Chapter 4
Management of the Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte, Using Transgenic Bt Maize

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Abstract The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is an important pest of maize, *Zea mays* L., in the United States and Europe. In the United States, transgenic maize hybrids that express Cry endotoxin proteins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) have been developed to limit feeding damage caused by corn rootworm larvae.

Transgenic maize expressing the Cry3Bb1 protein was first registered for commercial sale in 2003, and two additional products expressing different proteins were registered for commercial sale in 2005 (Cry34/35Ab1) and 2006 (mCry3A). More recently a fourth Cry protein, eCry3.1Ab, was registered as a pyramid with mCry3A in 2013. Although the United States Environmental Protection Agency has mandated that all registrants of Bt crops submit an insect resistance management (IRM) plan prior to registration, the long-term viability of transgenic Bt maize for control of western corn rootworm remains uncertain. Under laboratory conditions, selected western corn rootworm populations have developed resistance to all commercially available Bt products as well as the eCry3.1Ab protein. In addition, field resistance to transgenic Bt maize has been documented in certain fields in the United States. To extend the lifetime of this management option for the future, additional improvements in resistance management plans will be needed.

4.1 Introduction

Maize, *Zea mays* (L.), is an economically important crop grown for a variety of purposes (e.g., food, animal feed, biofuel) throughout the world. Although maize plantings can be severely impacted by a diverse assemblage of major agricultural pests, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is

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considered one of the most economically damaging. Yield losses and control costs due to this pest are estimated at over $1 billion annually in the United States (Metcalf 1986) and €0.472 billion in Europe (Wesseler and Fall 2010).

Native to North America, the western corn rootworm was first recorded in Kansas, USA, in 1867 (LeConte 1868), though it was not recognized as a pest of maize until 1909 (Gillette 1912). It is thought that this pest species originated in Guatemala and reached the southwestern United States approximately 3,000 years ago with the spread of maize production (Melhus et al. 1954; Krysan and Smith 1987). Its range expansion and importance as a pest in the Unites States increased dramatically within the last half century largely because of continuous maize production and improving irrigation systems throughout its native range (Chiang 1973). By 2005, western corn rootworm could be found infesting maize plantings in much of the eastern United States (Gray et al. 2009). In 1992, the first detection of western corn rootworm in Europe occurred near the Surcin International Airport in Belgrade, Serbia (Baca 1994). Within 15 years it had been recorded in 20 European countries (Gray et al. 2009) through at least five independent introductions across the continent (Ciosi et al. 2008).

The ability of western corn rootworm to invade new areas, and its capacity to adapt to various control measures, has necessitated development of additional pest management tactics that can serve as stand-alone practices or be readily allied with other pest management approaches. In the United States, transgenic maize that express Cry endotoxin proteins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) have been increasingly used to mitigate the economic damage caused by this pest. The benefits of transgenic Bt maize include effective management of the target pest (Storer et al. 2006; Hibbard et al. 2011; Clark et al. 2012), decreased use of broad-spectrum insecticides (Phipps and Park 2002), and reduced impact to nontarget species (Siegel 2001; Al-Deeb and Wilde 2003; Ahmad et al. 2005; Lundgren and Wiedenmann 2002; Mullin et al. 2005; Hönemann et al. 2008). To understand why transgenic Bt maize is effective at managing western corn rootworm, we must first understand the biology and behavior of this insect. We must then consider other management practices that are associated with controlling this pest. Efficient control of western corn rootworm requires a broad understanding of current management tactics and the limitations involved with each.

4.2 Western Corn Rootworm

The western corn rootworm is a univoltine beetle species in the family Chrysomelidae (Fig. 4.1). The insect overwinters as eggs, which are typically laid in the soil of maize fields from mid- to late summer. Three larval instars characterize western corn rootworm development following egg hatch in the spring. Larvae are the most economically damaging life stage, feeding almost exclusively on the roots of maize plants, though a limited number of other closely related plant species in the family Poaceae may also serve as hosts (Branson and Ortman 1967, 1970; Clark

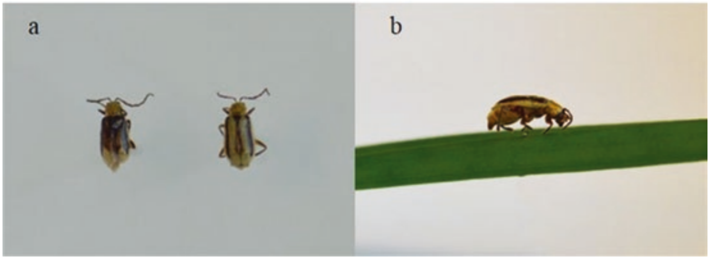

Fig. 4.1 (**a**) Adult male (*left*) and female (*right*) western corn rootworm, (**b**) Adult western corn rootworm on maize leaf

