Dispersal and Mating Behavior of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in *Bt* Cornfields

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ABSTRACT Understanding of the mating and dispersal behavior of the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), is essential to predicting potential resistance to *Bt* corn technologies recently deployed to combat this pest. To quantify movement of male beetles, field studies were conducted during 2006 and 2007 in commercial *Bt* cornfields that included the Environmental Protection Agency–mandated 20% refuge acreage. Wing traps containing a single virgin female beetle were placed along transects throughout these fields. Male beetles were collected from the sticky bottoms of traps to compare the mean number of beetles captured at the different transect distances. Gut contents of each captured male were assayed with protein test strips to determine if the *Bt* protein (*Cry3Bb1*) was present. This provided an estimate of the distances traveled by males to reach virgin females. These data indicated that the mean number of males captured ≈200 m from refuge corn was not significantly different than the mean number of males captured close to the refuge (<22 m). Complementary field observations were conducted to determine how far newly emerged female beetles moved before mating and the time of day mating occurs. Results indicated that females do not move far from the site of emergence before mating and do not mate within the first 4 h of adult life. The implications of these data for movement and mating patterns of adult rootworms in *Bt*/refuge environments are discussed.

KEY WORDS *Bt* corn, dispersal, insect resistance management, western corn rootworm

Understanding the movement and dispersal behavior of the western corn rootworm has become increasingly important in field crops. Interfield movement and oviposition behavior of the western corn rootworm has been a topic of both research and conjecture, especially since the discovery of the variant population of western corn rootworm in eastern Illinois and northwestern Indiana during the late 1980s (Levine and Oloumi-Sadeghi 1996, Sammons et al. 1997, Levine et al. 2002). Female variant beetles are more likely to move outside cornfields (Levine and Oloumi-Sadeghi 1996), a behavior that facilitates variant western corn rootworm oviposition in surrounding noncorn fields, thereby enabling the variant western corn rootworm to circumvent crop rotation and forcing many producers to use soil insecticide in first-year corn or, beginning in 2003, plant rootworm–resistant *Bt* corn hybrids to manage this pest (Spencer et al. 2003, Wilson et al. 2005).

Since its wide commercial release in 2003, adoption of *Bt* corn technology to help prevent economic loss caused by western corn rootworm damage has increased steadily (NASS 2006). However, some aspects of insect biology that influence the long-term efficacy of this type of control remain unclear. The rootworm insect resistance management (IRM) plan mandated by the Environmental Protection Agency (EPA), is designed to slow *Bt* resistance evolution within western corn rootworm populations. However, the current plan using a 20% refuge requirement is based largely on the established, successful European corn borer (*Ostrinia nubilalis* Huber) IRM plan for *Bt* corn, coupled with mathematical models and computer simulations of western corn rootworm movement and mating. These models and simulations are, in turn, based on a range of biological assumptions (and not empirical data) concerning mating behavior and dispersal (Hurley et al. 2006, Mitchell and Hurley 2006). Grower compliance with the 20% refuge requirement has also been problematic; planting the refuge area is inconvenient, and the hybrids without rootworm-resistant *Bt* traits often display inferior yields, presenting a disincentive for grower compliance (Hurley et al. 2006).

The current IRM plan mandates planting refuge areas consisting of corn that do not contain the rootworm-resistant trait in each rootworm-resistant *Bt* field. The purpose of the refuge is to produce susceptible western corn rootworm beetles that have not been exposed to the *Bt* toxin during larval development. Male in-field dispersal is one possible mechanism to promote the genetic mixing necessary for the IRM plan to succeed. Unfortunately, there is little

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published research regarding male dispersal, primarily because of difficulties marking large numbers of insects. A novel approach to track western corn rootworm movement was developed by Spencer et al. (2003) using ingested *Bt* corn tissue gut contents as an internal marker. With the advent of this novel tracking technique in combination with the use of traps baited with live virgin western corn rootworm females, describing male western corn rootworm in-field dispersal behavior has become more feasible.

