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We write as a group of public-sector corn entomologists to provide commentary about western corn rootworm (*Diabrotica virgifera virgifera*) resistance to Cry3Bb1, specifically the repeated reports of "greater than expected damage" to Cry3Bb1 rootworm-protected transgenic corn. We are troubled about the immediate implications of these observations for the durability of pyramid toxin rootworm-protected corn, as well as their potential long-term impact on corn production. This letter articulates our concerns about Cry3Bb1 resistance and then assesses the issue of resistance to Bt in the context of integrated pest management (IPM) of corn rootworm.

Executive Summary

Bt technology has been valuable in terms of reducing insecticide use and increasing farm income. The first documented case of field-evolved resistance to a Bt transgenic hybrid in the continental U.S. provides an opportunity to assess and respond to the current situation, one that should be acted upon carefully, but with a sense of some urgency. On-farm planting and other rootworm management decisions will alter the future course of resistance evolution, and we believe it is critical for industry, regulatory agencies and university and government scientists to work together to provide science-based, practical information to corn growers, consultants and the agricultural industry.

Likely contributing factors to the problem include: the widespread use of Bt corn hybrids (or Bt corn + insecticide) where it is not economically justified, the repeated deployment of hybrids expressing the same toxin in the same fields year after year, violation of stewardship requirements for refuges, and decreased options to employ alternative forms of pest management. Effective long-term corn rootworm management and sustainable use of Bt hybrid technology require an integrated approach that is not overly reliant on any single tactic.

Experience with commercialized rootworm-protected transgenic corn

Rootworm-protected transgenic corn hybrids were initially marketed to prevent economic yield loss while simultaneously reducing the use of broad-spectrum soil insecticides. They have proven to be an effective and environmentally responsible means of controlling corn rootworm.

Hybrids expressing the first rootworm Bt, Cry3Bb1 (trade named YieldGard® RW), were commercially planted in 2003, followed by those expressing Cry34/35Ab1 (trade named Herculex® RW) in 2005 and mCry3A (trade named Agrisure® RW) in 2006. All of these hybrids had a required 20% non-Bt refuge. More recently, hybrids with pyramids of rootworm traits Cry3Bb1 + Cry34/35Ab1 (trade named SmartStax®) and mCry3A + Cry34/35Ab1 (trade named Agrisure 3122™ Refuge Renew) were approved with a reduced refuge size. Delivery of the refuge "in the bag" had previously been approved for Cry34/35Ab1 (trade named Optimum AcreMax™ RW) at 10% refuge and is now approved for Cry3Bb1 + Cry34/35Ab1 at 5% refuge.

Convergence of evidence on field-evolved resistance

Greater than expected damage to Bt corn hybrids expressing the Cry3Bb1 protein was first observed across a wide geographic area during the 2009 growing season. By 2011, problem areas had been reported in northwestern and north-central Illinois, northeastern Iowa, southern Minnesota, northeastern Nebraska, and eastern South Dakota. Common features of affected fields in these areas included a history of continuous planting to corn and the use of Cry3Bb1-expressing hybrids for multiple years.

The first published report of field-evolved resistance by western corn rootworm to a Bt toxin, Cry3Bb1, also appeared in print in 2011. In this peer-reviewed paper, Gassmann et al. (2011) confirmed rootworm resistance to Cry3Bb1 corn and demonstrated that this was not accompanied by an increase in tolerance to Cry34/35Ab1 corn.

The circumstances surrounding the appearance of field-evolved resistance and its documentation by Gassmann et al. (2011) are consistent with laboratory selection studies, which revealed rapid evolution of resistance to Cry3Bb1 in nine of nine experiments (Meihls et al. 2008, Meihls 2010, Oswald et al. 2011). All available evidence thus converges in implicating field-evolved resistance to Cry3Bb1 as the most likely cause of "greater than expected damage" in rootworm problem fields.

