

2015

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## Early Detection and Mitigation of Resistance to *Bt* Maize by Western Corn Rootworm (Coleoptera: Chrysomelidae)

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J. Econ. Entomol. 1–14 (2015); DOI: 10.1093/jee/tov238

**ABSTRACT** Transgenic *Bt* maize that produces less than a high-dose has been widely adopted and presents considerable insect resistance management (IRM) challenges. Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, has rapidly evolved resistance to *Bt* maize in the field, leading to local loss of efficacy for some corn rootworm *Bt* maize events. Documenting and responding to this resistance has been complicated by a lack of rapid diagnostic bioassays and by regulatory triggers that hinder timely and effective management responses. These failures are of great concern to the scientific and agricultural community. Specific challenges posed by western corn rootworm resistance to *Bt* maize, and more general concerns around *Bt* crops that produce less than a high-dose of *Bt* toxin, have caused uncertainty around current IRM protocols. More than 15 years of experience with IRM has shown that high-dose and refuge-based IRM is not applicable to *Bt* crops that produce less than a high-dose. Adaptive IRM approaches and pro-active, integrated IRM-pest management strategies are needed and should be in place before release of new technologies that produce less than a high-dose. We suggest changes in IRM strategies to preserve the utility of corn rootworm *Bt* maize by 1) targeting local resistance management earlier in the sequence of responses to resistance and 2) developing area-wide criteria to address widespread economic losses. We also favor consideration of policies and programs to counteract economic forces that are contributing to rapid resistance evolution.

**KEY WORDS** *Bt* resistance, *Diabrotica virgifera virgifera*, insect resistance management, integrated pest management, western corn rootworm

Transgenic crops that produce insecticidal toxins (Cry proteins) from *Bacillus thuringiensis* Berliner (*Bt*) have been widely adopted in maize and cotton cropping systems in the United States, representing 80 and 84%, respectively, of the area planted to these two crops in 2014 (US Department of Agriculture–National Agricultural Statistics Service [USDA-NASS] 2015). *Bt* rootworm-protected maize was introduced in 2003

(Vaughn et al. 2005) and was quickly embraced by farmers because it provided excellent protection of corn roots from larval rootworm, while simplifying production by eliminating soil-applied insecticides (Rice 2004). Although some *Bt* traits have proven durable, failures of corn rootworm *Bt* maize to control its main target, western corn rootworm, *Diabrotica virgifera virgifera* LeConte, have increased rapidly after field-evolved resistance was first documented (Gassmann et al. 2011). Presently, this insect is the most important pest of cultivated maize in North America, with yield loss and control expenditures estimated to exceed US \$1 billion per annum (Sappington et al. 2006; Gray et al. 2009; Dun et al. 2010; Tinsley et al. 2012, 2015).

The rapid development of resistance to corn rootworm *Bt* maize can be attributed to multiple causes, including 1) an insect resistance management (IRM) strategy based on the “high-dose/ refuge” concept that proved inapplicable because of inaccurate assumptions about pest biology, 2) definitions of resistance that allowed lengthy delays in response to field observations, and 3) economic incentives and government policies that inadvertently increased selection pressure by encouraging continuous planting of maize instead of crop rotations.

In an open letter to the United States Environmental Protection Agency (USEPA), entomologists with

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expertise in the ecology and management of North American maize pests wrote that the situation requires urgent action to preserve efficacy of corn rootworm *Bt* technologies (Porter et al. 2012). Current attempts to mitigate the economic consequences of resistance often rely on tactics that create conflict between the goals of IRM and integrated pest management (IPM; Cullen et al. 2013, Sappington 2014). Yet IRM and IPM should be integrated to complement each other by reducing selection for resistance, extending the effective life of the *Bt* technology, and simultaneously reducing the risk of economic losses from rootworms (Porter et al. 2012, Cullen et al. 2013, Devos et al. 2013, Wangila et al. 2015). Here we review the causes of field-evolved resistance and suggest actions that could prolong the efficacy of existing and future technologies.

### Old Assumptions Meet New Evidence

The IRM plans implemented by the USEPA for the first several corn rootworm *Bt* maize products were similar to the high-dose refuge (HDR) strategy successfully used for European corn borer, *Ostrinia nubilalis* (Hübner), with the modification that refuge maize must be adjacent to CRW *Bt* maize (USEPA 2007, 2010a,b). High-dose was defined as 25× the amount of toxin needed to kill 99.99% of a susceptible population (USEPA 2001). In its simplest form, the HDR-IRM strategy assumes 1) that resistance alleles are rare, 2) that susceptible adults emerging from nearby non-*Bt* refuges move far enough and in large enough numbers to mate with nearly all rare resistant individuals emerging from *Bt* fields, and 3) that plants produce *Bt* toxin at a dose sufficient to kill heterozygous resistant insects (i.e., a high-dose = functionally recessive resistance; Alstad and Andow 1995, Gould 1998, Carrière et al. 2004, Devos et al. 2013). These attributes lead to substantial delays in resistance, because most resistant individuals will mate with a susceptible individual, their heterozygous progeny will not survive on the *Bt* crop, and thus resistance alleles will be purged from the population. In retrospect, the rootworm IRM plan manifestly did not protect sufficiently against western corn rootworm resistance to *Bt* maize. The reasons for the failure are described below and are important to understand because they clearly show that future plans should emphasize a more adaptive, integrated IRM and IPM approach.