Quantifying female postemergence dispersal is also important in understanding the effect of the IRM plan. After a successful mating, female beetles can move great distances (<1 m/flight to 24 km/flight) (Coats et al. 1986, Grant and Seevers 1989), yet postemergence dispersal is believed to be minimal (1–5 m) before mating (Ball 1957, Cates 1968, Lew and Ball 1979). Hammack (1995) found that female beetles release pheromone within a few hours after emergence, and peak pheromone release was 48 h after emergence. However, the location of female mating relative to emergence sites is not well studied. Because adults pupate and emerge near their natal host plant, field studies should address the question of where female western corn rootworm mate relative to their emergence sites and when mating occurs.

The objective of this study was to determine the in-field, short-range dispersal and mating behavior of western corn rootworm and provide data that can help biologists, modelers, and policy-makers alike predict the flight behavior and mating patterns of the western corn rootworm. Understanding these elements of western corn rootworm biology and behavior could help to assess the efficacy of the IRM plan for western corn rootworm.

Materials and Methods

Beetle Rearing Techniques. Western corn rootworm larvae were collected from the field and reared in the laboratory. The larval collection technique used was similar to that described by Mabry et al. (2004). Plastic cups (PK32T 136; Fabri-Kal, Kalamazoo, MI), 1,025 ml in size, were labeled with the date of collection. Roots were dug at each site (details follow), and the stalks were cut to a length of ≈20 cm. An entire root and partial stalk were placed inside each of the 1,025-ml cups along with any attached soil. Soil that fell from the roots was examined for the presence of larvae or pupae; these were collected and placed into the respective cups by hand if found. Cups were filled with field soil to completely cover the root and watered before being transferred to the laboratory. In 2006, ≈350–400 non-*Bt* corn roots were collected from Throckmorton-Purdue Agricultural Center (TPAC) south of Lafayette, IN, and in 2007, a total of 350–400 non-*Bt* corn roots were collected from TPAC and Pinney Purdue Agricultural Center (PPAC) west of Wanatah, IN.

Cups were taken to the laboratory and placed on plastic cafeteria trays (Cambro Manufacturing, Huntington Beach, CA). Each cup was held at a 14 L:10 D photoperiod at 24°C and 65% RH. Approximately 15–25 larvae were estimated to be in each cup based on the number of larvae placed in the cups during the field collection and from previous research (Mabry et al. 2004). At the onset of beetle emergence, each corn stalk was cut off at the soil level. A plastic lid (Fabri-Kal) with a 2-cm-diameter nylon mesh ventilation window was placed over each cup to prevent emerging adults from escaping. In 2007, 120 cups were placed into four environmental chambers (I30BLLC8; Percival Scientific, Perry, IA) to extend the emergence peak of the reared beetles over time. Two chambers were set at 20°C, and two chambers were set at 27°C. Relative humidity in all four chambers was set at 75% to simulate natural July weather conditions in west-central Indiana. The remaining cups were held at ambient laboratory conditions, which were ≈24°C with relative humidity at 65%. We used these chambers to avoid problems encountered in 2006 when all of the rearing cups were held under the same conditions in the laboratory, resulting in a compressed emergence peak in 2006 that only produced enough females to conduct field experiments for 1 wk. Each morning, the cups were sprinkled lightly with water using a hand-operated pump sprayer (model 20020; Chapin International, Batavia, NY) to keep the topsoil moist and were checked two to three times per day for adult emergence.

Emerging beetles were aspirated from each cup, separated by sex, and placed into separate 1,025-ml plastic cups. Each cup was labeled with the beetle emergence date and contained fresh corn silk, pollen, and immature ears collected from non-*Bt* DeKalb DKC60–17 RR2 corn hybrid in 2006 and non-*Bt* Specialty 4961 RR hybrid in 2007. Beetles were supplied with fresh water daily, and food was replaced every 2 d. In 2006, cups were stored outdoors near the Lily Life Sciences building at Purdue University campus in West Lafayette, IN, to acclimate female beetles to ambient weather conditions. The cups were held on cafeteria trays, which were placed inside a gated concrete-floored enclosure. The enclosure was completely shaded by surrounding trees, and the cups were protected from rain with plastic tarps. In 2007, the cups were held in the laboratory at room temperature to avoid the increased cup maintenance required when cups were stored outdoors, including replacing cups soaked in water caused by inadequate rain protection and frequently moving supplies back and forth from the outdoor enclosure to the laboratory. Only females 72 h postemergence or younger were used in this study.