Resistance to Cry3Bb1 threatens hybrids carrying two toxins

Confirmation of Cry3Bb1 resistance in field populations of western corn rootworm raises deep concerns about the durability of the Cry3Bb1 + Cry34/35Ab1 toxin pyramid in SmartStax hybrids. The appearance of Cry3Bb1 resistance is particularly troubling given the decreased non-Bt refuge requirements (from 20% to 5%) for these hybrids.

Reduction in refuge size was, of course, predicated on the effectiveness of both toxins against corn rootworm. Under these conditions, evolution of resistance to either single toxin would be slowed in fields having doubly susceptible insects. But this assumption is no longer valid in problem areas such as those described above, which are characterized by reduced efficacy of Cry3Bb1. Here Cry34/35Ab1 receives only partial protection from Cry3Bb1 and is vulnerable to insects quickly evolving resistance, especially with only a 5% refuge.

Continued reliance on smaller refuges in conjunction with pyramids planted in problem areas may slow the spread of Cry3Bb1 resistance into susceptible areas. This would occur because smaller refuge size reduces the total number of rootworms carrying resistance alleles. Thus, the Cry34/35Ab1 toxin serves to decrease the size of the local population where Cry3Bb1 resistance is building, in the process limiting the total number of Cry3Bb1 resistance alleles that can be spread by emigrating beetles.

However, use of the smaller refuge size in problem areas to slow resistance evolution to Cry3Bb1 would likely have the opposite effect of hastening evolution of resistance to Cry34/35Ab1. This is highly undesirable, because it would compromise the durability of the Cry34/35Ab1 proteins in both current and future non-pyramided and pyramided Bt corn hybrids. We strongly recommend that this possibility be taken into account in determination of the appropriate refuge size for SmartStax corn in problem areas of resistance to Cry3Bb1. It is crucial that susceptibility to Cry34/35Ab1 be preserved, in part because it has now been approved in pyramid with mCry3A and is the common toxin in two different pyramids from two registrants. A third registrant is also seeking to register mCry3A+Cry34/35Ab1.

The ultimate impact of increasing the SmartStax refuge requirement in problem areas hinges on allele frequency and fitness costs, and more research is required before we can assess the impact. If the Cry3Bb1 resistance allele frequency is low and fitness costs are high, then planting a larger refuge would likely manage both Cry3Bb1 resistance as well as delay Cry34/35Ab1 resistance evolution. However, if the frequency of Cry3Bb1 resistance alleles is high and fitness costs are low, then planting a larger refuge in problem areas could lead to a population density increase of Cry3Bb1 resistant insects over time, because resistant insects are not exposed to Cry34/35Ab1 on non-Bt plants. This in turn would accelerate spread of local Cry3Bb1 resistance. Additionally, in this scenario if there is cross resistance to Cry3Bb1 and mCry3A then resistance to mCry3A could be accelerated.

What have we learned and what can be done to protect future transgenics

The appearance of resistance to Cry3Bb1 corn has reinforced some of our underlying concerns about the role of Bt hybrids in IPM of corn rootworm. Rootworm-protected transgenic corn was introduced into an existing IPM-based system that promoted multiple practices to control the insect. These include crop rotation, scouting, and application of insecticides when and where necessary.

Although many factors come into play, the always “on” nature of transgenic toxins means they cannot be deployed or withdrawn in response to changing pest densities. Selection for resistance thus occurs wherever Bt corn is grown and susceptible insects are present. The response to this selection depends on many factors including resistance allele frequency, fitness costs, toxin dose etc. Avoidance of undue resistance risk forms the rationale for Insect Resistance Management (IRM) plans that are required for Bt crops. The current toxins deployed for control of corn rootworm are considered low to moderate dose, and it is more difficult to prevent resistance to low to moderate dose toxins than it is to the high dose Bt toxins deployed against Lepidoptera. For some Lepidoptera, especially the stalk borers European corn borer and southwestern corn borer, the toxins in Bt corn are truly high dose. However, they are less than high dose for other key Lepidoptera species like fall armyworm, corn earworm and western bean cutworm.

