Rootworm Management with Genetically Modified Corn: Current Status, Potential for Resistance, and a Look Toward the Future

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The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is one of the most serious crop pests in North America and Europe. WCR feeding causes reduced water and mineral uptake. Yield loss is most severe when water is limiting. Grain from damaged plants is also more likely to be left in the field due to increased lodging of plants with reduced brace root support. WCR are univoltine, with eggs overwintering in the soil. There are three larval instars, with the bulk of the damage being caused by third instar.

Transgenic corn (Bt corn[1]) that controls WCR damage by producing a Cry endotoxin (Cry3Bb1) from the soil bacterium *Bacillus thuringiensis* (Bt) was commercialized in 2003 and two additional products expressing different proteins were registered for commercial sale in 2005 (Cry34/35Ab1) and 2007 (mCry3A). The Environmental Protection Agency (EPA) has mandated that all registrants submit an Insect Resistance Management (IRM) Plan prior to registration of any Bt crop. IRM (i.e., maintaining pest susceptibility to control measures) can extend the lifetime of management options. The high-dose/refuge strategy for IRM proscribes planting of Bt crops that produce a very high concentration of toxin (25 times the amount needed to kill 99% of the susceptible insects) to ensure that individuals that are heterozygous for resistance do not survive on the Bt crop, thus making resistance functionally recessive (EPA 1998). In addition, a nearby refuge is maintained where the pests do not encounter Bt toxin. It is expected that a large number of susceptible pests emerging from the refuge will mate with any resistant individuals emerging from the Bt field to produce heterozygous susceptible offspring and thus delay the evolution of pest resistance.

Although even within a transgenic event different hybrids can provide drastically different control (Gray et al. 2007), overall, transgenic corn targeted toward corn rootworm management has been extremely effective as indicated by its increasing market share (USDA-ERS 2008). It is clear that each of the three registered rootworm transgenic products have met the original criteria to provide rootworm protection “as good or better than soil insecticides”.

The predicted duration of susceptibility of insect pests to Bt toxins depends on many factors including dose of the toxin. The Bt corn currently registered for control of WCR is not high-dose, but rather is considered low-to-moderate (Siegfried et al. 2005). Similarly, Cry1Ac targeting the lepidopteran *Heliocoverpa zea* in cotton is not high-dose (EPA 1998). An increase in resistance allele frequency has been reported for several field populations of *H. zea*

[1]Throughout the proposal, Bt corn refers to corn targeted toward corn rootworms, *not* transgenic corn targeted to the European corn borer. Isoline corn refers to non-transgenic (non-Bt) corn of similar genetic background.

(Tabashnik et al. 2008a; though the conclusions are controversial – see Moar et al. 2008, Tabashnik et al. 2008b), but not for *Heliothis virescens* or most other major lepidopteran pests targeted by Bt cotton or other crops where the high-dose standard is met. This supports modeling predictions that pests are at greater risk of evolving resistance if managed by Bt crops that are not high-dose (Tabashnik et al. 2004). Although rootworm-targeting Bt corn provides good protection of grain yield, it is common to observe adult WCR emerging from all of the rootworm Bt products currently available. WCR beetle emergence from plots of Bt corn expressing Cry34/35Ab1 proteins averaged 3.53% that of isoline plots (Storer et al. 2006). Emergence from transgenic plots expressing the mCry3A protein or the Cry3Bb1 protein, which are also currently registered for rootworm control, were similar (unpublished data, BEH and colleagues). Clearly, none of the transgenic events currently registered for WCR control expose larvae to a level considered high-dose. It is not known what proportion of survivors of WCR-targeted Bt corn have a susceptible genotype through escaping lethal exposure to the toxin or what proportion, if any, are genetically resistant.

We (Meihls et al. 2008) devised a greenhouse method of rearing WCR on transgenic corn expressing the Cry3Bb1 protein was used in which ~25% of previously unexposed larvae survived relative to isoline survival (compared to 1 to 4% in the field). After three generations of full larval rearing on Bt corn (Constant-exposure colony), WCR larval survival was equivalent on Bt corn and isoline corn in greenhouse trials, and in diet bioassays with Cry3Bb1 protein on artificial diet, the LC50 was 22-fold greater for the Constant-exposure colony than the Control colony. After six generations of greenhouse selection, the ratio of larval recovery on Bt corn to isoline corn in the field was 11.7-fold greater for the Constant-exposure colony than the Control colony. Removal from selection for six generations did not decrease survival on Bt corn in the greenhouse. The results of Meihls et al. (2008) suggest that rapid response to selection is possible in the absence of mating with unexposed beetles, emphasizing the importance of effective refuges for resistance management.