Pheromone Trap Design. The pheromone trap design used in this experiment used a modified wing trap (Wing trap; Great Lakes Integrated Pest Management, Vestaburg, MI). To create a beetle cage, an 8-cm-long by 4-cm-diameter cylinder of 1-mm nylon mesh was glued to 30-ml plastic condiment cup lids (Comet Products, Chelmsford, MA). Two 1-cm holes were made in one end of the cylinder to allow insertion of a moistened cotton wick and part of a corn leaf into the mesh cylinder. A 30-ml condiment cup (Comet Prod-

Table 1. Distance between each trap transect and refuge corn–transgenic corn interface for each refuge design and planter size in 2006 and 2007

Year	Planter size (rows)	Transect	Block (m)	Strip (m)	Adjacent (m)
2006	12	1	7.5	7.5	7.5
		2	15	7.5	15
		3	30	7.5	30
		4	≈200	7.5	≈200
2007	8	1	5	5	5
		2	10	5	10
		3	20	5	20
		4	≈200	5	≈200
2007	18	1	11	11	11
		2	22	11	22
		3	44	11	44
		4	≈200	11	≈200

ucts) filled with water was attached to the bottom of the cylinder to keep the cotton wick saturated. This method provided water to western corn rootworm without exposing an open water source that could drown the beetle. Once in the field, a female beetle was placed inside the nylon cylinder through the 1-cm opening in the top of the wing trap. A rubber stopper was inserted into the opening to contain the beetle. Each trap was hung from a 0.75-m rod in the corn rows.

Transect Protocol. In 2006 and 2007, pheromone traps were placed in six ≈20-ha commercial YieldGard Rootworm MON863 cornfields northwest of Lafayette, IN in Tippecanoe and Benton counties. Fields were planted and maintained by the producer. In 2006, a 12-row planter was used in all six fields, but in 2007, three fields were planted with an 8-row planter, and three fields were planted with an 18-row planter. Standard management practices, including herbicide treatments, were used throughout the season. Areas of refuge corn were treated with a planting time application of a granular soil insecticide (Aztec, cyfluthrin; Bayer CropScience, Research Triangle Park, NC). Four trap-lines or transects of modified pheromone traps were placed in the *Bt* area of each field. Each transect contained six traps spaced 90 m apart.

Each of the three EPA-mandated refuge configuration options were represented in the experiment (block, strip, and adjacent). The block refuge configuration placed the 20% refuge corn blocked at one end of the *Bt* cornfield. The strip refuge configuration placed the 20% refuge corn throughout the *Bt* cornfield in alternating strips with *Bt* corn. The adjacent configuration used an adjacent refuge field (separated by a road or a ditch) as the 20% refuge. During the 2006 and 2007 seasons, a total of 12 commercial fields were used (4 of each refuge configuration). Table 1 provides transect distance information for the three refuge configurations.

A virgin female beetle was placed inside each trap at ≈1400–1800 hours on Monday and Wednesday of each week. Trap bottoms were checked daily for trapped western corn rootworm between 1400 and 1800 hours, because this time period corresponds with a low point in published accounts of daily western corn rootworm activity cycles (Cates 1968, Hammack

1995, Isard et al. 2000). If any western corn rootworm were present, the trap bottom was collected at that time; if not, the trap bottom was collected with the rest of the trap the next day. On Wednesday of each week, the female beetle was replaced with a fresh <72-h-old virgin female in a newly placed trap and the process repeated for 2 more d. Because Hammack (1995) observed that a majority of calling from female beetles occurs in the morning, with peak calling occurring during the second day after emergence, traps that contained calling females were used for only a single 2-d trapping period to avoid any risk of pheromone contamination of the trap. There were a total of nine 2-d trapping periods during the study. On Friday, all beetles were removed from the traps, along with the trap bottoms, until fresh females were put out on the following Monday.

