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On-Plant Movement and Feeding of Western Bean Cutworm (Lepidoptera: Noctuidae) Early Instars on Corn

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ABSTRACT Western bean cutworm, Striacosta albicosta (Smith), has undergone a recent eastward expansion from the western U.S. Corn Belt to Pennsylvania and parts of Canada. Little is known about its ecology and behavior, particularly during the early instars, on corn (Zea mays L.). There is a narrow treatment window for larvae, and early detection of the pest in the field is essential. An understanding of western bean cutworm larval feeding and early-instar dispersal is essential to understand larval survival and establishment in corn. Studies were conducted in 2009 through 2011 in Nebraska to determine the feeding and dispersal of early-instar western bean cutworm on corn. The treatment design was a factorial with three corn stages (pretassel, tassel, and posttassel) and five corn plant zones (tassel, above ear, primary ear, secondary ear, and below ear) in a randomized complete block design. The effects of different corn tissues on larval survival and development were investigated in laboratory studies in a randomized complete block design during 2009 and 2011. Treatments were different corn tissues (leaf alone, leaf with developing tassel, pollen, pollen plus silk, and silk alone). Results demonstrated that neonate larvae move to the upper part of the plant, independent of corn stage. Larval growth was optimal when fed on tassel tissue. Overall results indicated a selective benefit for movement of the early instar to upper part of the plant.

KEY WORDS larval movement, larval feeding, Striacosta albicosta

The historical distribution of western bean cutworm [Striacosta albicosta (Smith)] comprises Colorado, Idaho, Kansas, Nebraska, western Iowa, Utah, Arizona, New Mexico, Texas, Alberta, and Mexico (Douglas et al. 1957, Hagen 1963, Appel et al. 1993). In 1970, reports of its distribution included South Dakota and Wyoming (Blickenstaff and Jolley 1982). Since 1999, there have been records of this pest in field and sweet corn (Zea mays L.) in the U.S. Corn Belt from Iowa to Pennsylvania and parts of Canada (O’Rourke and Hutchison 2000; Rice 2000, 2006; Cullen and Jytikka 2008; DiFonzo and Hammond 2008; Michel et al. 2010; Tooker and Fleischer 2010).

Because the western bean cutworm previously was only a sporadic pest in the west central United States (Hoerner 1948, Douglas et al. 1957), little is known about its ecology and behavior in corn, particularly across ecoregions. In part because of its recent range expansion, there is an increased demand for the development of fundamental integrated pest management (IPM) tools for this pest in corn. Western bean cutworm is univoltine (Hagen 1962), and in corn the larva feeds on several vegetative and reproductive tissues and can be described as having a mixed feeding behavior (Zalucki et al. 2002). Besides feeding on whorl, silk, and corn ear tissue during the fourth and fifth instar (Seymour et al. 2004), Douglass et al. (1957) indicate that western bean cutworm larvae can feed on all parts of the corn plant, including leaves, stems, tassels, ear shanks, husks, kernels and cobs. At the end of the fifth instar, the larva drops to the ground, burrows 12–25 cm below the surface, and becomes a prepupa (Douglass et al. 1957, Michel et al. 2010). The next spring the insect pupates, and adults emerge in July, mate, and oviposit on the top surface of leaves on the upper part of the corn plant (Douglass et al. 1957, Seymour et al. 2004).

One of the less understood aspects of western bean cutworm ecology is related its dispersal behavior, defined as any adult movement away from the initial population habitat or in the case of neonate larvae, movement from its egg mass (Southwood 1978). Stinner et al. (1983) affirm that pest movement received little attention until its importance was realized in relation to the rate of pest colonization of hosts. Knowledge of adult and larval movement is critical to apply pest management strategies, such as sampling...
protocols (Ross and Ostlie 1990), the release of natural biological control agents (Spangler and Calvin 2001), and other tactics.