Fig. 4.2 Undamaged maize root system (*left*) and maize root systems damaged by western corn rootworm larval feeding (*right*)

and Hibbard 2004; Oyediran et al. 2004). Root injury from larval feeding (Fig. 4.2) can reduce the uptake of water and nutrients by maize plants (Kahler et al. 1985; Sutter et al. 1990; Godfrey et al. 1993) and increase susceptibility to root diseases (Palmer and Kommedahl 1969; Godfrey et al. 1993; Kurtz et al. 2010). In addition, larval feeding can result in lodging of plants because of reduced brace root support, which can lead to direct yield losses because of problems associated with mechanically harvesting fallen plants (Spike and Tollefson 1991).

Host location by western corn rootworm larvae is facilitated by carbon dioxide gradients formed in the soil by respiring roots (Strnad et al. 1986). Once larvae locate root tissue, additional primary and secondary plant metabolites are likely used during the host selection process (Hibbard and Bjostad 1988; Robert et al. 2012a, b). When exposed to maize roots, larvae exhibit a localized foraging behavior consisting of decreased speed of movement and increased turning frequency within the soil, which facilitates larval establishment by allowing a more restricted area of search for food resources (Hibbard and Bjostad 1988; Strnad and Dunn 1990). Conversely, in the absence of proper host stimuli, larvae will exhibit ranging behavior, which involves relatively quick and straight movement through the soil to allow a greater area of search (Strnad and Dunn 1990). When exhibiting ranging behavior, larvae can travel distances of at least 100 cm through the soil to locate host tissue (Suttle et al. 1967; Short and Luedtke 1970), though factors such as soil type, soil moisture, and soil bulk density can influence distances traveled (Gustin and Schumacher 1989; Turpin and Peters 1971; MacDonald and Ellis 1990). Quickly locating and recognizing suitable host tissue is important for survival of western corn rootworm larvae because starvation for 72 h can significantly increase the rate of larval mortality (Branson 1989; Oloumi-Sadeghi and Levine 1989).

During initial establishment, western corn rootworm larvae feed in the cortex of seminal and nodal maize roots that are 2 mm or less in diameter (Strnad and Bergman 1987). Over the course of a growing season, older larvae move throughout the root zone to feed on newly developed nodal roots of larger diameter, which are required to complete their development (Hibbard et al. 2008). Western corn rootworm larvae may also move between plants after initial establishment if available food resources are lacking (Hibbard et al. 2003, 2004).

After development through the larval and pupal stages, western corn rootworm adults emerge from the soil to feed on the leaf tissue, silks, and pollen of maize plants (Bryson et al. 1953; Ball 1957). Several flowering weed species are also attractive to foraging beetles (Hill and Mayo 1980), which may help to facilitate their spread and survival when preferred maize resources are insufficient (Moeser and Vidal 2005). Although adults are not generally considered as economically important as larvae, injury from adult feeding can significantly affect maize seed production at high population densities (Culy et al. 1992; Capinera et al. 1986). Longevity of adults is typically between 45 and 57 days and females lay on average 289–356 eggs (Ball 1957). Female beetles often mate shortly after emergence, but do not typically begin laying eggs until they are approximately 20–23 days old (Short and Hill 1972).

4.3 Management of Western Corn Rootworm

Major options for the management of western corn rootworm involve insecticides, crop rotation, or planting of transgenic Bt maize.

4.3.1 Insecticides

Since its emergence as a major pest of maize in the mid-1900s, insecticides have played a large role in the management of western corn rootworm (Metcalf 2005). The application of persistent, broad-spectrum soil insecticides at planting has traditionally been used to protect maize roots from larval feeding injury in continuous maize production systems (Levine and Oloumi-Sadeghi 1991). Alternative strategies involving the use of insecticidal seed treatments (Gray et al. 2006; Cox et al. 2007) and application of foliar insecticides to suppress emerging adults and reduce egg laying (Pruess et al. 1974) have also been used with variable success. However, the widespread adoption and overuse of several primary insecticides has led to the rapid spread of resistance in western corn rootworm populations (Ball and Weekman 1963; Ball 1968; Meinke et al. 1998; Wright et al. 2000; Parimi et al. 2006).