Nearly all high-dose toxins have remained effective against their targeted pests, including European corn borer, despite widespread use (Huang et al. 2011, Tabashnik et al. 2013, Siegfried et al. 2014). In contrast, toxin levels that are less than high-dose increase the risk of resistance evolution because of an associated increase in the functional dominance of resistance (Carrière et al. 2010, 2015; Brévault et al. 2013; Tabashnik 2013); this has been a frequent contributing factor to instances of field-evolved resistance with concomitant control failures (e.g., van Rensburg et al. 2007, Storer et al. 2010, Farias et al. 2014) or increases in resistance allele frequency (e.g., Tabashnik et al. 2009, Tabashnik and Gould 2012, 2013; Dhurua and Gujar 2011). None

of the corn rootworm *Bt* events currently registered is high-dose, and this allows for some larval survival and adult emergence (e.g., Table 1; Storer et al. 2006; Meihls et al. 2008; Binning et al. 2010; Hibbard et al. 2010, 2011; USEPA 2012, Gassmann 2012; Head et al. 2014; Frank et al. 2015; Hitchon et al. 2015; Keweshan et al. 2015). The level of susceptible western corn rootworm killed by single-toxin *Bt* maize can range from ca. 70% to as high as 99% (Gassmann 2012; Petzold-Maxwell et al. 2013a,b). Thus, mortality seems to fall in the range of that expected for “moderate-dose” (“less than high-dose” in the current paper) toxins, which can increase rate of resistance evolution over that of either high-dose or low-dose toxins (Tabashnik and Croft 1982, Gould 1998, Tabashnik et al. 2013). A USEPA Scientific Advisory Panel tasked with examining possible IRM plans for corn rootworm recognized the risk of relying on an HDR strategy for less than high-dose events and recommended a non-*Bt* refuge of 50% to compensate (USEPA 2002). However, the USEPA chose not to follow that recommendation, and instead the final IRM plans mandated a 20% refuge.

Furthermore, several critical initial assumptions about western corn rootworm biology, which aligned with the assumptions underlying the HDR strategy, have now been refuted. For example, it was assumed that resistance in western corn rootworm populations is rare, likely caused by a single locus, and would require a large change in susceptibility to impact efficacy in the field, as was observed for other *Bt* toxins (e.g., 100- to 500-fold resistance; Caprio et al. 2000). The rapid response to laboratory selection for resistance was an early sign of trouble (Lefko et al. 2008, Meihls et al. 2008), and we now know that *Bt* resistance can evolve rapidly (Meihls et al. 2011, 2012; Oswald et al. 2012; Devos et al. 2013), implying that resistance alleles are relatively common. This was followed by the finding that western corn rootworm populations with only a

**Table 1. Mean root injury ratings for susceptible corn rootworm attacking rootworm-protected *Bt* maize and control hybrids without rootworm *Bt* traits**

| Corn rootworm <i>Bt</i> toxin           | Location                |                         |                                 |
|---|-------------------------|-------------------------|---------------------------------|
|   | DeKalb, IL <sup>a</sup> | Urbana, IL <sup>a</sup> | Crawfordsville, IA <sup>b</sup> |
| Cry3Bb1                                 | 0.08a                   | 0.15a                   | 0.03a                           |
| mCry3A                                  | 0.50b                   | 0.40b                   |                                 |
| Cry34Ab1/Cry35Ab1                       | 0.17ab                  | 0.05ab                  | 0.05a, 0.06a                    |
| Pyramid: Cry3Bb1 +<br>Cry34Ab1/Cry35Ab1 | 0.03a,<br>0.01a         | 0.05a,<br>0.02a         | 0.02a                           |
| Untreated control                       | 0.98c                   | 0.87c                   | 0.90b                           |
| Untreated control                       | 1.55d                   | 1.70d                   | 1.34c                           |
| Untreated control                       | 1.65d                   | 1.15c                   | 1.36c                           |

0–3 Node injury scale (Oleson et al. 2005) used to evaluate all roots.

Means within a column followed by the same letter are not significantly different, as reported in the original analyses (not all treatments in the original studies are presented here).

<sup>a</sup> From: Tinsley, N., R. Estes, and M. Gray. 2011. Preliminary root ratings for 2011 University of Illinois corn rootworm trials, <http://bulletin.ipm.illinois.edu/article.php?id=1560>

<sup>b</sup> From: Gassmann, A., and P. Weber. 2010. Iowa State University 2010 evaluation of insecticides and plant-incorporated protectants, p. 18, [http://www.ent.iastate.edu/pest/rootworm/2010\\_Ent\\_Report.pdf](http://www.ent.iastate.edu/pest/rootworm/2010_Ent_Report.pdf)

3- to 6-fold increase in resistance caused substantial feeding injury and yield loss in the field (Gassmann et al. 2011, 2014). Because it was not anticipated that resistance monitoring would have to detect smaller changes in resistance than occur for high-dose *Bt* crops, methods to detect such changes were not used or sought.

Assumptions about the interplay of adult movement and local mating also proved to be incorrect (Spencer et al. 2012). While it was known that many females mate near their emergence site, the effect of this behavior on local mating structure was not clear, and data suggested that male movement was extensive enough to ensure adequate encounters of susceptible refuge adults with adults emerging from the *Bt* maize (Quiring and Timmons 1990). The finding that the optimal male mating period is shorter than previously believed (Kang and Krupke 2009) implies that the distance that males move before mating is more limited than formerly thought. This promotes positive assortative mating, where resistant individuals are more likely to encounter and mate with one another. Limited interfield dispersal among adults (Marquardt and Krupke 2009, Spencer et al. 2009) also promotes positive assortative mating, as does later average emergence of adults from *Bt* maize than from refuge maize (Storer et al. 2006, Murphy et al. 2010, Hibbard et al. 2011, Frank et al. 2015, Hitchon et al. 2015, Keweshan et al. 2015). In aggregate, current evidence suggests that positive assortative mating occurs frequently, the effect of which is to greatly accelerate resistance evolution (Deitloff et al. 2015). Consequently, fields planted to the same *Bt* trait for as few as three consecutive years have become foci of selection for resistance in western corn rootworm (Gassmann et al. 2011, 2012, 2014; Wangila et al. 2015).