With the advent of transgenic corn that expresses Bt toxins, information on pest dispersal is important because the mobility of the species at different life stages impacts larval exposure to lethal and sublethal concentrations of Bt toxins. Larval feeding behavior and plant-to-plant movement can influence how to design strategies to manage resistance (Gould 1998, Dirie et al. 2000). Short and long-range movement patterns, including larval on-plant and plant-to-plant movement (International Life Sciences Institute 1998, Shelton et al. 2002); dispersal of adults among fields (Caprio 1998, 2001); premating adult dispersal, as well as other behaviors, also need to be considered.

The western bean cutworm adult is a strong flyer (Seymour et al. 2004, Dorhout 2007), with preference for corn plants that are just beginning to tassel (Holtzer 1983) where they deposit eggs on the upper leaves of the plant (Douglass et al. 1957). The distribution of egg masses within a field follows a random pattern and there is no oviposition preference between current Bt and non-Bt corn hybrids (Paula-Moraes et al. 2011). This study investigated larval development and on-plant movement of early western bean cutworm in stars at different corn stages in the field, and the effect of different corn tissues on larval survival and development in the laboratory. The objectives were to determine the feeding behavior, dispersal, and establishment of early instar on the corn plant.

**Materials and Methods**

**On-Plant Larval Movement.** Larval survival, development, and distribution on the corn plant were characterized in the field at the Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE. A corn hybrid (DKC 61–72 RR) expressing *Bacillus thuringiensis* (Bt) protein Cry1Ab (YieldGard, Monsanto, St Louis, MO) that is not toxic to western bean cutworm (Catagui and Berg 2006) was used to minimize the confounding effect of European corn borer, *Ostrinia nubilalis* (Hübner).

The treatment design was a 3 by 5 factorial. There were three corn stages, pretassel, tassel, and posttassel, which correspond approximately to corn stages V18, VT, and silking (Ritchie et al. 1993), respectively. The corn plant was divided in five plant zones (tassel, above ear, primary ear, secondary ear, and below ear). The experimental area had three corn stages established using different planting dates. The three corn stages were randomly assigned in a Randomized Complete Block Design. Larval movement on the corn plant was evaluated based on the number of larvae in each plant zone. In 2009 and 2010 there were six replicates, and in 2011 four replicates. Each experimental plot was three rows by 3 m, with \( \approx 20 \) plants per row.

Artificial infestation was conducted in 2009 and 2010 by using egg masses collected from commercial cornfields. Leaf squares containing single egg masses were cut from leaves. The number of eggs per egg mass was determined by photographing each egg mass and counting the eggs in the laboratory. A small square of screen was fixed loosely behind the egg mass with staples forming a “sandwich” to keep the leaf from curling and dislodging egg masses. When the eggs turned dark purple, indicating imminent eclosion, the infestation was conducted.

A range of four to six plants in the central row of each plot was infested depending on the availability of egg masses. Every other plant received an egg mass. The egg mass was stapled to the leaf, and infested plants were marked with flagging tape. In 2009, egg masses were attached on the leaf just above the primary ear. In 2010, the egg mass was attached to an upper corn leaf, which is more typical of the female moth oviposition behavior. In 2011, wild moths were used for the infestation. Moths were collected in cages positioned under UV light traps. One male and two females were confined on the top part of the corn plant within a large flat mesh pollination bag (46 by 51 cm) (Delnet Pollination Bags, Middletown, DE). Two days after infestation, the presence of egg masses was determined and thinned so that only one egg mass was left per plant. Egg masses were photographed to later count the number of the eggs. Plants on which females did not lay egg masses were artificially infested by using the same methodology of 2010. Plots were inspected for wild western bean cutworm egg masses, but in all years no natural infestation was detected.

In 2009, plant sampling was done 4 and 6 d after infestation (eggs hatched within 24 h of infestation). In 2010, plant sampling was done 3 and 8 d after infestation. In 2011, the deposition of the egg masses was from oviposition, so it took longer for eggs to hatch after infestation, and sampling was done 6 and 9 d after infestation. One plant was measured in each plot, on each sampling day. Plant inspection took 15–20 min per plant. In 2010, the number of the hatched eggs per egg mass was recorded to determine egg mortality and initial larval infestation (Ross and Ostlie 1990). In 2011, egg survival was visually estimated, and based on the high number of unhatched eggs, was considered very low. Each corn plant zone was inspected for the presence of larvae (destructive sampling). A step ladder was used to access different parts of the corn plant. Based on the difficulty of inspection of the tassel zone, in 2010 and 2011 larvae from the tassel zone were recovered using Berlese funnels (24-h recovery period).

