2001). This yields 0% efficacy when the pest density is the same in the pesticide treatment and the untreated control, and 100% efficacy when the pesticide reduces the pest density to zero. Using analogous calculations, one could also evaluate pesticide efficacy as the percentage reduction in pest damage caused by a pesticide.

The decrease in efficacy associated with resistance can be calculated as the efficacy of a pesticide against a susceptible population minus the efficacy of the

Table 3. Mechanisms and modes of resistance to one or more pesticides

Behavioral resistance: resistance conferred by changes in behavior that reduce exposure to a pesticide
Cross-resistance: resistance to a pesticide caused by exposure of a population to a different pesticide
Mechanism of resistance: a genetically based change in a particular phenotypic trait that decreases susceptibility to a pesticide, such as a change in physiology, morphology, or behavior
Metabolic resistance: resistance conferred by enhanced enzymatic transformation of a pesticide to make it less toxic
Multiple resistance: resistance to more than one pesticide in a single organism; can be caused by cross-resistance, by independent evolution of resistance to two or more pesticides used sequentially or simultaneously, or by a combination of cross-resistance and independent evolution of resistance
Reduced penetration: resistance conferred by reduced entry of a pesticide into an organism
Sequential resistance: evolution of resistance at different times to different pesticides in the same population
Sequestration: resistance conferred by increases in the extent to which a pesticide that enters an organism is kept away from target sites, yet remains inside the organism (Pittendrigh et al. 2008, Yu et al. 2010)
Target site: the part of an organism a pesticide interacts with to kill or harm the organism; it can be a specific molecule or portion of a molecule
Target site resistance (= target site insensitivity): resistance conferred by changes in the target site that reduce the toxicity of the pesticide (e.g., changes in pesticide binding sites that reduce binding of the pesticide)

Table 4. Resistance monitoring and management

Bioassay: a test in which a group of live organisms is exposed to a pesticide to evaluate their susceptibility
Concentration: amount of pesticide per unit of another substance (e.g., micrograms of pesticide per milliliter of a suspension, milligrams of pesticide per gram diet, or nanograms of pesticide per square centimeter of a plant surface)
Diagnostic concentration (or dose): concentration (or dose) of pesticide in a particular bioassay that kills all or nearly all susceptible individuals but few or no resistant individuals
Dose: amount of pesticide eaten by or administered to an organism, such as milligrams eaten per gram of the organism or grams of pesticide injected into an organism
EC_{50} (= median effective concentration): concentration of pesticide that causes a specific response (such as failure to emerge as an adult) in 50% of the individuals in a population
IC_{50} (=median inhibitory concentration): concentration of a pesticide that inhibits an essential process such as growth or feeding in 50% of the individuals in a population
LC_{50} (=median lethal concentration): concentration of a pesticide that kills 50% of the individuals in a population
LD_{50} (=median lethal dose): dose of a pesticide that kills 50% of the individuals in a population
Refuge: a place where organisms are not exposed to a pesticide or a time during which organisms are not exposed to a pesticide
Resistance management: tactics implemented to delay evolution of resistance in pest populations
Resistance monitoring: systematic testing of organisms with bioassays, biochemical tests (e.g., enzyme assays), or molecular tests (e.g., DNA screening) to assess the frequency, magnitude, and spatial pattern of resistance
Resistance ratio: an index of the magnitude of resistance often calculated as the LC_{50} for a resistant population divided by the LC_{50} for a susceptible population; it can also be calculated analogously for other parameters that specify the amount of pesticide that causes a response in a specified percentage of a population such as LC_{95}, LD_{50}, LD_{95}, IC_{50}, or IC_{95} (but a ratio is usually not useful if it is based on the percentage mortality or percentage inhibition at a single pesticide concentration)

pesticide against a resistant population. Within the category of practical resistance, the loss in efficacy caused by resistance can vary from a statistically significant but minor decrease (e.g., 10% decrease in efficacy) to a complete failure of the product to control the pest (0% efficacy). Although the meaning is similar for the terms "practical resistance" and "field resistance" (Brent 1986), we prefer "practical resistance" because it emphasizes resistance that has practical consequences for pest control and avoids the inevitable confusion between the terms "field resistance" and "field-evolved resistance" (e.g., Van den Berg et al. 2013).