### Current IRM Plans and Definitions of Resistance

Registration of the first rootworm-active *Bt* maize (Cry3Bb1) in 2003 included an IRM plan that had four components: 1) a 20% structured refuge, 2) a resistance monitoring program, 3) a remedial action plan, and 4) a grower compliance and education program (USEPA Reg. No. 524-528). This framework is similar to that used for lepidopteran-active *Bt* crops (USEPA 2001). US registrants are required to routinely monitor for resistance, with the goal of detecting resistance before widespread economic crop losses occur. Resistance monitoring for rootworms involves testing arbitrarily selected populations for a change in susceptibility and relying on farmers to report unexpected root injury. Resistance management proceeds through four sequential steps (USEPA 2007, 2010a,b): performance inquiry, unexpected damage, suspected resistance, and confirmed resistance (Fig. 1a). Meeting the definitions of the latter three steps is necessary for triggering remedial action.

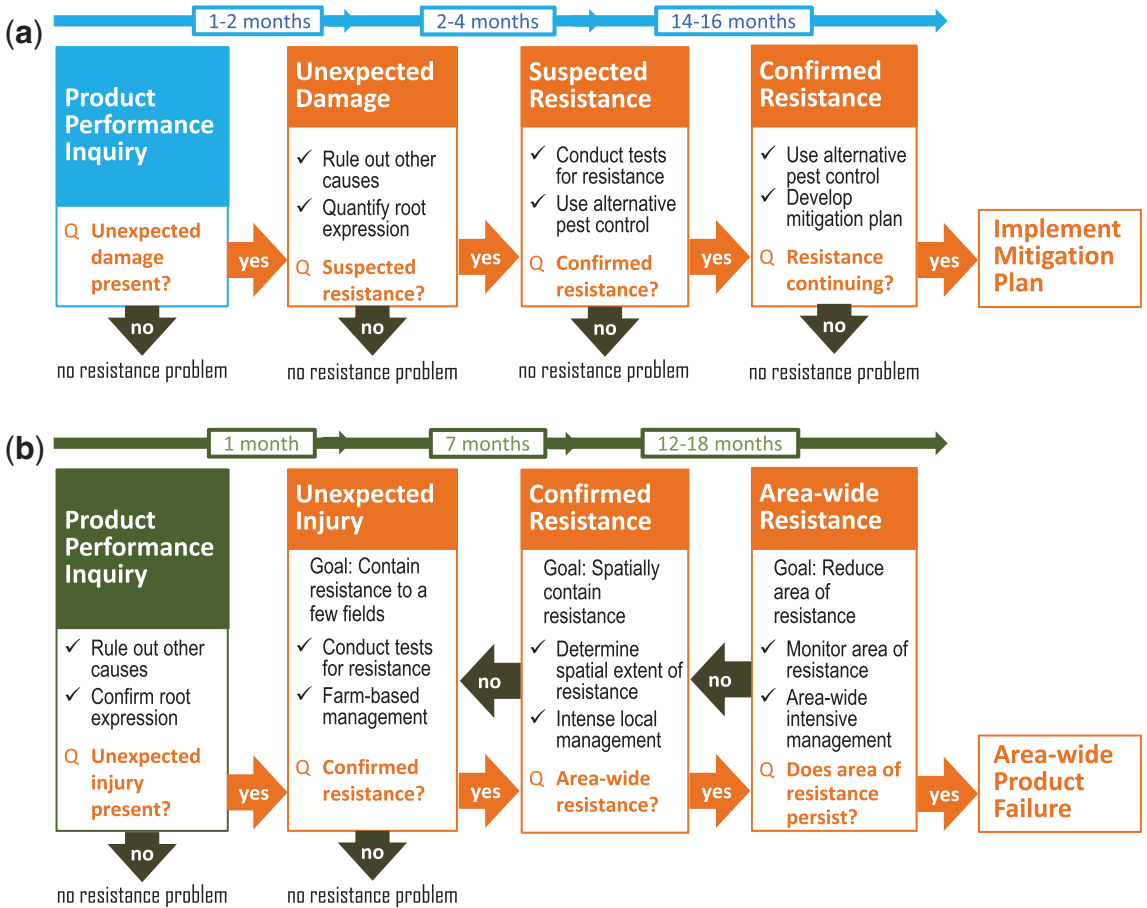
A product performance inquiry is initiated by a farmer who perceives a problem in a rootworm *Bt* maize field and contacts the technology provider. Each report is investigated by the technology provider to

determine the nature of the problem, which sometimes is unrelated to product performance, e.g., planting errors, non-rootworm pests, weather, or other factors. The technology provider samples roots to determine whether the degree of corn rootworm feeding injury is unusually high, i.e., whether unexpected injury has occurred. Once spurious causes of injury are ruled out and normal production of *Bt* toxin is verified quantitatively in the laboratory of the technology provider, the field is considered to have suspected resistance. Adult corn rootworms are then collected from this or adjacent fields as soon as possible (typically in the following cropping season), and offspring assayed to confirm resistance. Registrants could advise farmers to implement local remedial management during the current or following season, (i.e., management of adults, crop rotation, or an alternative pest management method) to reduce the population of potentially resistant insects (USEPA 2012).

According to the current regulatory definition, resistance is operationally confirmed for a single *Bt* protein if 1) the LC<sub>50</sub> from a diet-based bioassay on progeny of the sampled beetle population exceeds the 95% confidence interval of the mean historical LC<sub>50</sub> for susceptible pests, or 2) over 50% of *Bt*-expressing plants have  $\geq 1.0$  root nodes destroyed by suspected resistant populations under controlled laboratory conditions. Western corn rootworm produces one generation annually and has an obligate egg diapause of several months, so the process from product performance inquiry to confirmed resistance may require several years to complete (Fig. 1a; USEPA 2007; 2010a,b). If resistance is confirmed, registrants are required to take several actions. These include steps to characterize the spatial extent of the resistance problem, recommend measures to reduce the local population, reduce selection pressure, and develop a case-specific remedial action plan (USEPA 2010a,b).

Definitions of resistance, especially the regulatory definition of confirmed resistance, have contributed to lengthy delays in responding to problems in the field (Tabashnik and Gould 2012). Indeed, USEPA scientists (2012) identified serious shortcomings associated with the artificial diet bioassays used to detect resistance and concluded that “the current regulatory definition of ‘confirmed resistance’ for corn rootworm is flawed.” They indicated that these shortcomings could lead to circumstances where farmers and extension entomologists can see that there is an extensive resistance problem, even as the definition of resistance remains unmet and remedial action is withheld. Improved methods of verification have been proposed (USEPA 2014), but in practice, farmers are reacting to the perception of field failure with actions that may promote neither IRM nor IPM.