Egg hatch and larval survival were calculated. Larval survival was based on recovered larvae. In 2010 and 2011, larval head capsule width was measured. The data were separately analyzed by year, and tested for normality of the residuals and homogeneity of variance (PROC GLIMMIX PLOT = RESIDUAL PANEL) (SAS Institute 2009). The normal distribution assumption was violated, and the distribution with the best fit is lognormal. This was implemented via the DISTRIBUTION option in PROC GLIMMIX (SAS Institute 2009). The dependence among plant zones...
was also tested and the best fitting model, as measured by Akaike’s information criterion corrected, was the one that assumed constant correlation or covariance among the locations. Larval survival as a function of corn stage, and the relationships and interactions between number of the larvae in different plant zones and corn stages were examined. The Dunnett procedure was performed to detect differences from a control (plant zone where the egg mass infestation was done) in situations where it was possible to estimate the means in a lognormal scale.

Larval Feeding. Larval survival and development were measured in the lab during 2009 and 2011. Different corn tissues were tested from the same Bt transgenic corn hybrid expressing Cry1Ab used in the field study.

The larval feeding treatments were based on the availability of corn tissue when corn was at pretassel (whorl leaf with developing tassel), tassel (pollen and pollen plus silk), and posttassel stage (silk). The different plant tissues were removed from corn plants in the field when needed and cleaned with alcohol to remove foreign material from leaf surface (e.g., dust) (Dorhout 2007). Pollen was collected by collecting tassels from the field and sifting pollen through a 0.5-mm mesh screen to remove anthers and other tassel material (Hellmich et al. 2001). Neonate larvae were obtained from egg masses kept in growth chambers at 25°C until hatch. The egg masses were from wild females that were collected from light trap cages and allowed to oviposit on corn leaves placed in cages and held in the lab.

The study was conducted as a Randomized Complete Block Design with three replications (growth chambers). In 2009, three different corn food sources were evaluated: silk, pollen, and leaves from the whorl. One hundred neonate larvae were placed in a 10-cm-diameter container and held at 25°C and a photoperiod of 24:0 (L:D) h for 13 d in each growth chamber. Every 2 d larval survival was evaluated by recording the number of dead larvae and fresh corn tissue provided.

In 2011, four different corn food sources were evaluated: leaves from the whorl plus tassel, silk, pollen, and silk plus pollen. Five neonate larvae were placed in a 10-cm-diameter container and held at 25°C and a photoperiod of 24:0 (L:D) h for 7 d. The reduced number of neonates per container allowed for easier manipulation of the insects, reduced evaluation time, and more accurate evaluation. Five containers per food source were placed in each growth chamber (three replications), and mortality and weight were recorded after 7 d. The larvae were removed from containers and weighed live on the same day.

Survival was measured by comparing the percentages of remaining live larvae. Development was measured by comparing the larval weights and head capsule widths at the end of the experiment. Data were analyzed using analysis of variance separately for 2009 and 2011 (Proc Glimmix, SAS Institute 2009). Larval-instar classification was based on Antonelli (1974).