This study was conducted from 17–25 July 2006 and 9–26 July 2007; dates that fall within the peak emergence and mating periods for western corn rootworm adults (Levine and Oloumi-Sadeghi 1991, Isard et al. 2000). During the trapping period, the corn was tasseled and actively pollinating. Trap bottoms were frozen in the field inside a cooler of dry ice on collection. Trap bottoms were stored in the laboratory at −80°C until processing. During processing, the bottoms were brought to room temperature, and each captured male beetle was placed in a 1.5-ml microcentrifuge tube and crushed in buffer using a Branson Sonifier (model S-250A; Branson Ultrasonics, Danbury, CT) and tested for the presence of ingested *Cry3Bb1* protein using QuickStix for YieldGard Rootworm corn (Envirologix, Portland, ME) according to the methods developed by Spencer et al. (2003). Each beetle was categorized as testing positive or negative for the presence of *Cry3Bb1.* Spencer et al. (2003) found that beetles that had previously fed on *Bt* corn within the previous 32 h tested positive for the presence of *Bt* For the purposes of our studies; if a male tested positive for *Cry3Bb1,* we concluded that it had fed on *Bt* corn within the previous 24 h. We adopted this shorter interval to provide a conservative interpretation of our results based on our assay methods.

The total number of males captured per trap was log-transformed to normalize the data. The proportional data were not transformed. Two-way analysis of variance (ANOVA) was performed on the total number of males captured per trap and the proportion of beetles testing negative for the presence of *Cry3Bb1* protein, and significantly different means were separated using Tukey's studentized range test (JMP version 6.0.2; SAS Institute 2006). Main effects included planter size and transect distance. Planter size was used because the effect was nested within year, and the refuge configuration was nested within planter size indicating planter size and transect distance as the most important effects. Only data from baited traps were analyzed. To compare refuge configurations, the initial analysis included all three of the refuge configurations. To compare trap capture data and increasing transect distance from the refuge, the strip refuge configuration data were eliminated from the analysis,

because refuge strips occur regularly throughout the entire field. Data were pooled from 2006 and 2007, and a two-tailed *t*-test ($\alpha = 0.05$) was used to compare the number of male beetles captured on baited traps versus control traps.

Female Emergence Observations. Cups containing roots, larvae, and pupae used in this portion of the experiment were selected from the same set of cups used in the previously described male dispersal study. Western corn rootworm are protandrous (Ball 1957), and males emerge before females. Here all emerging adults were discarded for 1 wk before the cups being transferred to the field. This allowed for a majority of the male beetles to emerge before peak female emergence. The cups were transferred from the laboratory and taken to a non-*Bt* cornfield. In 2006, this field was located at TPAC. In 2007, the field was located northwest of Lafayette, IN, at the Purdue Agronomy Center for Research and Education (ACRE). In the field, the lids and mesh covers were removed, and each cup was trimmed so that the rim of the cup was ≈1 cm above the soil level in the cup.

In 2006, three observation sites were used: two non-*Bt* sites and one *Bt* site. Fifteen cups were buried around the base of one marked corn plant at each observation site so that the rims were flush with the surface of the field soil. The cups were buried on 12 July at the non-*Bt* sites and 25 July at the *Bt* site. In 2007, a single non-*Bt* observation site was used. Thirty cups were buried around the plants at this site on 8 July. We estimated ≈10 female western corn rootworm would emerge from each cup (based on results obtained in the laboratory).

The observation sites selected for this experiment were located ≈20 m from the edge of a one ha cornfield. Three adjacent corn plants in the same row, including the marked plant, were selected at each observation site. Two of the plants were cut off at ground level and a circular piece of black-mesh landscaping fabric with a 5-cm hole cut in the center was carefully pulled over the remaining plant. The fabric allowed some light through, and visual observations could be made of beetles below the mesh from above. The edges of the fabric were secured to the ground with field soil. Once affixed around the base of the remaining plant, this fabric created a funnel from the ground up to the stalk of the corn plant. The funnel covered the buried emergence cups and the cut plants so that any emerging beetles would be directed toward the remaining plant where they would emerge from beneath the fabric at the base of that plant. At the end of each observation period, the fabric was removed to allow for natural light and temperatures at the soil surface. Two meter sticks were taped end to end and pushed into the soil 0.5 m away from the remaining corn plant. This served as a vertical reference point for the location of beetle behaviors on the plants. Visual observations of behavior were recorded as occurring either on the stalk, ear, leaf, whorl/tassel, or ground. The straight line distance the female moved in relation to her emergence site was recorded using a measuring tape.