A Web of Science search (conducted on 10 January 2014) for the topic "field-evolved resistance" identified 66 publications, including 45 published from 2010 to 2013. Because this search identified only papers in which "field-evolved resistance" appears in the title, abstract, or key words, it underestimates the use of this term. For example, the term "field-evolved" and either "resistance" or "resistant" occurs in at least 18 additional publications, including seven highly cited articles about insecticide or herbicide resistance: Roush and McKenzie (1987); Holt et al. (1993); Roush (1994); Tabashnik et al. (2002, 2008a); Bates et al. (2005); and Powles and Yu (2010). According to the Web of Science, the 84 publications mentioned above were authored by >200 academic, government, and industry scientists from >20 countries and have been cited >2,500 times.

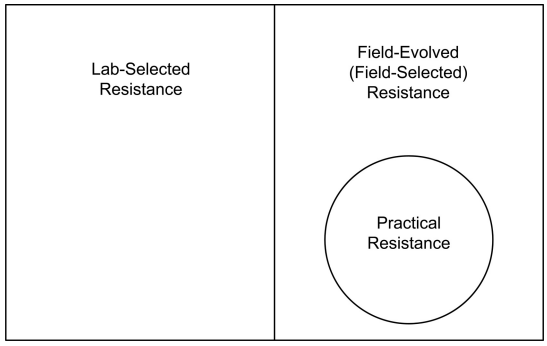

Fig. 2. Field-evolved resistance, laboratory-selected resistance, and practical resistance. Resistance, defined as a genetically based decrease in susceptibility, can evolve in the laboratory or field. Practical resistance is field-evolved resistance that reduces pesticide efficacy and has practical consequences for pest control. See text and Table 1 for details.

Despite the widespread and growing use of the term "field-evolved resistance," some industry scientists prefer definitions of resistance that include failure of the product (reviewed by Whalon et al. 2008, Tabashnik et al. 2013). However, we agree with Brent and Holloman (2007), who concluded: "attempts to restrict in this way the meaning of such a broadly used term as 'resistance' are bound to fail and to create more confusion." For example, the Insecticide Resistance Action Committee (IRAC), composed of members from more than a dozen major agrochemical and biotechnology companies, defines resistance as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (IRAC 2013). The first part of the IRAC definition, "a heritable change in the sensitivity of a pest population," is similar to the definition of resistance provided here (Table 1). The rest of the IRAC definition sets additional criteria that are problematic for objectively identifying resistance and for proactive detection and responses to resistance (Whalon et al. 2008, Tabashnik et al. 2013). By the time a product has failed repeatedly, it is too late to respond most effectively to resistance. The "expected level of control" is not specified, which allows for variation in interpretation. Moreover, this definition excludes resistance in any species that are not on the label.

Compared with the IRAC definition of resistance, the definition of "field-evolved resistance" provided here has several advantages. It explicitly recognizes

that resistance results from evolution that occurs in the field, enables objective identification of resistance, includes nontarget pests and beneficial species, and most importantly, it facilitates proactive detection and management of resistance (Tabashnik et al. 2013). Brent (1986), Brent and Holloman (2007), and Whalon et al. (2008) provide additional discussion of the history of various definitions of resistance as well as their advantages and disadvantages.