| Table 1. Corn stage and plant zone effects on mean number of western bean cutworm larvae |
|---------------------------------|-------|-------|
| Effect                          | DF    | P value |
| First sampling date             |       |        |
| 2009<sup>a</sup>                |       |        |
| Corn stage                      | 2     | 0.41   |
| Plant zone                      | 4     | 0.05   |
| Corn stage × plant zone interaction | 8     | 0.54   |
| 2010<sup>b</sup>                |       |        |
| Corn stage                      | 2     | 0.55   |
| Plant zone                      | 4     | 0.02   |
| Corn stage × plant zone interaction | 8     | 0.42   |
| 2011<sup>b</sup>                |       |        |
| Corn stage                      | 2     | 0.28   |
| Plant zone                      | 3     | <0.01  |
| Corn stage × plant zone interaction | 6     | 0.92   |
| Second sampling date            |       |        |
| 2009<sup>a</sup>                |       |        |
| Corn stage                      | 2     | 0.07   |
| Plant zone                      | 4     | 0.59   |
| Corn stage × plant zone interaction | 8     | 0.70   |
| 2010<sup>b</sup>                |       |        |
| Corn stage                      | 2     | 0.32   |
| Plant zone                      | 4     | 0.72   |
| Corn stage × plant zone interaction | 8     | 0.50   |
| 2011<sup>b</sup>                |       |        |
| Corn stage                      | 2     | 0.34   |
| Plant zone                      | 3     | 0.51   |
| Corn stage × plant zone interaction | 6     | 0.51   |

<sup>a</sup> Infestation of egg mass on leaf close to primary ear.
<sup>b</sup> Infestation of egg mass on leaf in tassel zone.

Results

On-Plant Larval Movement. In 2009 and 2010, egg hatch was ≥85%. In 2011, visual evaluations after infestation detected low egg hatch (<5%), which likely resulted from a combination of events (high temperatures with lack of irrigation) in the experimental area. The overall larval recovery in all years was low when considering number of the eggs infested and hatched. A decrease in the number of larvae recovered also was observed from the first to the second sampling date. Larval recovery in 2009 was 7.3% on the first sampling date (94 recovered larvae), and 3.9% on the second sampling date (50 recovered larvae). In 2010, the larval recovery was 16% (183 recovered larvae) and 4% (28 recovered larvae) on the first and second sampling dates respectively. In 2011, besides the low egg survival, the larval survival ranged from 7.7% (50 recovered larvae) to 2% (nine recovered larvae) on the first and second sampling dates, respectively. Following Antonelli (1974), all larvae recovered on the first sampling date were classified as first instar, and on the second sampling date all larvae were second instar.

In all years, there was no significant corn stage effect for either sampling date (Table 1). Likewise, there was no significant interaction between corn stage and plant zone (Table 1). There was a significant plant zone effect on the first sampling date in 2009 (P = 0.05), 2010 (P = 0.02) and 2011 (P < 0.01) (Table 1).

The mean number of the larvae recovered in each plant zone is presented in Table 2. Overall, there was a lower number of larvae in the below ear zone with the concentration of larvae in the upper part of the
The mean comparison on the first sampling date is presented in Table 3. The mean comparison is between the mean number of larvae recovered in the plant zone where the egg mass was placed and the other plant zones. In 2009, the mean number of larvae recovered in the primary ear zone (where the egg mass was placed) was significantly higher than in the below ear zone ($P = 0.03$). However, the number of larvae recovered in the primary ear zone was the same as in the above ear zone ($P = 0.57$). The low number of larvae recovered in 2009, especially in the tassel zone, and the log scale used in the model did not allow estimation of the difference between the mean number of larvae in the primary ear and tassel zones. The assessment of the early larvae inside the tassel was improved by using Berlese funnels in 2010 and 2011 (Table 2).

Because of the extremely low number of larvae in 2010, there was a consequent lack of variability in the mean number of larvae in the tassel, secondary, and below ear zone, so it was not possible to estimate and compare means in the model (Table 3). However, it was possible to observe a concentration of larvae in the upper part of the plant. There was a significant lower number of the larvae in the primary ear zone compared with the tassel zone ($P = 0.01$) (Table 3).

In 2011, the low number of larvae recovered and the use of log scale in the model did not allow estimation and comparison of differences. However, the mean number of larvae recovered in the upper part of the plant was greater than in the below ear zone (Table 2).

### Larval Feeding

In 2009, there were no significant differences in larval survival between cohorts reared for 13 d on pollen or silk (Table 4). Larvae reared on silk had a significantly higher weight than those reared on pollen (Table 4). For both corn tissues, most of the larvae were classified as fourth instar (Antonelli 1974) (Table 4). Survival of larvae reared on leaves from the whorl was significantly lower than on the other two corn tissues. Only four larvae were recovered, which were too small to be weighed or measured.