Additionally, the definition of confirmed resistance has become entangled with the concept of yield loss. This was first proposed by the Insecticide Resistance Action Committee, which advocated for demonstration of economic loss (Tomlin 1997), a position recently reiterated by others (Moar et al. 2008, Sumerford et al. 2013). This is problematic because the causes of economic loss are complex and not directly linked to the



**Fig. 1.** Current (USEPA 1998, 2007, 2010b) (a) and proposed adaptive (b) IRM response sequences, beginning with a corn rootworm *Bt* maize product performance inquiry from a farmer. If the question (Q) in the box is answered affirmatively, the orange arrows indicate the next step in the sequence. If not, the black arrows are followed. Months above the boxes indicate approximate time needed to advance from one stage (box) to the next (not cumulative). Definitions are in the text.

biological basis for the evolution of resistance (Tabashnik et al. 2013, 2014). For example, when population sizes are small, even a highly resistant pest population is unlikely to cause economic loss because there may be minimal feeding injury. Conversely, if the initial density of a susceptible pest population is high, enough individuals may survive the less than high-dose crop so that economic loss occurs. Thus, requiring a demonstration of economic loss adds an additional burden of proof without increasing the accuracy with which resistance is determined. IRM should aim to detect resistance at earlier stages, so that timely actions can be taken to avoid economic loss and preserve the long-term utility of a *Bt* trait (Whalon et al. 2008). Indeed, the goal of IRM monitoring should be to identify increased risk of resistance before it can cause yield loss, enabling an effective response to reduce that risk (Tabashnik et al. 2013, 2014).

For corn rootworm *Bt* maize, economic loss also is reflected in the regulatory use of the term “unexpected crop damage.” Damage measures loss of crop yield from pests (Pedigo et al. 1986) and can be determined

in maize only at the end of the growing season when the crop is harvested. Furthermore, yield loss varies greatly, even for the same level of crop injury (Dun et al. 2010, Tinsley et al. 2012). A better indicator is “unexpected crop injury,” a measure of the effect of the pest’s activities on host physiology and morphology (Pedigo et al. 1986). This can be assessed earlier in the maize growing season. Although it is possible that crop damage will occur before resistance can be confirmed, identifying injury that is likely due to resistance offers a practical advantage by allowing farmers to take action that year, and during following cropping seasons, to avoid more extensive damage.

### A New Sequence of Management Responses

Reassessment of current IRM plans seems warranted after more than a decade of experience with corn rootworm and *Bt* maize (Tabashnik and Gould 2012). Future plans should be adaptive, acknowledging that toxin production by corn rootworm *Bt* maize is less than high-dose. They should also be decoupled from



definitions of resistance that are based on economic damage in the field, and they should de-emphasize the importance of “confirmed resistance” as the trigger for rapid responses to likely resistance. Such an approach fosters a balance between management responses and severity of the problem (Andow and Ives 2002; Tabashnik et al. 2013, 2014). We propose three levels of management to follow the initial step of performance inquiry (Fig. 1b).

**Unexpected Injury.** *Risk Factors.* Several risk factors may be used qualitatively to help determine if a portion of a field should be investigated for unexpected injury. These include continuous cultivation of maize producing the same *Bt* toxin, late planting date the previous year (which can attract a large number of adults from neighboring fields), lodging, high adult density, informal root evaluations, and other signs of poor plant health such as water stress. A field history of three or more consecutive years with the same *Bt* toxin favors both unexpected injury and confirmed resistance (see below, Gassmann et al. 2011, Wangila et al. 2015). Late-planted and late-flowering maize acts as a trap crop for corn rootworm adults (Darnell et al. 2000), which are highly attracted to fresh maize silks and pollen (Chiang 1973, Prystupa et al. 1988, Meinke et al. 2009, Spencer et al. 2009), resulting in increased egg laying in that late-maturing field (Hill and Mayo 1974), and high larval populations and potentially high root injury in the following year. Unexpectedly high adult corn rootworm densities emerging from a field can indicate moderate to severe root injury (Branson et al. 1980). Although varietal differences in rootworm susceptibility may arise (Urías-López and Meinke 2001, Ivezić et al. 2009), lodging of maize after the late whorl stage may indicate severe root injury from corn rootworms (Branson et al. 1980, Reidell 1990, Spike and Tollefson 1991, Godfrey et al. 1993). However, high winds can lodge maize without rootworm injury, especially on saturated soils (Sutter et al. 1990) or following herbicide injury, and so these possibilities also should be considered.

Roots can be rapidly evaluated by excavating several maize plants, and looking for signs of feeding injury. If rootworm resistance were present in a number of fields in a region, trap crops the previous year may concentrate resistant beetles into a relatively small area. Other signs of root injury, relative to nearby healthy plants, may include stunting or earlier leaf curling under drought stress conditions.

*Quantifying Injury Level.* In the case of less than high-dose events, the target pest will cause some crop injury even when there is no resistance, so presence of injury is not by itself diagnostic of a *Bt* trait performance problem. Unexpectedly high injury can be a simple, rapidly assessed, and reliable early warning indicator of resistance that can be evaluated in the field within a few hours. Two critical criteria are: 1) there must be a threshold above which observed injury is considered unexpected, and 2) expression of the requisite *Bt* toxin(s) in the maize plants must be confirmed.

A simple root injury index (Oleson et al. 2005) can be used to quantify the level of root protection

provided by a given *Bt* technology. The root injury index is related to the number of severely injured primary root nodes and ranges from 0 to 3. A standardized sampling approach is important. We suggest that maize roots be excavated after peak root injury has occurred (i.e., coincident with or shortly after peak adult emergence). Because there can be variation in rootworm density and associated feeding injury among plants (Meinke et al. 2009), at least 12 plants should be sampled from the area of the maize field showing indications of unexpected injury, such as lodging. The plants should be separated from one another by at least 2 meters, but otherwise sampled randomly within the area of suspected injury.