4.3.2 Crop Rotation

Crop rotation has been used as an effective alternative to insecticides throughout much of the United States and is the primary pest management option practiced throughout Europe (Kiss et al. 2005). Because the western corn rootworm has a close association with maize, planting a substitute nonhost crop in maize fields in alternating years can disrupt the life cycle of this pest species and decrease its presence (Shaw et al. 1978; Levine and Oloumi-Sadeghi 1991). In the United States, maize is typically rotated with soybean, *Glycine max* (L.), to control corn rootworm species and provide yield advantages. However, in certain regions of the Midwestern United States, a rotation-resistant variant of the western corn rootworm has developed where females lay eggs outside of maize fields and in soybeans (Levine et al. 2002; Gray et al. 2009). It is thought that this new strain of western corn rootworm is not necessarily attracted to soybeans, but instead lays eggs in a more general fashion outside of maize (Gray et al. 1998). Therefore if maize is planted in these areas the following year, or other alternate host plants are present, opportunities exist for larval establishment and survival to the adult stage.

4.3.3 Transgenic Bt Maize

Transgenic maize that express *Bacillus thuringiensis* Berliner (Bt) proteins toxic to western corn rootworm have been developed by several seed companies as an additional management tactic to limit damage caused by this pest (Moellenbeck et al. 2001; Ellis et al. 2002; Schnepf et al. 2005; Vaughn et al. 2005; Walters et al. 2008). Various Bt strains naturally produce insecticidal crystal (Cry) proteins known as δ-endotoxins, which are highly specific to certain insect species. When Cry proteins are ingested and solubilized in the midgut of susceptible insects, an active toxin is produced that binds to epithelial cells causing perforations, which results in cell lysis and ultimately death of the insect (Gill et al. 1992; Bravo et al. 2007). Genetic engineering has allowed the ability to incorporate novel genes encoding these insecticidal Cry proteins into specific locations in the plant genome (a process collectively termed a transformation event). These genetically modified, or transgenic, plants are then able to express Bt proteins within their tissue. Transgenic Bt maize can offer protection from aboveground lepidopteran (caterpillar) pests and/or belowground corn rootworm species. Bt maize that offer protection against one of the above pest complexes are termed single-trait hybrids, while those that target both pest complexes (in addition to providing herbicide tolerance) are termed stacked hybrids. Pyramided Bt maize hybrids contain genes expressing more than one Cry protein that targets the same pest complex.

In 1995, the United States Environmental Protection Agency (USEPA) approved the first registration of transgenic Bt maize for the management of lepidopteran pests. In the years following, the USEPA registered several other Bt maize events for lepidopteran pests and in 2003 approved the first registration of transgenic Bt maize for the management of western corn rootworm. The first rootworm-active Bt maize hybrids contained event MON863 (developed by Monsanto under the trade name YieldGard® RW) and expressed the Cry3Bb1 protein. Additional Bt maize hybrids expressing genes for different Cry proteins were subsequently commercialized for control of western corn rootworm. The binary Cry34/35Ab1 proteins expressed in maize event DAS59122-7 (co-developed by Dow AgroSciences and Pioneer Hi-Bred under the trade name Herculex® RW) was registered in 2005, and the mCry3A protein expressed in maize event MIR604 (developed by Syngenta under the trade name Agrisure® RW) was registered in 2007. Several stacked and pyramided maize hybrids expressing one or more of these proteins have since been developed. More recently, a new Bt protein, eCry3.1Ab expressed in maize event 5307, was registered as a pyramid with mCry3A in 2013 (developed by Syngenta under the trade name Agrisure Duracade™). No additional Bt proteins targeting western corn rootworm have been registered, although the Cry3Bb1 protein began transitioning from event MON863 to event MON88017 after its registration in 2005.

Adoption of transgenic Bt maize has increased dramatically in the United States since its commercialization. However, the ability of western corn rootworm to evolve resistance to various control measures has made the long-term viability of this technology uncertain. Under laboratory and greenhouse conditions, selected western corn rootworm populations have developed resistance to all four commercially available Bt proteins: Cry3Bb1 (Meihls et al. 2008; Oswald et al. 2011), Cry34/35Ab1 (Lefko et al. 2008), mCry3A (Meihls et al. 2011), and eCry3.1ab (Frank et al. 2013). In addition, field resistance to Cry3Bb1 and mCry3A has been documented in specific fields in the Midwestern United States (Gassmann et al. 2011, 2014).