In 2011, larvae were reared for 7 d on the respective diet tissues, which is more representative of their feeding choices in the field. The larvae reared on tassel tissue enclosed in leaf (late whorl with developing tassel) had significantly higher weights and survival than those reared on other diet tissues (Table 4). There were no significant differences in weight or survival between larvae reared on the other diets. The larvae reared on tassel tissue had significantly higher weights than those reared on other diet tissues (Table 4). For both corn tissues, most of the larvae were classified as fourth instar (Antonelli 1974) (Table 4). Survival of larvae reared on leaves from the whorl was significantly lower than on the other two corn tissues. Only four larvae were recovered, which were too small to be weighed or measured.

### Discussion

Hagen (1962) indicated that under field conditions, 97% of western bean cutworm eggs hatch from natural oviposition. In our study, during 2009 and 2010, overall egg hatch was typically over 90%, and never lower than 85%, demonstrating the high rate of egg survival, and also the efficiency of the infestation methods used. Low egg hatch in 2011 was likely the result of plant
stress because of a nonfunctional irrigation system coupled with uncommonly high temperatures. In the period between infestation and neonate emergence in 2011, the average temperature was ~26°C in Concord, NE, with a high of 35°C (High Plains Region Climate Center 2011). Hence, there was an extremely dry and unfavorable microclimate, especially on the surface of the leaf (leaves were curling and in some cases desiccating) where eggs and neonates initially reside, resulting in overall low egg hatch.

The literature indicates that relatively few western bean cutworm neonates survive to maturity (Seymour et al. 2004, Eichenseer et al. 2008, Paula-Moraes 2012), even in field cages. For example, in Nebraska 3.3% survival was reported in experimental field cages (Appel et al. 1993). The results from the current study support low larval survival of this species, and indicate that the early instars are the critical stages for establishment in corn. Low larval survival in Lepidoptera has been reported previously, especially in early instars (Zalucki et al. 2002). In the case of western bean cutworm, plant factors, environmental effects, and an array of potential natural enemies all contribute to that mortality.

Early-instar movement toward the tassel is discussed in the literature as a function of larval feeding behavior (Hagen 1962, Seymour et al. 2004, Eichenseer et al. 2008). Neonates move to the tassel forming within the whorl to feed on developing pollen (Hagen 1962), the flag leaf, and other tissues (Seymour et al. 2004). For the western bean cutworm that movement differs according to corn plant stage (Hagen 1962). Larvae move toward the tassel until the latter emerge from the whorl. In corn fields where plants are tasseled or are in the silking stage, larvae are reported to migrate to the silk rather than to the tassel zone (Hagen 1962, Seymour et al. 2004). However, the results from this study indicate significant on-plant larval movement, with a concentration in the upper part of the plant (Table 2), regardless of plant stage.

The results in 2009, 2010, and 2011 did not indicate an effect of corn stage or an interaction between corn stage and plant zones on the distribution of the larvae (Table 1). Overall, the highest concentration of early instars was on the upper part of the plant, independent of the pretassel, tassel, or posttassel stage (Table 2).

In 2009, the larvae were also more evenly distributed among plant zones (Table 2), but the 2009 differences in larval distribution from the 2010 and 2011 results were possibly because the 2009 egg mass infestation was done close to the primary ear, instead of higher on the plant, which is more typical of western bean cutworm oviposition. However, even though the egg mass was infested near the primary ear zone, no differences were detected in larval recovery between the primary ear and above ear zone (Table 3), indicating that larvae moved to the upper part of the corn plant. In addition, some larvae may have been missed during the visual inspection of the tassel zone in 2009. The use of Berlese funnels in 2010 and 2011 improved the ability to detect the larvae in the tassel zone. In other plant zones, visual inspection of removed and dissected plants was adequate to assess larval presence.