Beetle emergence was observed beginning between 0500 and 0600 hours each morning, 1 d after the cups were placed in the field. This interval was chosen based on laboratory and field studies indicating that both western corn rootworm emergence and peak mating occurs during the morning hours (Cates 1968, Quiring and Timmins 1990, Hammack 1995). Beetles were sexed at the time of emergence based on both elytral coloration and antennal length (Krysan and Miller 1986), because there is the possibility of error by visually sexing western corn rootworm beetles based exclusively on elytral coloration alone (Kuhar and Youngman 1995).

Female beetles were observed for up to 4 h or until mating occurred (i.e., insertion of the aedeagus) or the females left the observation area. A 10-cm-diameter mirror, attached to the end of a wooden dowel at a 45° angle, was used to observe the females if they moved to the opposite side of the corn stalk. This allowed observations to be made with the least amount of disturbance to the female.

Data collected included duration of time between emergence and mating, the location and timing of the initiation of calling, and the location of initiation of copulation relative to the emergence site. A female was classified as calling based on female body position observations as reported by Hammack (1995), in which a calling female holds her body elevated from, and parallel to, the substrate and remains relatively motionless as she calls without feeding or grooming. Data were also collected on mate acceptance/rejection, based on the total number of courting males that approached a female, which can be identified as agitated movement and rapid antennal movement (Lew and Ball 1979). Flight of the female before any observed courtship/copulation and/or female feeding behavior during the study period was also recorded.

Means were generated for each of the following categories: time of emergence, the time from emergence to mating, and the distance and height at copulation from the emergence site.

Results

Male Trapping Study. Comparison of baited versus control traps showed that significantly more male western corn rootworm were caught on the baited traps ($t = 12.90$; df = 1509; $P < 0.0001$; $n = 2,592$ traps; Fig. 1). There was no significant difference between the total number of males captured per trap ($F = 1.80$; df = 3; $P = 0.1460$) or the proportion of males that tested negative for *Bt* ($F = 2.26$; df = 3; $P = 0.0804$) with respect to female trap distance from the refuge in the various field configurations. There was a significant difference between the mean number of males captured per trap and the planter size used ($F = 8.28$; df = 2; $P = 0.0003$). There was also a significant difference between the proportion of captured males that tested negative for *Bt* and the planter size used ($F = 20.81$; df = 2; $P < 0.0001$).

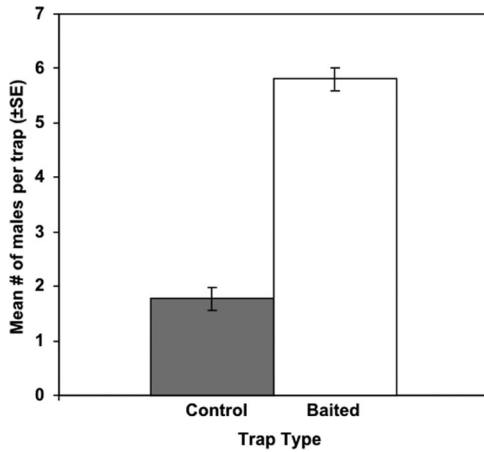Comparisons were made between planter size and transect distance using only data from the block and

Fig. 1. Mean number of male western corn rootworm captured in female-baited ($n = 1246$) and unbaited control ($n = 1346$) traps in *Bt* cornfields.