Categories of Field-Evolved Resistance to Bt Crops

The impact of field-evolved resistance on pest control can vary from none to severe, depending on many factors such as the frequency and magnitude of resistance, the pest's population density, the geographic distribution of resistant populations, and the availability of alternative controls (Tabashnik et al. 2009, 2013). Recognizing this spectrum, Tabashnik et al. (2013) described and applied criteria for four categories of field-evolved resistance to classify 24 cases involving Bt crops, with each case representing responses to one Bt toxin of one pest species in one country. These criteria explicitly acknowledge that field-evolved resistance is not "all or none," which facilitates objective classification of monitoring data and may help to appropriately gauge management actions depending on the severity of resistance (Tabashnik et al. 2013).

We can readily identify the two opposite ends of the spectrum of susceptibility and resistance: no decrease in susceptibility and resistance that causes complete failure of a product to control a pest. However, characterizing the various levels of resistance between these two extremes is challenging. Moreover, with intermediate levels of resistance, the impact on pest control of any given level of resistance varies from situation to situation. Although the particular criteria of Tabashnik et al. (2013) may not be optimal for other sets of resistance monitoring data, the concept of specifying objective criteria to describe the level of field-evolved resistance is widely applicable. To illustrate this concept, we review and extend the four categories of field-evolved resistance described by Tabashnik et al. (2013), all of which entail statistically significant, genetically based decreases in susceptibility in field populations: 1) incipient resistance, <1% resistant individuals; 2) early warning of resistance, 1–6% resistant individuals; 3) >50% resistant individuals and reduced efficacy expected, but not reported; and 4) >50% resistant individuals and reduced efficacy reported. Only cases in the last category meet the criteria for practical resistance. To provide a comprehensive classification, we add a fifth category here: >6–50% resistant individuals, which was not seen in any of the 24 cases of resistance monitoring data for Bt crops reviewed by Tabashnik et al. (2013).

In each case, the percentage of resistant individuals was estimated from survival at a "diagnostic concentration" of the relevant Bt toxin that kills all or nearly all susceptible individuals (Tabashnik et al. 2013). Large increases in the concentration of pesticide killing 50% (LC_{50}) of insects tested also indicate that >50% of the individuals in a population are resistant. The resistance ratio, typically calculated as the LC_{50} for a resistant population divided by the LC_{50} for a susceptible population (Table 3), reflects the magnitude of resistance. A resistance ratio ≥10 has been used as a standard for categorizing cases of resistance (Mota-Sanchez et al. 2002). When some populations are highly resistant, LC_{50} values and survival at a diagnostic concentration tend to be correlated (Tabashnik et al. 1993). In the early stages of resistance evolution, however, detection of resistance is more sensitive with diagnostic concentration tests than evaluations of LC_{50} (Roush and Miller 1986). F_1 and F_2 screens can be especially useful for detecting rare recessive resistance alleles (Gould et al. 1997, Andow and Alstad 1998).

Of the 24 cases based on resistance monitoring data from eight countries for responses to six Bt toxins by 13 major pest species (12 lepidopterans and 1 coleopteran), 11 cases showed no statistically significant decrease in susceptibility after 2–15 yr (median = 7 yr) of exposure to Bt crops (Tabashnik et al. 2013). Below we review the other 13 cases (Table 5), which all meet the criteria for field-evolved resistance, but only five cases meet the criteria for practical resistance.

Downes et al. (2010) used the term "incipient resistance" to describe a statistically significant increase in the frequency of alleles conferring resistance to Bt toxin Cry2Ab in *Helicoverpa punctigera* (Wallengren) from Australia. All three cases of incipient resistance are from Australia, where a rigorous, proactive monitoring program has enabled early detection of resistance to Bt toxins in *H. punctigera* and *Helicoverpa armigera* (Hübner) (Downes et al. 2010; Downes and Mahon 2012a,b; Table 5). Based on results from the 2008–2009 field season, Downes et al. (2010) found that the frequency of alleles conferring resistance to Cry2Ab was eight times higher in areas where Bt cotton producing this toxin was grown compared with noncropping areas. They also detected an 11-fold increase from 2004–2005 to 2008–2009 in the frequency of resistance to Cry2Ab in populations exposed to this toxin. However, they estimated that the maximum percentage of resistant individuals was 0.2%, which is too low to reduce the efficacy of Bt cotton. Moreover, the frequency of resistance to Cry2Ab did not increase from 2008–2009 to 2010–2011 (Downes and Mahon 2012a). These results show that the statistically significant yet small increases in resistance allele frequency characteristic of incipient resistance do not necessarily indicate that further increases in resistance are imminent.