- *Rotate Bt corn hybrids to expose rootworms to different Bt toxins*

An essential component of IPM as practiced with conventional insecticides is the alternation of modes of action to avoid repeated selection, and a fundamental principle of resistance management is to cease use of an insecticide if resistance is developing. Rotation of toxins in hybrids as a strategy has been neglected as transgenic corn acreage has increased across the Corn Belt. In areas with significant corn rootworm pressure, hybrids expressing the same toxin(s) are often planted in the same field year after year. This practice is not a sound component of effective IPM.

- *Plant non-Bt corn and avoid prophylactic planting of Bt corn*

As described above, substitution of Cry34/35Ab1 or Cry3Bb1 + Cry34/35Ab1 hybrids for Cry3Bb1 hybrids may slow evolution of resistance to Cry3Bb1. Unfortunately, this could actually accelerate resistance development to Cry34/35Ab1, especially with reduced refuge. In short, planting more of a failing toxin and/or more of an effective toxin over a larger area poses significant risk.

Rootworm-protected Bt corn is being used prophylactically in areas with little or no need for it. This unwarranted use occurs in part because genes to produce rootworm Bt toxins (and toxins active against Lepidoptera) are incorporated into elite germplasm with the highest yield potential. Thus growers often have few options other than to plant stacks and pyramids if they wish to use the hybrids with best yield potential. When growers do not want to use Bt corn, many report increasing difficulty in obtaining non-transgenic seed. Scarcity of non-Bt seed may become more acute as the seed industry transitions to a refuge "in the bag" approach for resistance management.

Planting non-Bt corn can be profitable and should be one of the IPM tools to maintain susceptibility to rootworm-protected transgenic corn. After all, whether used in conjunction with soil-applied insecticides or not, conventional hybrids cause no selection for resistance to any Bt toxin. It is ironic that the decreasing availability of non-Bt hybrids erodes the ability of producers to move to a more integrated system of corn rootworm management, one that protects the value of Bt hybrids. As a component of effective IPM for corn rootworms, attention should be given to increasing the supply of elite hybrids that do not contain Bt.

- *Bt Resistance has real economic and environmental costs*

IPM emphasizes a minimal environmental footprint that is consistent with grower profitability, and it is crucial to note that resistance to Cry3Bb1 corn threatens both. Reduction in the use of broad-spectrum insecticides has been asserted to be one benefit of the registration of Bt rootworm corn. Yet in problem areas where Cry3Bb1 resistance is present, the management plan proposed by the registrant recommends the use of soil insecticides against larvae and/or foliar insecticide sprays against adult beetles in conjunction with Bt corn hybrids.

We can envision that multiple approaches might be necessary under special circumstances if, for example, growers in problem areas have purchased Cry3Bb1 seed for the coming season. But in general, treatment of rootworm protected transgenic corn with insecticides is not a recommended control strategy. It elevates production costs, reduces profits, selects for resistance to the insecticides, and masks the geographic extent and in-field severity of Cry3Bb1 resistance.

Conclusions

The widespread recommendations to apply insecticides to protect transgenic Bt corn rootworm corn strikes us as a clear admission that the Cry3Bb1 toxin is no longer providing control adequate to protect yield, and that economic value derived from the toxin is declining. Pyramided Cry3Bb1 + Cry 34/35Ab1 corn should not need insecticidal protection to protect yield given that the Cry34/35Ab1 toxin is still effective. Any insecticide use would therefore be for preservation of rootworm susceptibility to the toxins. It is unfortunate that the widespread adoption of transgenic technology has now left many growers without the equipment necessary to apply soil insecticides if needed. Similarly, Cry34/35Ab1 corn should not need insecticide other than as protection for the 20 percent refuge in areas with extreme rootworm populations.

Finally, we note that there is an escalating use of insecticides directed at western and northern corn rootworm in areas of the Corn Belt where rootworm densities are low and the likelihood of economic injury is minimal. When insecticides overlay transgenic technology, the economic and environmental advantages of rootworm-protected corn quickly disappear. We are concerned that high commodity prices and other factors may have fueled an insurance-based approach to corn rootworm management, one that violates many tenets of IPM and that will only increase insect resistance development in the long term.

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Signatures follow on the next page.

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