*Unexpected Injury Threshold.* In the case of susceptible populations, injury to hybrids expressing single traits historically has been higher than to hybrids expressing pyramided traits (i.e., more than one toxin effective against the same pest; Prasifka et al. 2013, Head et al. 2014). Consequently, the threshold value for pyramided hybrids should be set lower than that for single trait hybrids. Because large population densities of susceptible rootworms can cause average root injury indices nearing 1.0 on some single trait hybrids (Gray et al. 2007), injury thresholds of 1.0 for maize hybrids with a single rootworm *Bt* event and 0.5 for pyramided hybrids have been widely used and are scientifically justified (USEPA 2014). These thresholds enable conservative but realistic detection of unexpected injury based on historical performance of rootworm traits against susceptible rootworm populations (see Table 1).

*Confirming Expression of Bt Toxin.* All sampled plants must express the relevant *Bt* protein. Expression can be confirmed qualitatively using immunochromatography (such as QuickStix). In seed mixtures, 5 to 10% of sampled plants on average will be non-*Bt* refuge plants, and these plants should be discarded from the sample. While laboratory-based quantification of *Bt* toxin may ultimately be important for some purposes, it is not essential at this early step. Unexpected injury is confirmed when the presence of the relevant *Bt* protein is verified and the root injury threshold is exceeded, with both results available within a day.

**Confirmed Resistance.** Resistance is a genetically based decrease in pest susceptibility to a management tactic (Tabashnik et al. 2009, 2014; Box 1). In the case of western corn rootworm, small changes in susceptibility are sufficient to cause injury to *Bt* maize in the field; greater changes would be needed to cause injury to a high-dose *Bt* crop (Gassmann et al. 2014). A 3- to 6-fold increase in survival on Cry3Bb1 maize is sufficient to cause increased root feeding injury (Gassmann et al. 2011, 2012, 2014; Meihls et al. 2012; Wangila et al. 2015). Furthermore, because resistance can evolve within three years of continuous use of a trait (Gassmann et al. 2011, Wangila et al. 2015), whatever method is used to confirm resistance must be rapid, repeatable, and sensitive to changes in pest susceptibility. Confirmation of resistance in areas subject to unexpected injury requires evidence of reduced susceptibility within an insect population, and of its genetic basis.

### Box 1. Resistance

**“Resistance”** is a genetically based decrease in pest susceptibility to an insecticide (Tabashnik et al. 2009). It is a phenotype, a trait, and a characteristic of an *individual* organism (Crow 1960, Andow 2001). “Resistance” also can be employed, usefully, in the sense of being a characteristic of a population as described below (see also Tabashnik et al. 2014). But it is important to remember that the fundamental unit of resistance is the individual, and that population-level resistance is an emergent property of the frequency of resistance among whatever group of individuals constitutes the population of interest. Likewise, evolution of resistance is a population-level phenomenon, but the unit of selection is the individual based on its resistance phenotype. Terms for the underlying genetics of resistance include “resistance allele” and “resistance locus.”

**“Frequency of resistance in a population,”** or simply the **“frequency of resistance,”** is the proportion of individuals in a population that is resistant. The “frequency of resistance alleles” and, equivalently, the “genetic frequency of resistance” are scientifically rigorous terms to describe the underlying genetics.

**“Evolution of resistance”** describes a change in the frequency of resistance alleles within a population. Because resistance evolution is typically studied with respect to the dynamics of directional selection, most studies on the evolution of resistance implicitly assume that the frequency of resistance alleles is increasing, although a decrease in frequency also is possible.

**“Field-evolved resistance”** is an increase in the frequency of resistance alleles that occurs in a wild population. This follows directly from classical definitions of evolution as any change in allele frequencies, and it recognizes that resistance evolution is a response to selection for higher resistance (lower susceptibility). This definition does not differ much from an alternative: “genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field” (Tabashnik et al. 2009, 2013, 2014), but it emphasizes the response to selection (and not the selective differential), allows for dispersal to be a contributing cause of resistance evolution, and keeps clear distinctions between individual phenotype and population characteristics. Moreover, an increase in resistance allele frequency may have different impacts on the frequency of resistant individuals in that population, which depends on dominance relationships among alleles, levels of incomplete resistance [i.e., difference in fitness of resistant individuals on a *Bt* crop relative to corresponding non-*Bt* crop (Carrière et al. 2010)], and the magnitude of the increase in allele frequency. Fitness costs associated with the resistant phenotype relative to susceptible individuals on a non-*Bt* crop also can affect the frequency of resistance over the larger landscape. Neither the detection of “resistance” in individuals nor “field-evolved resistance” in populations necessarily implies that there will be economic losses or even detectable changes in field efficacy (WHO 1957, Crow 1960, Brent 1986, Sawicki 1987, Tabashnik et al. 2013, 2014).

In principle, resistance to *Bt* toxins in rootworms can be measured by any diet-based or plant-based laboratory bioassay sensitive enough to distinguish *Bt*-resistant from *Bt*-susceptible individuals (Alves et al. 2006, Meihls et al. 2008, Gassmann et al. 2011, Huang et al. 2011). The progeny of pests sampled from a field with unexpected root injury are pooled and their corrected survival measured (Abbott 1925) on *Bt* plants (Siegfried et al. 2005, Nowatzki et al. 2008, Gassmann et al. 2012). If the population harbors individuals with heritable resistance, corrected survival will be higher on *Bt* plants than for susceptible control populations. Currently, only laboratory methods using *Bt* maize plants, such as a whole-plant assay (Gassmann et al. 2011) or a seedling-mat assay (Nowatzki et al. 2008), are sufficiently sensitive to achieve this, but ongoing efforts will likely improve the utility of diet-based bioassays. A sensitive assay is necessary to avoid misclassifying a resistant population as susceptible (i.e., obtaining a false negative), and is desirable for IRM purposes. Any assay used to confirm resistance of field populations must be freely available to all public-sector

scientists, and ideally, all public- and private-sector scientists would use the same assay method.