Zhang et al. (2011) used the phrase "early warning" of resistance to describe a statistically significant increase in the percentage of individuals with resistance to Bt toxin Cry1Ac in *H. armigera* from northern China. Their 2010 survey showed that survival at a diagnostic concentration of Cry1Ac was significantly higher for 13 field populations from northern China where exposure to Bt cotton was extensive, relative to

Table 5. Field-evolved resistance to Bt crops in nine pest species classified into categories ranging in severity from incipient resistance to practical resistance[a]

Pest[a]	Crop	Toxin	Country	Practical resistance
Incipient resistance (<1% resistant individuals)				
H. armigera[b]	Cotton	Cry1Ac	Australia	No
H. armigera[b]	Cotton	Cry2Ab	Australia	No
H. punctigera[c]	Cotton	Cry2Ab	Australia	No
Early warning (1–6% resistant individuals)				
D. saccharalis[d]	Corn	Cry1Ab	United States	No
H. armigera[e]	Cotton	Cry1Ac	China	No
O. furnacalis[f]	Corn	Cry1Ab	Philippines	No
P. gossypiella[g]	Cotton	Cry1Ac	China	No
>50% resistant individuals and reduced efficacy expected				
H. zea[h]	Cotton	Cry2Ab	United States	?[i]
Practical resistance (>50% resistant individuals and reduced efficacy reported)				
B. fusca[j]	Corn	Cry1Ab	South Africa	Yes
D. v. virgifera[k]	Corn	Cry3Bb	United States	Yes
H. zea[l]	Cotton	Cry1Ac	United States	Yes
P. gossypiella[m]	Cotton	Cry1Ac	India	Yes
S. frugiperda[n]	Corn	Cry1F	United States	Yes

[a] Adapted from Tabashnik et al. 2013; no cases occurred with >6% to 50% resistant individuals.
[b] Downes and Mahon 2012b, Tabashnik et al. 2013.
[c] Downes et al. 2010, Downes and Mahon 2012a.
[d] Huang et al. 2012.
[e] Zhang et al. 2011, 2012; Jin et al. 2013.
[f] Alcantara et al. 2011.
[g] Wan et al. 2012.
[h] Ali and Luttrell 2007; Tabashnik et al. 2009, 2013.
[i] Practical resistance is expected, but has not been reported.
[j] Van Rensburg 2007, Kruger et al. 2011, Van den Berg et al. 2013.
[k] Gassmann et al. 2011, 2012.
[l] Luttrell et al. 2004; Ali et al. 2006; Tabashnik et al. 2008a,b.
[m] Monsanto 2010, Dhurua and Gujar 2011.
[n] Storer et al. 2010, 2012.

two field populations from northwestern China where exposure to Bt cotton was limited. For the populations from northern China surveyed in 2010, the mean survival at the diagnostic concentration was 1.3% (range: 0–2.6%) compared with 0% for the populations from northwestern China and a susceptible laboratory strain (Zhang et al. 2011). Results of screening in 2009 and 2011 also support the conclusion that exposure to Bt cotton increased the frequency of *H. armigera* resistance to Cry1Ac in northern China, with up to 5.4% resistant individuals in a population (Zhang et al. 2012, Jin et al. 2013).