Fig. 2. Mean number of captured males and proportion of captured males testing negative for *Bt* in traps deployed in four trapping transects. Data only represent trap transects within the block and adjacent refuge configurations. Trap transect 1 indicates traps deployed 5, 7.5, and 11 m from the refuge, trap transect 2 indicates traps deployed 10, 15, and 22 m from the refuge, trap transect 3 indicates traps deployed 20, 30, and 44 m from the refuge, and trap transect 4 indicates traps deployed mid-field or ≈200 m from the refuge.

adjacent refuge configurations. There was a significant difference between the mean number of males captured per trap and the planter size used ($F = 11.12$; df = 2; $P = 0.0001$). There was also a significant difference between the proportion of captured males that tested negative for *Bt* and the planter size used ($F = 21.33$; df = 2; $P < 0.0001$). The tests indicated no significant difference between the total number of males captured per trap ($F = 2.54$; df = 3; $P = 0.0556$) or the proportion of males that tested negative for *Bt* ($F = 1.50$; df = 3; $P = 0.2149$; Fig. 2).

Female Emergence Observations. The mean time of emergence for females was 0659 ± 0014 (SE) hours ($n = 30$). The beetles that displayed calling behavior ($n = 11$) began calling an average of 43 ± 10 min after emerging from the soil. Of the 30 female beetles that were observed after emergence, none accepted a male during the observation period. Sixteen males approached females and attempted to mount and rapidly antennate the observed females. All females spent the majority of time on the leaf surface (approximately equal time was spent on the upper and lower surface of the leaf) at an average height of 77.84 ± 9.5 cm above ground level. This height was 0.5–1 m below the whorl and tassel of the corn plant. Of the 30 females observed, 22 moved vertically onto the observation plant. Beetles that did not fly remained on the observation plant for the entire 4-h observation period ($n = 11$) or walked outside the observation area ($n = 19$).

Observations were also made to quantify the activity of the natural population of western corn rootworm beetles around the observation site. The number of mating pairs within view at the observation point was recorded per day. Of the 32 mating pairs observed nearby (all were observed in 2006), 30 of the pairs had a teneral female (i.e., a female without fully sclerotized cuticle) in the pair (these were similar in appearance to the females emerging in our study). In 2007, there were no mating pairs observed in the area of the observation site, because beetle populations overall were much lower in the study area.
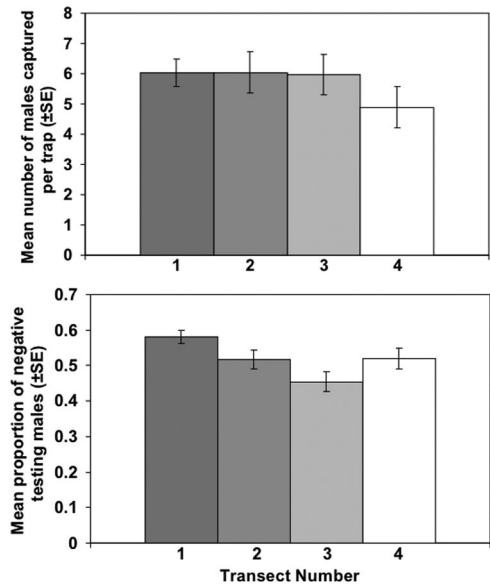
Discussion

Our data indicate that the in-field dispersal of male western corn rootworm responding to reproductive females is extensive. The dispersal of western corn rootworm beetles is influenced by pheromone release of western corn rootworm females and available food sources (Darnell et al. 2000, Ventura et al. 2001), but this is the first documented analysis of movement of males in response to virgin females within refuge/*Bt* cornfield environments. These two factors may be key in determining the number of male beetles moving into *Bt* blocks of cornfields. Because more beetles are generated in the non-*Bt* refuge, females that are closer to the refuge/*Bt* interface should attract more males, based on probabilities alone. However, our data suggested that even females in the center of *Bt* cornfields have an equal probability of attracting the same number of males as the females near the refuge/*Bt* interface. In addition, we found no significant difference between the proportion of negative males captured close to the refuge as opposed to the center of the *Bt* block of the field. This indicated that negative males could be found throughout the cornfield (even long distances from any refuge corn). We note here that we do not know the frequency with which adult male beetles feed, so that male beetles not feeding at all for a 24-h period would be classified the same as those that fed only on non-*Cry3Bb1* corn.