For any type of assay it is important to test several susceptible strains to increase sensitivity for comparison with populations from fields with unexpected injury (Siegfried et al. 2005, Gassmann et al. 2012, Wangila et al. 2015). Several strains were brought into the laboratory prior to 2003, the year that corn rootworm *Bt* maize was commercialized, and they are the best available control populations. These populations are maintained by the USDA-ARS North Central Agricultural Research Laboratory and have been used for research to quantify baseline susceptibility to *Bt* toxins (Siegfried et al. 2005). Preserving these USDA-ARS strains is critical, because widespread planting of corn rootworm *Bt* maize will make it problematic to find populations unexposed to selection for future research.

**Area-Wide Resistance.** Under the current USEPA framework, confirmed resistance to a specific *Bt* maize event is the last stage in the response sequence for corn rootworm (Fig. 1a). Confirmation of resistance to a specific event at a specific location does not preclude



the economic value of this event to farmers in other locations. We propose area-wide resistance as a landscape-level stage of resistance management to link confirmed resistance with economic loss at an appropriate spatial scale (Fig. 1b). Hence, there is a need for a clear definition of the “area of resistance” that includes scientifically sound criteria for determining the degree of lost efficacy and the spatial extent of resistance. Adopting the concept of area-wide resistance has an advantage in that pre-established remedial actions (see below), which may eventually include product withdrawal, are not implemented until clearly needed in a well delineated geographic space.

Conceptually, the spatial dimensions of area-wide resistance will reflect the tendency of farmers in an area with confirmed resistance to avoid a product with locally declining or failing efficacy. Many affected or concerned farmers will quickly adopt hybrids having other *Bt* toxins, supplement *Bt* maize with chemical insecticides, or take other measures to attain the immediate goal of suppressing pest injury. These immediate pest management actions can make it difficult to measure the spatial dimensions of resistance in an area. The protocol for defining an area of resistance should incorporate incentives for its rapid determination at a reasonable cost, with unambiguous specification of management responses when some threshold value is reached. Research to develop the methodology for accomplishing this is sorely needed, and will require both field work and modeling.

### Management Options for Mitigation

Consideration should be given to several levels of mitigation tactics (Fig. 1). We define mitigation as making the consequences of resistance less severe, which is a central goal of IPM. This is in contrast to remediation, which aims to restore susceptibility within a population by decreasing the frequency of resistance alleles. The goal of IRM is to slow the evolution of resistance, and while remediation is consistent with this goal, it is more elusive. Thus, to integrate IRM and IPM, mitigation tactics must also slow the rate of resistance evolution.

The first cases of unexpected injury from corn rootworm occurred in isolated fields on single farms (Gassmann et al. 2011, Wangila et al. 2015). Farmers would be expected to respond quickly to a determination of unexpected injury in their fields and reduce the risk of crop losses in the current and following year (Fig. 1). Consequently, the IRM plan should identify and recommend responses that both limit yield loss and reduce evolution of resistance (Table 2). Delaying mitigation until resistance is confirmed likely will prolong selection pressure and may increase dispersal of resistance alleles making future efforts to mitigate resistance, and delay additional cases of resistance, more difficult. Essential steps to reduce such delays include 1) modifying regulatory requirements to allow use of the most sensitive bioassay available, such as those that are plant-based (e.g., Nowatzki et al. 2008, Gassmann et al. 2011), 2) adopting a more practical definition of

confirmed resistance, and 3) implementing effective mitigation measures once unexpected injury is confirmed. These steps will enable the rapid suppression of a locally resistant population and reduce additional selection for resistance. Timely action is especially important where the problem is still localized, because it capitalizes on the potentially fleeting opportunity to reduce the frequency of resistance alleles and contain their spread. Such an opportunity is exemplified by the crop rotation resistance phenotype of western corn rootworm that oviposits in soybean fields as well as cornfields, which spread outward from a 3-km<sup>2</sup> point source in eastern Illinois at a rate of 10–30 km/year from 1986–1997 (Levine and Oloumi-Sadeghi 1996, Onstad et al. 1999, Meinke et al. 2009).

Confirming resistance with a bioassay provides an assessment of resistance in a single field rather than a region. It does not imply that the technology has lost its utility at a broader landscape scale or even to that farmer. Moreover, early detection of resistance followed by localized mitigation may sustain the viability of a *Bt* trait in an area where resistance has been confirmed. The management responses to confirmed resistance must be commensurate with the risk and should help preserve efficacy of the *Bt* maize event.

Several management tactics are available to mitigate *Bt* resistance in western corn rootworm (Table 2). The most favorable tactic is crop rotation, which has been used by farmers to manage rootworm for over a century (Gillette 1912, Schaafsma et al. 1999, Spencer and Levine 2008, Miller et al. 2009). In most instances the biology of corn rootworms—laying eggs in the preferred crop (maize) during late summer, hatching in the subsequent year, little or no larval survival in non-maize crops—enables rotation to destroy the greatest number of resistant individuals simply and effectively. Early detection and immediate implementation of crop rotation may be particularly useful to suppress newly forming foci of resistance alleles in areas outside the Corn Belt where selection pressure has not been as intense and adult population densities as great. Crop rotation is not always a viable option for a farmer, as when, for example, maize production is critical to feed livestock, the landlord or lender disallows it, or rotation-resistant rootworms predominate (Schaafsma et al. 1999, Sappington 2014). Volunteer *Bt* maize in the rotated crop can allow survival of rootworms and even accelerate resistance evolution (Krupke et al. 2009), and should be eliminated if possible.