In total, four cases of "early warning" of resistance show a statistically significant increase in resistance, with the percentage of resistant individuals between 1 and 6% (Table 5). The other three cases are field-evolved resistance to Cry1Ac in Bt cotton by *Pectinophora gossypiella* (Saunders) in China (Wan et al. 2012), and resistance to Cry1Ab in Bt corn by *Ostrinia furnacalis* (Guenée) in the Philippines and *Diatraea saccharalis* (F.) in the southern United States (Huang et al. 2012).

As with incipient resistance, the four cases of "early warning" of resistance entail a frequency of resistance that is too low to substantially reduce the efficacy of Bt crops. However, field-evolved resistance with >1% resistant individuals detected warrants consideration of enhanced actions to manage resistance, such as increases in monitoring, refuge requirements, and alternative methods of control. It will be instructive to see what actions, if any, are taken in these four cases and how this affects the trajectory of resistance.

In the five most severe cases of field-evolved resistance to Bt crops, one or more pest populations had >50% resistant individuals and reduced efficacy of the Bt crop was reported (Table 5). These five cases entail practical resistance to Bt corn in three pests: *Busseola fusca* (Fuller), *Diabrotica virgifera virgifera* LeConte, and *Spodoptera frugiperda* (J.E. Smith); and practical resistance to Bt cotton in two pests: *Helicoverpa zea* (Boddie) and *P. gossypiella.*

In the U.S. territory of Puerto Rico, *S. frugiperda* (fall armyworm) evolved resistance to Bt corn producing Cry1F in 3 yr, which is the fastest documented case of field-evolved resistance to a Bt crop with reduced efficacy reported (Storer et al. 2010, 2012). This is also the first case of resistance leading to withdrawal of a Bt crop from the marketplace. In 2011, 4 yr after Dow Agro-Sciences and Pioneer Hi-Bred International voluntarily stopped selling Cry1F corn in Puerto Rico, high levels of resistance persisted in the field (Storer et al. 2012).

Practical resistance to Bt corn producing Cry1Ab occurred in *B. fusca* (maize stem borer) in South Africa in 8 yr (Van Rensburg 2007, Tabashnik et al. 2009, Van den Berg et al. 2013), with striking parallels

to *S. frugiperda* resistance to Cry1F corn. In both cases, proactive resistance monitoring was not conducted and observations of reduced efficacy in the field preceded documentation of resistance with bioassays (Kruger et al. 2009, 2011, 2012; Storer et al. 2010, 2012; Van den Berg et al. 2013). In South Africa, however, Cry1Ab corn was not withdrawn from sales, with 1.8 million hectares planted in 2012 (James 2012). This yielded widespread resistance and hundreds of reports of product failure during the 2010–2011 and 2011–2012 seasons (Kruger et al. 2009, Van den Berg et al. 2013). Monsanto, the company that developed the predominant type of Cry1Ab corn grown in South Africa, compensated growers for their insecticide sprays on this Bt corn (Kruger et al. 2009). Large scale planting of two-toxin Bt corn producing Cry1A.105 (similar to Cry1Ab; Tabashnik et al. 2009) and Cry2Ab began during the 2012–2013 season in South Africa (Van den Berg et al. 2013).

Field and laboratory data show that control problems in the field during 2009 and 2010 were associated with resistance to Cry3Bb in Bt corn in some Iowa populations of *D. v. virgifera* (western corn rootworm; Gassmann et al. 2011, 2012; Gassmann 2012). In "problem" fields, which had severe damage to Cry3Bb corn caused by rootworms, Cry3Bb corn had been planted for 3 to 7 yr (Gassmann et al. 2011, 2012). A 2011 field study of two of the problem fields identified in 2009 found that *D. v. virgifera* emergence did not differ significantly between Cry3Bb corn and non-Bt corn (Gassmann 2012).

