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**Rootworm behavior and resistance in Bt cornfields**

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**Introduction**

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte, WCR) is the most economically important U.S. corn pest (Gray et al. 2009). Together with the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence, NCR), it was estimated that these pests annually cost producers $1 billion in yield losses and control costs (Metcalf 1986). Today the cost is thought to be much greater (Dun et al. 2010; Tinsley et al. 2013).

Corn rootworm life cycles are closely tied to that of corn (Levine and Oloumi-Sadeghi 1991). WCR eggs hatch in late May or early June. The tiny newly-emerged “neonate” larvae use the CO$_2$ emissions from growing corn roots to locate nearby hosts. Larval root-feeding causes most of the yield losses and costs associated with this species, while the adults feed on corn foliage, tassels, silks, and kernels (Branson and Krysan 1981, Moser and Vidal 2005). Adult emergence begins in late June. Mate-seeking males locate newly-emerged females and mate in cornfields. Within a few days of mating, a portion of the females disperse from the field where they emerged; they may be carried long distances on prevailing winds to distant cornfields. The remaining females feed and within a week or two begin laying the first of several 100's of eggs in the soil. Egg-laying females use cracks and crevices to locate moist, protected locations. Most eggs overwinter, or diapause, in the top 10 inches of soil.

**Managing corn rootworms**

A strong egg-laying fidelity to cornfields and the larval dependence on corn roots led to the original recommendation of annual crop rotation with a non-host crop (like soybean) as a corn rootworm management tactic (Forbes 1883, Gillette 1912). Corn rootworm larvae emerging in soybean fields will starve and die. Crop rotation remains one of the most effective methods of rootworm control in the Corn Belt and elsewhere, with some notable exceptions.

Their long history of field-evolved resistance is a key reason why rootworms are such a threatening pest (Gray et al. 2009). Where crop rotation could not be practiced, growers relied on soil-applied and broadcast insecticides to control the root-feeding larvae and egg-laying adults beginning in the late 1940s. From the late 1950s to the present, rootworms have evolved resistance to multiple classes of insecticides. Most recently, WCR resistance to the pyrethroid insecticide, bifenthrin, was documented in Western Nebraska and Kansas (Pereira et al. 2015).

In the mid-1980s, the great efficacy of crop rotation against WCR lead to its undoing. By 1995, the corn and soybean rotation had selected for females with reduced egg-laying fidelity to cornfields. The result was a pest that laid eggs everywhere, including the soybean fields where corn would grow the following year. Some Illinois and Indiana growers experienced 50% yield loss in corn after soybeans in 1995 (Levine et al. 2002). Decades earlier, the NCR had evolved resistance to crop rotation by delaying the normal time of egg hatch by prolonging egg diapause during the winter (Chiang 1965). Rather than hatching the next summer, a portion of the eggs remained in diapause for two winters (or more) and hatched when corn was again planted in the field. The evolution of WCR behavioral resistance to crop rotation resulted in expanded use of soil-applied insecticide on millions of rotated corn acres (Levine et al. 2002). The alarming escalation of soil-applied insecticide use was one of the motivating factors driving interest in rootworm management alternatives—like Bt corn.
Bt corn hybrids for rootworm management

Commercial sales of Bt corn hybrids expressing Bt toxins that were protected from corn rootworm larval injury were first approved by the U.S. EPA in 2003 (EPA 2003). Additional Bt toxins have since been registered and commercialized in other Bt corn hybrids (Tabashnik and Carrière 2017). In-plant expression of toxic proteins from the soil microbe Bacillus thuringiensis (Bt) provided targeted control of corn rootworm larvae with an efficacy equivalent to that of soil insecticides, but without the human health risks and environmental concerns associated with broad-spectrum insecticides (Rice 2004).

The threat of rootworm resistance to Bt technology factored into discussions of how best to deploy Bt corn to delay the inevitable evolution of resistance (Tabashnik and Gould 2012). Data on WCR biology and behavior from the published literature were used in computer models to evaluate the durability of Bt corn under a variety of deployment scenarios. Rapid evolution of insect resistance to Bt toxin(s) would be slowed by requiring that a contiguous block or broad strips of each Bt cornfield were planted with a “refuge” of non-Bt corn (initially 20% of total field area). Refuges of non-Bt corn would provide places where rootworms with susceptibility to Bt toxins could survive.

The Bt-susceptible refuge rootworm beetles were expected to disperse into nearby Bt corn where they would mate with any rare, potentially resistant beetles that had survived on Bt plants (Tabashnik and Gould 2012). Because Bt-susceptible refuge beetles would vastly outnumber any Bt-resistant survivors, it would be highly unlikely that pairs of Bt-resistant beetles would ever mate, and thus few resistant offspring would be produced. Planting refuges was a key element of the Insect Resistance Management (IRM) plan designed to delay the evolution of rootworm resistance to Bt corn. Other expectations about rootworm biology/behavior in Bt cornfields (e.g., resistance alleles were initially rare in rootworm populations) and characteristics of the Bt plants (e.g., Bt toxin levels in plant roots would kill a very high proportion of larvae) were also integral to the plan’s success. In addition, growers were expected to adhere to refuge requirements and plant the required percentages of refuge acres.