Pioneer scientists reported survivorship of two WCR populations on Cry34/35Ab1 (Lefko et al. 2008). Survivorship increased 15.1 and 58.5 fold for populations from Rochelle, IL and York, NE, respectively, selected to survive on event DAS-59122-7 containing the Cry34/35Ab1 protein, though survivorship never exceeded 30% isoline surviviorship. Lefko et al. concluded that "major resistance gene(s) are rare in populations of *D. v. virgifera* in the United States, and that a minor trait(s) conferring a low level of survival on DAS-59122-7 maize was present. The tolerance trait identified in this study was considered minor with respect to its impact on DAS-59122-7 maize efficacy, and the role this trait may play in total effective refuge for major resistance genes with recessive inheritance is the basis of future work." Raw data from Storer et al. (2006) indicated that 3.53% of feral beetles survives Cry34/35Ab1 relative to isoline survival. Initial survivorship relative to isoline survivorship in Lefko et al. (2008), was initially 0.4% and 1.3% on the seedling corn used for rearing (root tips have a higher protein level and more root tips are present in seedling corn). With selection, this increased to 23.4% and 19.7%, respectively. If field survivorship increased on the order of magnitude as did survivorship under colony conditions, we would expect damage to increase in a similar fashion.

Planting of Bt crops imposes intense selection on some key agricultural pests to evolve resistance. Recently, cases of field evolved resistance to Bt crops have been documented for several crop pests (van Rensburg 2007, Matten et al. 2008, Tabashnik et al. 2008a). Planting of transgenic Bt corn for control of corn rootworm is increasing rapidly, and in 2007, Bt corn for control of rootworm and other corn pests covered 50% of all corn acres in the United States (James et al. 2007). Thus, WCR may evolve resistance to Bt corn unless appropriate IRM plans are in place.

Few insect pest problems have presented more challenges to U.S. growers than rootworms. In the past, more hectares of crop land received insecticide applications for the two major species of *Diabrotica* corn rootworms than for any other U.S. agricultural pest (Suguiyama and Carlson 1985). Yield losses and control costs have long exceeded $1 billion each year (Metcalf 1986). Because of recent changes in their distribution (now in Europe, Sivcev et al. 1994), biology (resistance to crop rotation, Levine et al. 2002, Krysan et al. 1986), and increased continuous corn due to the push for biofuels, the impact of corn rootworms is growing. Until rootworm-targeted Bt corn was registered for commercial sale in 2003, no practical alternatives to the use of insecticides for corn rootworm control were available in areas with resistance to crop rotation or in continuous corn (Levine and Oloumi-Sadeghi 1991). Now, with Cry3Bb1, Cry34/35Ab1 and mCry3A Bt corn, the registration of insecticidal seed treatments such as Poncho™ (clothianidin) and Cruiser® (thiamethoxam), and many of the traditional management tools still available in most areas, it could be argued that never before in the history of corn rootworm management have corn producers been able to select from such a diverse variety of rootworm management tactics.

Unfortunately, most of the current options for rootworm management, including crop rotation, controlling adults to prevent egg laying, granular and liquid soil insecticides, insecticidal seed treatments, and Bt corn, have issues reducing their effectiveness or do not work in certain areas. Crop rotation is no longer an option over an area that is expanding each year (Gray et al. 2009). WCR have developed resistance to the primary insecticides which have been used for adult control in the region of the Corn Belt employing this control tactic (Meinke et al. 1998). In this region, WCR larvae are also resistant to some of the soil insecticides (Wright et al. 2000). Two other issues are affecting granular insecticides as a management option. First, John Deere's best-selling new planters no longer come with the option of granular insecticide boxes, hindering that option for some growers in all regions. Second, it is possible that several of the current rootworm granular insecticides could be lost due to the Food Quality and Protection Act of 1996. Even if granular insecticides are not pulled from the market and are still effective, they are not the preferred option of many growers. In a survey of 400 growers who grew at least 100 acres of corn (200 grew Bt corn in 2003 and 200 did not, average farm size 550 acres), the number one reason given for using Bt corn for rootworm control was grower safety (Stevan Madjarac, The Context Network, Des Moines IA, personal communication). The response was the same from growers with Bt corn and those without. Lastly, although insecticidal seed treatments work relatively well under moderate infestations, such as those in Missouri in 2004 (Wayne Bailey, personal communication), root protection is less adequate under high pressure often seen in Illinois (Estes 2004). It seems that many have been assuming that Bt corn will address the above problems. The results of Meihls et al. (2008) and Lefko et al. (2008) suggest that rapid response to selection is possible in the absence of mating with unexposed beetles, emphasizing the importance of effective refuges for resistance management.

The future of rootworm management with transgenic corn remains uncertain. EPA's Office of Pesticide Programs (OPP) has received an application of registration from Pioneer Hi-Bred International, Inc. for registration of Optimum® AcreMax™ 1 Insect Protection, which is a corn seed blend containing seeds that express the Bt toxins Cry34Ab1, Cry35Ab1, and Cry1F in a stack for corn rootworm and lepidopteran protection mixed with seeds that express only Cry1F for lepidopteran protection. Pioneer proposes to use a seed blend mixture containing ≥2% refuge seeds. Monsanto company and Dow Agrisciences have also submitted application materials to register SMARTSTAX™ (*Bacillus thuringiensis* (B.t.) CRY1A.105, CRY2Ab2, CRY1F, CRY3Bb1, CRY34/35Ab1 proteins and the genetic material necessary for their production in SMARTSTAX corn). Regulatory decisions on these two applications are expected to be made this year.