In a letter to the EPA, 22 public sector corn entomologists stated that "greater than expected damage" to Cry3Bb1 corn was first seen widely during 2009, and problem areas had been reported in Illinois, Iowa, Minnesota, Nebraska, and South Dakota by 2011 (Porter et al. 2012). They concluded that all available evidence "converges in implicating field-evolved resistance to Cry3Bb1 as the most likely cause of 'greater than expected damage' in rootworm problem fields." This urgent problem has been addressed in several recent publications (Tabashnik and Gould 2012, Cullen et al. 2013, Devos et al. 2013, DiFonzo et al. 2013, Gray 2013) and by a Scientific Advisory Panel convened in December 2013 by the EPA (2013b). In addition, Monsanto (2013) has sponsored a new competitive grant program that includes research on managing corn rootworm resistance to Bt corn.

Both cases of practical resistance to Cry1Ac in Bt cotton (*P. gossypiella* in India and *H. zea* in the United States; Table 5) have been controversial, stimulating discussion about bioassay data based on insects derived from Bt crops (Tabashnik and Carrière 2010, Tabashnik et al. 2013). Sampling insects from Bt crops is essential for resistance monitoring (Tabashnik et al. 2008a,b; 2009; 2013) and has been important in documenting all three cases of practical resistance to Bt corn (Van Rensburg 2007; Storer et al. 2010, 2012; Gassmann et al. 2011, 2012; Kruger et al. 2011; Gassmann 2012; Van den Berg et al. 2013). The primary goal of resistance monitoring is to detect resistance soon enough to enable proactive management; failure to sample insects from Bt crops can delay detection of resistance (Tabashnik et al. 2009, 2013). Although survival on a Bt crop alone does not constitute evidence of resistance, bioassays of progeny derived from such survivors can determine if the survivors were resistant. For example, bioassays showed that *B. fusca* and *S. frugiperda* surviving on Bt corn were resistant (Van Rensburg 2007, Storer et al. 2010), but *Helicoverpa* surviving on Bt cotton in Australia during 2006 and *D. v. virgifera* surviving on Bt corn in Missouri during 2005 and 2006 were not (Hibbard et al. 2010, Downes and Mahon 2012a). Documentation of field-evolved resistance also requires evidence that the frequency of resistance alleles has increased in response to selection. Data provide strong evidence of field-evolved resistance if they show that the frequency of resistance alleles is higher in insects derived from Bt crops (or from any population with a history of exposure to Bt crops) relative to insects from conspecific susceptible populations.

Resistance of *P. gossypiella* (pink bollworm) to Bt cotton producing Cry1Ac was first detected with laboratory bioassays of the offspring of insects collected from non-Bt cotton fields in 2008 in the state of Gujarat in western India (Dhurua and Gujar 2011). India ranks second in cotton production, behind only China, and Gujarat accounted for one-third of India's cotton production in 2009–2010, which is equivalent to about half of the annual cotton production in the United States during 2009 and 2010 (FAO 2011, Desh Gujarat 2013). Monsanto (2010), the company that developed Cry1Ac cotton, reported in a press release that its monitoring of the 2009 cotton crop "confirmed" *P. gossypiella* resistance to Cry1Ac in four districts of Gujarat. This widespread resistance documented with laboratory bioassays was associated with unusually high abundance of both larvae on Cry1Ac cotton (Monsanto 2010) and moths caught in pheromone traps (India's Genetic Engineering Approval Committee [GEAC] 2010).

As far as we know, the details of Monsanto's methods and results remain unpublished. Nonetheless, a presentation at a scientific meeting by Monsanto scientists (Dennehy et al. 2010) indicated that most of their bioassay data from populations sampled in 2009 were obtained from insects derived from Bt cotton. A recent summary of this work coauthored by Monsanto scientists (Sumerford et al. 2013) concluded that, in laboratory bioassays of *P. gossypiella* populations sampled in 2009, median survival was 70% at a diagnostic concentration of Cry1Ac (≈500 times higher than the LC_{50} of susceptible populations). Sumerford et al. (2013) added, "During 2010, resistance also was detected in populations collected from non-Bt cotton."