Studying WCR biology in Bt cornfields with refuges

In 2010, a three-year study began to document the abundance, behavior, and biology of WCR adults in large field plots of refuge and Bt corn at the University of Illinois (Hughson 2017, Hughson and Spencer 2015). The goal was to learn whether WCR beetles from refuges really would emerge, move into Bt corn and mate, as expected. Four configurations of refuge and Bt corn were used study the impact of different refuge designs on WCR biology and behavior. Refuge was deployed in contiguous blocks (i.e. Bt cornfields with 20% and 5% structured refuge blocks on one side of a field), as a seed blend where 5% of seeds in a bag of Bt seed were non-Bt refuge seeds (i.e. 5% seed blend refuge) and as a no-refuge control (i.e. 0% refuge). Nearly 35,000 individual beetles and almost 900 pairs of mating beetles were collected. Dissection and analyses revealed unexpected details of WCR abundance, movement patterns and reproductive biology.

The reality of WCR behavior was very different from the optimistic expectations of the rootworm IRM plan, especially regarding beetle movement. Before corn pollination began, an average of 17 to 25% of adults left refuges for Bt corn each day. However, once corn began pollinating and female emergence peaked in the refuges, movement of WCR of both sexes nearly stopped. Though moving beetles traveled at substantial rates (up to 31 m/day), they represented just 3 to 10% of the population during and after pollination.

Mate-seeking refuge males also did not live up to IRM expectations; they did not rapidly disperse into Bt corn. Instead, they stayed in refuge blocks where newly emerged, unmated females were already nearby and abundant. The normally delayed emergence of WCR females versus males (called protandry) was further delayed for beetles that developed on Bt plants. Thus, many refuge males emerged weeks before the females with whom they were intended to mate in Bt corn. Even if those refuge males had moved into
Bt corn, they would have been too old to mate by the time females emerged. This made mating between potentially-resistant individuals from Bt corn more likely than expected.

The distribution of mating activity mirrored overall beetle abundance; both were concentrated in blocks of refuge corn. In fields with separate refuge blocks of refuge and Bt corn, there were few (ca. 9%) matings between partners from both sides of the fields, a.k.a. “mixed matings”. When these desired pairings occurred, they were found within a few rows of the interface between refuge and Bt corn areas. Where refuge plants were growing among Bt plants (i.e. seed blends), the distribution of mating pairs across fields was uniform.

If poor dispersal of mate-seeking beetle into Bt corn was the problem with block refuges, integrating refuge plants into Bt corn should improve population mixing. However, analyses of beetles from those mating pairs revealed that regardless of whether the 5% of refuge plants were randomly scattered among Bt corn plants or deployed as a single refuge block on one side of a field, the percentage of mixed-matings between refuge and Bt beetles did not differ. Use of a seed blend to integrate refuge plants in to the Bt corn did not promote mixed-matings as anticipated. Movement and feeding data suggest that concentrations of beetles may persist in the near vicinity of isolated refuge plants in seed blends. Increasing the percentage of refuge plants in seed blends may be needed to put more refuge beetles much closer to Bt plants.

These analyses indicated that expectations for rootworm behavior in Bt cornfields were wrong at nearly every turn. Unfulfilled assumptions were compounded by other failings. Critically, corn plants did not express Bt toxins at doses high enough to kill the expected proportion of WCR larvae in Bt corn and rootworm populations had higher than expected initial levels of Bt resistance. Another critical “man-made” problem was poor compliance with refuge planting. Only 75 to 80% of growers complied with rootworm refuge requirements (Jaffee 2010, Gray 2011). As a result, even before the susceptible beetles had a chance to “do their part” to ensure well-mixed populations of mixed-mating beetles, a myriad of problems with the plan had already compromised chances for its success.

Field-evolved Bt resistance

Given the problems with refuge function, it is perhaps not surprising that field-evolved WCR resistance to Bt corn was first documented in Iowa during 2009 (Gassmann et al. 2011). Since then, rootworm resistance to multiple Bt toxins has been found across the U.S. Corn Belt. Beginning in 2012, Illinois WCR populations with resistance or significantly reduced susceptibility to multiple Bt toxins were documented from counties across the state. During 2013, broad areas in Livingston, Kankakee, and Ford counties (i.e. in east- and north-central Illinois) experienced severe injury in rotated Bt corn due to Bt-resistance in rotation-resistant WCR populations.

Heightened awareness of Illinois Bt resistance, combined with greater adoption of pyramided Bt hybrids have likely prevented subsequent “surprises”. The role of extremely wet conditions during 2015 was also important; rootworm populations were reduced to historically low levels. They continue to slowly rebuild; however, abundance is still below levels likely to inflict economic injury in most areas. In spite of low population years, bioassays show that Illinois’ WCR beetles retain high levels of resistance to some Bt toxins. There is evidence of reduced susceptibility to all commercialized Bt toxins that have been used against corn rootworms (Tabashnik and Carrière 2017).

In the coming year, a completely new mode of action (MOA) for rootworm management will be commercialized (Moar et al. 2017). Based on RNA interference (RNAi), the new MOA will kill larvae by interfering with expression of critical rootworm-specific genes. It will be pyramided with two Bt MOAs and deployed as a 5% seed blend (or with a 20% block refuge in cotton-growing areas) (EPA 2017). Hopefully, the lessons that rootworms have taught us will be applied to promote the long-term durability of RNAi corn hybrids for rootworm control.
References