The overall results from the field study demonstrate a behavior of initial larval movement to the tassel area. It could be presumed that this would be in part because of feeding preference, and indeed, the greatest larval survival in the feeding study was in tassel tissue enclosed in leaf (Table 4). These results help to explain the benefits of this upward movement of western bean cutworm during the early instars.

There is a lack of information about the feeding requirements of western bean cutworm, so the early larval feeding studies were done to improve the understanding of how feeding on selected corn tissues affects larval survival and development. The diets were based on possible feeding scenarios considering different corn stages available in the field. In 2009, only leaf tissue from the whorl was demonstrated to be an unsatisfactory source of food for young larvae. Two days after infestation, there was ~50% mortality. Due to the size of the larvae that remained, the head capsules were not measured, but visual observation indicated they were first instar. However, in this initial study the larvae were kept on the diets for 13 d (Table 4), with
2 d evaluation intervals until larvae reached the fourth instar. Within a 13-d period in the field, larvae would be able to move to other tissues (e.g., developing kernels) to feed after the early instar feeding period. Therefore, in 2011 additional tissues were tested and the feeding period was reduced to 7 d, which gave the larvae time to reach second instar. This scenario was considered to be more representative of field conditions. In this way, the feeding study focused only on initial larval feeding.

The results from 2011 confirmed the late whorl plus tassel tissue as the best food source for early instars. It is possible that late whorl plus tassel tissue is beneficial to early instars because the tassel, with the associated developing pollen, satisfies their nutritional requirements. Mature pollen alone did not provide the same benefit (Table 4). In addition, the late whorl stage probably plays a role in providing shelter and a favorable microclimate for small larvae, resulting in better survival and development.

The results from the field together with the lab studies demonstrated that the behavior of neonates is to move to the upper part of the corn plant, and the benefits of the tassel zone providing food and shelter could be a selective factor for this movement. The selection of the late whorl stage for oviposition, as well as the tendency to oviposit on the upper leaves (Blickenstaff 1979, Holtzer 1983) are other indications of the importance of the tassel zone for western bean cutworm. The female moth is responsible for the selection of the most suitable corn stage for neonate survival (Renwick and Chew 1994), and larvae would have the predetermined behavior to move to the tassel. Similarly, oviposition site selection by O. nubilalis resulted in neonates being close to their most suitable feeding site (Spangler and Calvin 2001). Moreover, western bean cutworm larval movement could be a function of a mixed feeding behavior, which follows a sequence of feeding, instead of being a function of corn stage. Such behavior could also contribute to the high intrinsic rate of larval mortality. Mixed feeding behavior requires larval movement to the different zones of the plant, which results in larval exposure to biotic and abiotic mortality factors (Zalucki et al. 2002).

These results contribute to the understanding of an important part of the western bean cutworm early-instar period, when the larva is small, hiding inside the tassel, and before colonization of the corn ear. Insecticide application, when necessary, should be done before larvae colonize the ear because during that period larvae are moving on the corn plant and would be exposed to insecticidal control.

Understanding larval on-plant movement and feeding behavior can be useful for developing western bean cutworm resistance management strategies. In the current study, the corn hybrid (DKC 61–72 RR) expressing Cry1Ab, which does not affect western bean cutworm larvae (Catangui and Berg 2006, Eichenseer et al. 2008), was used. However, others hybrids are commercially available expressing Cry1 F (Herculex I, Dow AgroSciences and Pioneer Hi-Bred International), which is toxic to western bean cutworm (Eichenseer et al. 2008). More recently, Bt corn hybrids have been introduced with pyramided genes encoding Cry1 F/Cry1Ab (Optimum Intrasect Insect protection, DuPont/Pioneer), and Cry1A.105/Cry2Ab2/Cry1 F (SmartStax Genuity, Monsanto) (DiFonzo and Cullen, 2010). Some larval feeding has been detected on these hybrids, even in the case Cry 1 F-only hybrids (Eichenseer et al. 2008). The potential variability of Bt toxin expression in different corn tissues (Nguyen and Jehle 2007, Székács et al. 2010), and the mixed feeding behavior should be a focus of future studies on the evolution of resistance of western bean cutworm to Bt toxins.

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