To delay resistance development to transgenic Bt maize, attention must be focused on optimizing current resistance management plans for this pest.

4.4 Insect Resistance Management in Bt Maize

In the United States, the USEPA has required that all companies registering transgenic Bt crops submit an insect resistance management (IRM) plan prior to registration. The main goal of these IRM plans is to delay the evolution of resistance to this technology. IRM plans for transgenic Bt maize have involved the use of the "high-dose refuge strategy," which involves planting Bt crops that produce a high concentration of toxin (a dose 25 times the amount needed to kill 99 % of susceptible insects) combined with the planting of a non-Bt refuge. Essentially, non-Bt refuge plants promote survival of homozygous susceptible insects, which are available to mate with rare homozygous resistant insects that emerge from Bt plants. The high dose of toxin thus ensures that the hybrid progeny (i.e., heterozygous for resistance) do not survive, making resistance functionally recessive (Tabashnik et al. 2004).

Currently, the USEPA requires a 20 % structured refuge or 10 % blended refuge for single-trait maize hybrids targeting western corn rootworm. Structured refuges can be planted as blocks or strips within or adjacent to the 80 % Bt maize, whereas blended refuges involve seed mixtures that intersperse a 10 % blend of non-Bt seed with 90 % Bt seed in a bag sold to growers. These blended seed mixtures are called "refuge in a bag" and have been developed to help growers better comply with refuge requirements as well as allow for more efficient mixing of beetles emerging from Bt and refuge plants within maize fields. Unlike single-trait maize hybrids, pyramided hybrids only require a 5 % refuge, which can be structured or blended. Reduced refuge size for pyramided maize hybrids is predicated on simulation models that have shown that two independently acting toxins that kill the same pest can be more effective at delaying the evolution of resistance than each toxin by itself (Zhao et al. 2003; Onstad and Meinke 2010; Ives et al. 2011), though mortality of susceptible insects should be "nearly 100 %" for this to occur (Roush 1998).

Several assumptions must be met if the high-dose refuge strategy for IRM is to be successful including the following: frequency of resistant alleles is rare, mating is random between resistant and susceptible insects, and the dose of Bt toxin in plants is high enough to kill nearly all heterozygous progeny (Gould 1998; Tabashnik et al. 2004; Carrière et al. 2010). In the case of western corn rootworm, many of these assumptions are not necessarily valid. Currently, all individual Bt proteins targeting the western corn rootworm are not considered high dose as defined by the USEPA for single-trait events (Storer et al. 2006; Hibbard et al. 2010a, b; Clark et al. 2012). In addition, nonsynchronous emergence of beetles from refuge fields and Bt fields has regularly been documented (Storer et al. 2006; Murphy et al. 2010; Hibbard et al. 2011; Clark et al. 2012), which may contribute to assortative mating among Bt-resistant and susceptible insects. Furthermore, the frequency of resistant alleles is likely much higher than generally assumed (Tabashnik and Gould 2012). Current debate concerning IRM plans for western corn rootworm has centered on the appropriate size and placement of refuges needed to reduce the selection pressure of transgenic Bt maize (Murphy et al. 2010; Pan et al. 2011; Tabashnik and Gould 2012; Onstad et al. 2014). Consequently, additional research is needed to determine optimal refuge configurations for sustainable control of this pest.

4.5 Conclusion

The economic gains and environmental benefits associated with using transgenic Bt maize to manage western corn rootworm as well as its ease of incorporation have made this technology attractive to many growers. Although none of the registered individual Bt proteins targeted toward the western corn rootworm express a concentration of Cry proteins considered high dose, root protection from larval feeding due to Bt maize is frequently comparable or better than is possible with insecticides. Nevertheless, the durability of this technology can only be ensured with effective IRM plans. Continued research on western corn rootworm biology and IRM strategies is needed to determine an acceptable long-term approach to mitigate resistance development. In addition, Integrated Pest Management (IPM) can further delay the evolution of resistance and must also be incorporated into future IRM plans. IPM practices such as scouting, crop rotation, rotation of Bt maize hybrids that produce different Cry proteins, and judicious use of insecticides are all highly compatible with the goals of IRM for western corn rootworm. Although transgenic Bt maize is a valuable tool in insect control, it is important to remember that it is not the panacea of pest management.

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