The variability in our results resulted in no significant differences between the three IRM refuge de-

signs in terms of total capture of males on female-baited traps. The only significant difference in the data was produced by the different planter types used to plant the field—an unavoidable and unforeseen consequence of using large-acreage, commercial growing operations for our field studies. At the outset of this work, we expected the strip design to have the highest number of captured males compared with the other two refuge designs (block and adjacent), yet the data did not support this hypothesis. There may be several reasons for these findings.

First, male western corn rootworm fly within corn-fields and between corn and soybeans on a daily basis, yet exact dispersal distance is unknown (Coats et al. 1986, Spencer et al. 1998, 1999, 2003, Isard et al. 2000). Darnell et al. (2000) found that dispersal is influenced by the food sources available for adult western corn rootworm, yet this would only affect dispersal from refuge corn into *Bt* corn if the two hybrids (refuge and *Bt*) were phenologically different. The hybrids used in this study were planted at the same time and were phenologically similar, likely ruling out food quality as a primary factor in male western corn rootworm movement in this study.

Second, in the 2 yr of the study, there was no significant difference in the mean number of males captured per trap or the proportion of negative males captured in the middle of the *Bt* block ($\approx$200 m from the refuge) compared with the edge closest to the refuge. This result is counterintuitive and may illustrate that western corn rootworm males may be moving significant distances from emergence sites and/or that the males are not feeding on *Bt* corn 24 h before being captured. Because the gut contents of beetles 24 h before being caught are the target of the assay, negative testing beetles captured in the mid-field transects could be males emerging from the *Bt* area of the field that have not yet fed on *Bt* corn tissue. Literature regarding the feeding habits of adult males is scarce, although there is reason to believe that males may have to feed quite frequently. Quiring and Timmins (1990) found that male foraging and feeding on corn tissue was correlated with mating success, and males mating multiple times per day accumulated nutritional reserves by foraging on corn tissue.

Previous studies have modeled the correlation between refuge placement and the evolution of western corn rootworm resistance to *Bt* toxins (Crowder and Onstad 2005, Crowder et al. 2005a, b, Onstad et al. 2001, Storer 2003, Onstad 2006). A resistance evolution model designed by Onstad et al. (2001) analyzed how long western corn rootworm populations may take to reach a 3% *Bt*-resistance allele frequency. This model showed that refuge configuration could play a significant temporal role in resistance evolution but that *a priori* resistance allele frequencies in western corn rootworm populations had the most influence concerning evolution of resistance. Data from our study would add support to this model by indicating that refuge placement may not be a large factor in western corn rootworm resistance evolution because of the dispersal ability of male western corn rootworm.

It is also important to reiterate that our study was conducted in an area where variant western corn rootworm is present (northwestern Indiana). Dispersal behavior of variant western corn rootworm within refuge/*Bt* corn may be significantly different from that of nonvariant beetles (Knolhoff et al. 2006). The scope of our study did not include comparison between variant and nonvariant populations.

Female dispersal before mating could also play a significant role in the speed of resistance evolution. Although females may mate soon after emergence in a laboratory or confined setting as Ball (1957) reported, no newly emerged females were observed to mate during our study, although a number of the newly emerged females were observed exhibiting characteristic calling behavior while on the corn plants.

Our data indicate that some precopulatory dispersal occurs among females, and the ability of females to fly soon after emergence has not been described previously. This is important in that female dispersal is an important component of models predicting the evolution of resistance.

At this point, we can speculate on how these data will affect new and as yet untested resistance management strategies and products such as blending refuge and transgenic seed (often called "refuge in a bag") and corn containing multiple insecticidal traits (SmartStax) developed jointly by the Monsanto Company and Dow AgroSciences (Associated Press 2007). Although the introduction of multiple rootworm-resistant traits in the same plant may ultimately lead to an alteration of the current refuge configurations, an understanding of adult movement and mate-finding behavior in *Bt*/refuge systems will remain integral to developing IRM plans regardless of the toxins employed. Evaluation of the assumptions on which western corn rootworm IRM is presently grounded demands information about what individual beetles are doing before population-level models can be generated. These types of data are difficult to collect, yet the distortion of expectations and assumptions about western corn rootworm mating observed in this study suggest that there is much more to learn about the reproductive ecology of this insect.

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