Bagla (2010) reported in the journal "Science" that Dr. Keshav Raj Kranthi, Director of India's Central Institute for Cotton Research, questioned Monsanto's methods and its conclusion of field-evolved resistance to Bt cotton in *P. gossypiella.* According to India's GEAC (2010), Kranthi indicated that because Monsanto's bioassay data were derived from larvae collected from Bt cotton instead of conventional cotton,

their inferences about resistance were not correct. As explained above, however, testing insects derived from Bt crops is an essential component of resistance monitoring. Consistent with this principle, Bagla (2010) reported that Monsanto asserted that its methods (which include testing of insects from Bt cotton) are "standard practice." Furthermore, resistance in insects derived from non-Bt cotton was reported subsequently by Dhurua and Gujar (2011) and Sumerford et al. (2013).

Meanwhile, since 2008, farmers in India have almost completely switched from cotton producing only one Bt toxin (Cry1Ac) to cotton that makes two Bt toxins (Cry1Ac and Cry2Ab; Choudhary and Gaur 2010, Monsanto 2010). The main advantage of this two-toxin cotton against *P. gossypiella* is that Cry2Ab kills larvae resistant to Cry1Ac (Tabashnik et al. 2002, Dhurua and Gujar 2011).

As with *P. gossypiella* in India, documentation of practical resistance of *H. zea* to Cry1Ac in the United States includes evidence of resistance in samples from Bt crops and other sources. Eight strains of *H. zea* derived during 2003–2006 from field sources other than Bt crops had resistance ratios >100 (median = 630), including two strains with resistance ratios >1,000 (Ali et al. 2006, Luttrell and Ali 2007, Tabashnik et al. 2008b). In this case, the initial evidence of field-evolved resistance in the southeastern United States came in 2002, 6 yr after commercialization of Bt cotton in that region (Luttrell et al. 2004, Ali et al. 2006). The extensive evidence confirming this case of practical resistance includes >50% survival at a diagnostic concentration of Cry1Ac for four strains derived from the field in 2003 (Ali et al. 2006) and a significant association between larval survival on Bt cotton leaves and decreased susceptibility to Cry1Ac in bioassays (Tabashnik et al. 2008b). Similar to the evidence from India, the documentation of *H. zea* resistance includes "unacceptable levels of boll damage" in problem fields (Luttrell et al. 2004) as well as decreased susceptibility to Cry1Ac in laboratory bioassays (Ali et al. 2006, Luttrell and Ali 2007, Tabashnik et al. 2008b).

Despite the results summarized above, some scientists have challenged the conclusion of practical resistance to Bt cotton in *H. zea* (Moar et al. 2008, Luttrell and Jackson 2012, Sumerford et al. 2013). One of their principal arguments is that the documentation relies on bioassays of insects collected from Bt crops (Moar et al. 2008, Sumerford et al. 2013). However, testing insects derived from Bt crops is essential for resistance monitoring and *H. zea* resistance to Cry1Ac was detected in samples from sources other than Bt crops. In particular, Sumerford et al. (2013) stated that the data for *H. zea* demonstrate "strikingly elevated LC_{50} values, mostly from populations collected from non-Bt crops."

In the United States from 2003 to 2011, Cry1Ac cotton was progressively replaced by transgenic cotton making two Bt toxins, predominantly Cry1Ac and Cry2Ab (Brévault et al. 2013). Field-evolved resistance of *H. zea* resistance to Cry2Ab in the southeastern United States is categorized as >50% resistant individuals detected, with reduced efficacy of the Bt crop expected. Like both cases of field-evolved resistance to Bt cotton producing Cry1Ac, this case has been controversial.