If a farmer must plant continuous maize, *Bt* traits should be rotated to reduce selection pressure on any one trait. Rotating modes of action is a recommended IRM strategy before resistance develops, and is useful as a short-term response to reduce the size of resistant populations. However, switching to a different *Bt* trait after a heavily used one fails is equivalent to the chemical insecticide treadmill (Onstad 2008), which is unsustainable for transgenic crops in the long-term (McDougall 2011, Fuglie et al. 2012), so it is important to rotate traits before failures occur. Also, it is important to avoid rotating traits with cross-resistance, as is the case with Cry3Bb1 and mCry3A, where western corn

**Table 2. Suggested mitigation tactics for *Bt* resistance, including rationales, and associated risks for resistance evolution and pest management**

| Tactic   | Rationale   | Risks for resistance evolution  | Risks for pest management  |
|--|---|---|--|
| Crop rotation  | Eggs hatch the year after they are oviposited, and larvae die if maize is unavailable                               | Volunteer <i>Bt</i> maize   | Not effective with rotation-resistant rootworm populations           |
| Planting a different single <i>Bt</i> trait in subsequent years          | New trait kills resistant larvae, reducing selection pressure on compromised trait                                  | Cross resistance  | Different <i>Bt</i> trait is not effective                           |
| Planting a pyramid hybrid containing multiple <i>Bt</i> traits           | Additional trait kills resistant larvae   | Reduced refuge; pyramid acts as a single trait if it includes the compromised trait; cross resistance   | Pyramid hybrid is not effective                                      |
| Soil insecticide/seed treatments with non- <i>Bt</i> maize               | Insecticides kill resistant larvae, allow fitness costs to be incurred  | Potential reduction of effective refuge   | Soil insecticide or seed treatment not effective                     |
| Soil insecticide/seed treatments with a single-trait hybrid <sup>a</sup> | Insecticides kill some resistant larvae   | Reduction of effective refuge in cases where refuge and <i>Bt</i> seeds are blended; may mask continued poor performance of trait and thus continued resistance evolution | Insecticide provides no measurable improvement in pest control       |
| Soil insecticide/seed treatments with a pyramided hybrid <sup>a</sup>    | Insecticides kill some resistant larvae   | Reduction of effective refuge in cases where refuge and <i>Bt</i> seeds are blended; pyramid acts as a single trait; cross resistance                                     | Insecticide provides no measurable improvement in pest control       |
| Adult insecticide application  | Insecticides kill resistant adults  | Resistant adults disperse widely prior to application; resistance to the insecticide class used   | Poorly timed application will provide no improvement in pest control |
| Increase refuge size   | Increase susceptible population and reduce selection pressure; increase relative impacts of potential fitness costs | Resistant population will also increase and may increase resistance in nearby fields via dispersal  | Increase in population can increase risk of pest losses              |
| Continuous non- <i>Bt</i> maize without soil insecticide/seed treatment  | Increase susceptible population and reduce selection pressure; increase relative impacts of potential fitness costs | Resistant population will also increase and may increase resistance in nearby fields via dispersal  | Increase in population size can increase risk of pest losses         |
| Farm-wide management; Area-wide management                               | Reduce selection pressure, select against resistance, and limit spread of resistance                                | More complicated management increases likelihood of mistakes  | More complicated management increases likelihood of mistakes         |

*Bt* traits refer only to those targeting corn rootworm.

<sup>a</sup> These tactics are possible, but not recommended.

rootworms resistant to one toxin are also resistant to the other (Gassmann et al. 2014, Wangila et al. 2015).

Alternatively, farmers could replace a hybrid containing a single compromised trait with one that contains two corn rootworm *Bt* traits. Such pyramided hybrids are an excellent IRM practice to use before resistance develops to one of the traits. Currently, all commercial pyramided corn rootworm *Bt* hybrids contain either Cry3Bb1 or mCry3A (Carrière et al. 2015), so all are less than fully effective in delaying resistance in areas where resistance has developed. Reliance on pyramided hybrids after resistance has developed against one of the toxins is not as effective, because the already compromised trait exposes the second trait to direct selection for resistance (Roush 1998, Gould et al. 2006, Onstad and Meinke 2010). Nevertheless, in many cases where *Bt* resistance is suspected or confirmed, the compromised trait may not be wholly ineffective. In short, planting a pyramid containing a trait of declining effectiveness is better for IRM than planting an uncompromised single-toxin hybrid.

If a suitable pyramided hybrid is not available, it is advisable to plant a maize hybrid without a rootworm *Bt* trait, but with a soil insecticide to protect the crop from yield loss. Planting non-*Bt* maize may reduce resistance allele frequencies if a fitness cost is associated

with resistance (Gassmann et al. 2009). Unfortunately, such costs seem to be low in *Bt*-resistant western corn rootworm (Oswald et al. 2012; Petzold-Maxwell et al. 2012; Devos et al. 2013; Hoffmann et al. 2014, 2015). The alternative, treatment of *Bt* maize with a soil insecticide, does not decrease root injury or increase yield (Petzold-Maxwell et al. 2013a, Tinsley et al. 2015). This occurs because soil insecticides are applied in a band to protect only the root crown, but they do not substantially reduce the number of rootworm adults emerging from a field when typical density-dependent mortality is occurring (Gray et al. 1992). Petzold-Maxwell et al. (2013a) concluded that any additional mortality provided by the insecticide was too low to slow the evolution of resistance. Furthermore, use of a soil insecticide in *Bt* maize increases the usual delay in adult emergence in *Bt* maize relative to refuge, which can exacerbate assortative mating and accelerate resistance evolution (Petzold-Maxwell et al. 2013a, Frank et al. 2015). In short, the combination of soil insecticides and a corn rootworm *Bt* hybrid does not reduce selection on the *Bt* trait, promotes assortative mating of resistant individuals, and offers little or no short-term economic advantage to the farmer compared to growing a non-*Bt* hybrid protected with a soil insecticide.

Adulticides are sometimes used to protect maize from adults feeding on maize silks, or to reduce adult population size and thus oviposition in the field (Pruess et al. 1974, Meinke et al. 1998). This tactic may have value at the unexpected injury stage to reduce reproduction by and dispersal of resistant adults (Porter et al. 2012, Cullen et al. 2013), but timing of the adulticide application is critical to ensure that gravid females are optimally targeted before substantial oviposition occurs.