References Cited

Estes, R. 2004. 2004 Evaluations of Rootworm Control Products. The Bulletin, No. 22. (http://www.ipm.uiuc.edu/bulletin/article.php?issueNumber=22&issueYear=2004&articleNumber=2).

E.P.A. 1998. The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management (Environmental Protection Agency, Washington, D.C., U.S.A.).

Gray, M. E., K. L. Steffey, R. E. Estes, and J. B. Schroeder. 2007. Responses of transgenic maize hybrids to variant western corn rootworm larval injury. J. Appl. Entomol. 131: 386-390.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, and M. O. Bohn. 2009. Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Ann. Rev. Entomol. 54: 303-321.

James, C. 2007. Global Status of Commercialized Biotech/GM Crops: 2007. ISAAA Brief No. 37. ISAAA, Ithaca, NY..

Krysan, J. L., D. E. Foster, T. F. Branson, K. R. Ostlie, and W. S. Cranshaw. 1986. Two years before the hatch: rootworms adapt to crop rotation. Bull. Entomol. Soc. Am. 32: 250-253.

Lefko S. A., T. M. Nowatzki, S. D. Thompson, R. R. Binning, M. A. Pascual, M. L. Peters, E. J. Simbro, and B. H. Stanley. 2008. Characterizing laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae) selected for survival on maize containing event DAS-59122-7. J. Appl. Entomol. 132: 189-204.

Levine, E. and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. Ann. Rev. Entomol. 36: 229-255.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Gray. 2002. Adaptation of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), to crop rotation: Evolution of a new strain in response to a cultural management practice. Amer. Entomol. 48: 94-107.

Matten, S. R., G. P. Head, and H. D. Quemada. 2008. How governmental regulation can help or hinder the integration of Bt crops with IPM programs. *In* J. Romeis, A. M. Shelton and G. G. Kennedy [eds.], Integration of Insect Resistant Gentically Modified Crops within IPM Programs. Springer, New York.

Meihls, L. N., M. L. Higdon, B. D. Siegfried, T. A. Spencer, N. K. Miller, T. W. Sappington, M. R. Ellersieck, and B. E. Hibbard. 2008. Increased survival of western corn rootworm on

transgenic corn within three generations of on-plant greenhouse selection. Proc. Nat. Acad. Sci. 105: 19177-19182.
Meinke, L. J., B. D. Siegfried, R. J. Wright, and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.
Metcalf, R. L. 1986. Foreword, pp. vii-xv. *In* J. L. Krysan & T. A. Miller [eds], Methods for the study of pest *Diabrotica*. Spring-Verlag, New York.
Moar, W., R. Roush, A. Shelton, J. Ferre, S. MacIntosh, B. R. Leonard and C. Abel. 2008. Field-evolved resistance to Bt toxins. Nat. Biotech. 26: 1072-1074.
Siegfried, B. D., T. T. Vaughn, T. Spencer. 2005. Baseline susceptibility of western corn rootworm (Coleoptera: Crysomelidae) to the Cry3Bb1 *Bacillus thuringiensis* toxin. J. Econ. Entomol. 98: 1320-1324.
Sivcev I., B. Manojlovic, S. Krnjajic, N. Dimic, M. Draganic, F. Baca, Z. Kaitovic, R. Sekulic, and T. Keresi. 1994. Distribution and harmful effect of *Diabrotica virgifera* LeConte (Coleoptera, Chrysomelidae), a new maize pest in Yugoslavia. Zastita Bilja. 45: 19-26.
Storer, N.P., J.M. Babcock, and J.M. Edwards. 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99:1381-1387.
Suguiyama, L. F. and G. A. Carlson. 1985. Field crop pests: farmers report the severity and intensity. USDA, Economic Research Service, Agriculture Information Bulletin 487.
Tabashnik, B.E., F. Gould, and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J. Evol. Biol. 17: 904-12.
Tabashnik, B. E., A. J. Gassmann, D. W. Crowder, and Y. Carrière. 2008a. Insect resistance to Bt crops: Evidence versus theory. Nat. Biotech. 26: 199-202.
Tabashnik, B. E., A. J. Gassmann, D. W. Crowder, and Y. Carrière. 2008b. Field-evolved resistance to Bt toxins: reply to Moar et al. Nat. Biotech. 26: 1074-1076.
USDA-ERS 2008. Bt maize adoption in the U.S., 2000-2007. http://www.ers.usda.gov.
van Rensburg, J. B. J. 2007. First report of field resistance by stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. S. Afr. J. Plant Soil 24: 147-151.
Wright, R. J., M. E. Scharf, L. J. Meinke, X. Zhou, B. D. Siegfried, and L. D. Chandler. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93: 7-13.