The data documenting resistance to Cry2Ab include a significant increase in the proportion of populations screened that had an LC_{50} value greater than the diagnostic concentration of toxin (150 μg Cry2Ab per milliliter of diet), which indicates >50% survival at the diagnostic concentration (Ali and Lutrell 2007, Tabashnik et al. 2009). Based on this criterion, the percentage of *H. zea* populations tested that were resistant to Cry2Ab rose from 0% in 2002 to 50% in 2005, only 2 yr after commercialization of Bt cotton producing Cry2Ab and Cry1Ac (Ali and Lutrell 2007, Tabashnik et al. 2009). Three populations sampled from non-Bt plants in Arkansas in 2005 had such low mortality in bioassays that LC_{50} values could not be calculated, but were estimated to be >400 μg Cry2Ab per milliliter of diet (Ali and Luttrell 2007).

In addition, data from field populations in Arkansas show that mortality caused by a diagnostic concentration of Cry2Ab decreased substantially in 2010 compared with the previous 4 yr (Jackson et al. 2011). This evidence of field-evolved resistance to Cry2Ab coincided with higher abundance of *H. zea* in the field and increased insecticide sprays targeting *H. zea* on Bt cotton in 2010 (Jackson et al. 2011). In the United States from 1999 to 2011, the percentage of Bt cotton producing two toxins increased from 0 to 90%, while the sprays against *H. zea* on Bt cotton tripled (Williams 2012, Tabashnik et al. 2013). Although factors other than resistance could contribute to increased sprays against *H. zea* on Bt cotton, the data refute the alternative hypothesis offered by Luttrell and Jackson (2012) that the increased abundance of this pest in the midsouthern United States was associated with increased planting of corn (Tabashnik et al. 2013).

Overall, the data summarized above include some degree of field-evolved resistance to Bt crops in nine target pests, ranging from incipient resistance to practical resistance. Although Sumerford et al. (2013) expressed concern that claims of field-evolved resistance could "trigger unnecessary resistance remediation," we are not aware of any examples indicating this has occurred in the 18 yr since Bt crops were commercialized. Conversely, the five cases of practical resistance to Bt crops (Table 5) are associated with failure to comply with refuge requirements or inadequate refuge requirements (Storer et al. 2010, 2012; Kruger et al. 2012; Tabashnik et al. 2013; Van den Berg et al. 2013). Despite three cases of practical resistance to Bt crops in the United States (Table 5), the observed association between limited planting of refuges and rapid evolution of resistance, and recommendations from public sector scientists to maintain or increase refuge requirements (EPA 2002, Knight 2003, Alyokhin 2011, Tabashnik and Gould 2012), the EPA has greatly reduced refuge requirements for Bt crops since 2007. Currently in the United States, refuges of non-Bt corn can be as little as 5% of the total area planted to corn (EPA 2011a,b; 2013a). Refuges of

non-Bt cotton are not required for Bt cotton in most of the nation, primarily because of the presence of non-Bt host plants other than cotton that are considered "natural" refuges (EPA 2007).

Conclusion

We hope that the definitions provided here will facilitate improved understanding and management of resistance. Results from extensive resistance monitoring conducted for Bt crops demonstrate that increases in the frequency of resistance in pest populations can be detected before reduced efficacy of Bt crops occurs in the field. Although the term "practical resistance" is useful because it recognizes resistance that has practical consequences, the broader term "field-evolved resistance" is essential for proactive detection and management of resistance. In the absence of consensus, explicitly stating the definition used in a particular case and citing a relevant reference can avoid confusion.

To expedite progress, we urge scientists in the public and private sectors to publish and analyze their resistance monitoring data in conjunction with relevant information on management practices, including the history of pest exposure to the pesticide. Systematic analyses of such data can yield insights about the relationship between management practices and resistance evolution (Hutchison et al. 2010; Tabashnik et al. 2010, 2013; Carrière et al. 2012). In general, the sooner steps are taken to delay resistance, the more likely they are to succeed. Finally, rather than debating definitions of resistance, we encourage discussion and analysis on a case-by-case basis engaging resistance experts, agricultural economists, stakeholders, industry scientists, and regulators to determine the management actions that will be most useful in response to specific data on the magnitude, distribution, and impact of resistance.

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