Increasing refuge size substantially (Tabashnik and Gould 2012) and growing more continuous non-*Bt* maize without soil insecticide or seed treatment could be effective IRM tactics, because they will reduce the selection pressure for resistance. These tactics, however, will increase the rootworm population size and will likely contradict the goals of IPM because they increase the risk of pest injury and are potentially costly to a grower (Onstad et al. 2003, Tabashnik and Gould 2012).

In principle, farm-wide or area-wide management would increase the options available to a farmer, because any of the previously mentioned tactics could be used to optimize IRM and IPM across multiple fields on a farm or multiple farms across an area. A farmer could choose to balance IPM and IRM benefits by focusing IRM on the fields with highest resistance risk and focusing IPM on fields with highest pest risk. As a hypothetical example that remains to be tested, if a part of the farm has an area of unexpected injury, a farmer could plant a trap crop nearby to concentrate oviposition of resistant rootworms in a small field. Rotating that field to another crop the next year would kill the *Bt*-resistant offspring. Area-wide management of western corn rootworm may be difficult to coordinate, but could provide substantial benefits. For example, area-wide releases of sterile male pink bollworm in conjunction with near 100% adoption of pyramided *Bt* cotton in Arizona reduced pest abundance >99% without accelerating the development of resistance (Carière et al. 2001a,b, 2003; Tabashnik et al. 2010).

### Policies and Incentives

Economic forces and government policies influence pest management practices immensely, including those for IRM. High maize prices, commodity subsidies, crop insurance, and biofuels policies have created incentives to plant more maize and rotate crops less often. More importantly, corn rootworm *Bt* maize has enabled farmers to respond to these incentives, at least in the short-term, by substituting *Bt* maize for crop rotation and soil insecticides to manage corn rootworm (Osteen and Fernandez-Cornejo 2013). This substitution was particularly attractive because of the large nonpecuniary benefits and risk reduction provided by corn rootworm *Bt* maize (National Research Council 2010, Shi et al. 2013). Regulatory approval of seed mixtures (i.e., refuge in a bag) and the crop insurance biotech yield endorsement (BYE; only available 2008–2011) also contributed to increased adoption of corn rootworm *Bt* maize (Onstad et al. 2011, Hurley and Mitchell 2013).

As a consequence of all of these factors, US acreage planted to maize increased 21% between 2003, when corn rootworm *Bt* maize was introduced, and 2013 (USDA-NASS 2014). The most recently available public data also show the percentage of acres planted to continuous maize increased from 21% in 2000 to 29% in 2010 (Osteen and Fernandez-Cornejo 2013), with corn rootworm *Bt* maize constituting half of all maize acreage planted as of 2011 (Marra et al. 2012).

Sound policies are needed to counterbalance these economic forces, which are inadvertently contributing to the rapid evolution of resistance. We recommend experimentation with programs that use voluntary financial incentives to encourage farmers to rotate crops, management options, and/or transgenic traits, although the latter is constrained by cross-resistance issues. These could resemble the Natural Resources Conservation Service's Environmental Quality Incentives Program and Conservation Stewardship Program, company rebate programs, or the Risk Management Agency's previous crop insurance premium reductions through the BYE. More aggressive programs may ultimately be needed if these voluntary measures are not sufficient. For example, IRM, IPM, or both, could be added to the eligibility requirements for crop insurance or participation in other federal programs, similar to conservation compliance requirements for federal crop insurance programs authorized by the 2014 Farm Bill.

### The Future

IRM and IPM must be integrated to complement one another. Yet, farmers and crop consultants must, of course, manage corn rootworm within a season and for a given field in ways that are practical and economical. Nevertheless, they should be encouraged to develop management strategies that extend beyond one season and consider the entire farm, and to use tactics that meet the goals of both IPM and IRM (Table 2). Farmers should be encouraged to move away from a mentality of "what trait do I use" to a multifaceted pest management approach. This integrated approach should start as soon as a new technology is commercialized, so that it can be more effectively stewarded by reducing the rate of resistance evolution, especially for traits with less than a high-dose. New biotechnology traits take a long time to develop and register—an average of 12 yr for maize, with more than 5 yr needed to proceed through the regulatory process alone (McDougall 2011, Fuglie et al. 2012). Loss of a transgenic trait to resistance leaves a much bigger gap in the farmer's management options than did the loss of a chemical insecticide in earlier decades.

We propose a proactive adaptive IRM and IPM strategy to delay resistance evolution to the less than high-dose corn rootworm *Bt* maize hybrids (Fig. 1b). The need for multitactic approaches likely will become increasingly generalized, because future transgenic traits for management of corn rootworm also appear to be less than high-dose, and will likely have short durability in the field under current IRM practices. Maintaining the efficacy of less than high-dose traits over

the long term will require cooperation and integration of planning and managing at all levels—crop consultants, farmers, private technology providers, public extension, public-sector scientists, regulatory agencies, and seed companies.

### Acknowledgments

We thank Eileen Cullen, Mike Gray, Tom Hunt, Chuck Mason, Ken Ostlie, and Blair Siegfried for discussions early in the development of this paper. This work was supported by the USDA Regional Research Committee NC-205 and the USDA Multistate Research Coordinating Committee NCCC-46, and with funds from Agricultural Experiment Stations in Minnesota, Iowa, Illinois, and Nebraska. Mention of a proprietary product does not constitute an endorsement or a recommendation by the above Universities or USDA for its use.

### Author Contributions

D.A.A. organized and led workshops at meetings of the NCCC46 and NC205 corn insect technical committees and stakeholders to formulate the issues and recommendations to be presented in the manuscript; wrote the first draft; and coordinated early revisions among authors. T.W.S. coordinated later revisions; finished the final draft; and co-coordinated submission of the manuscript to the journal with D.A.A. All authors contributed significantly to the content and writing of the paper.

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Received 28 April 2015; accepted 17 July 2015.

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