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ECOLOGY AND INTEGRATED PEST MANAGEMENT OF WESTERN BEAN CUTWORM *Striacosta albicosta* (SMITH) (LEPIDOPTERA: NOCTUIDAE) IN FIELD CORN

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ECOLOGY AND INTEGRATED PEST MANAGEMENT OF WESTERN BEAN CUTWORM *Striacosta albicosta* (SMITH) (LEPIDOPTERA: NOCTUIDAE) IN FIELD CORN

by

Silvana Vieira de Paula Moraes

DISSERTATION

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ECOLOGY AND INTEGRATED PEST MANAGEMENT OF WESTERN BEAN CUTWORM *Striacosta albicosta* (SMITH) (LEPIDOPTERA: NOCTUIDAE) IN FIELD CORN

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University of Nebraska, 2012

Advisers: Thomas E. Hunt and Robert J. Wright

Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), is a native pest of dry beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.), and historically distributed in the western United States. The insect's recent expansion eastward is documented since 1999. The species is univoltine, the adults typically emerge in the middle of July, lay eggs on corn leaves, and the larvae feed on different corn tissues. In the fourth instar, larvae start colonization of the ear where they feed on kernels. There is a narrow window for management and early detection of the pest in the field is essential. Larval feeding and dispersal of early larval instar studies of western bean cutworm are necessary to improve the understanding about how feeding requirements affect larval survival and establishment on the corn plant. A refinement of economic injury levels (EIL) and economic thresholds (ET) are also required for management decision-making. Studies during 2008 - 2011, across three ecoregions of Nebraska, had the following objectives: (1) developing a binomial sequential sampling plans for egg masses, (2) determine the feeding behavior and dispersal of the early instars on corn, and (3) development of EILs and the ETs that incorporate the dynamics of corn price, management cost, and pest survival. The results from the present study demonstrated that the binomial sequential sampling plans developed significantly reduce the effort required for effective management of this pest, reducing sampling effort from 100 plants sampled to an average of 38 to 41 plants sampled per field. The results indicate low larval survival of this species and that the early instars of western bean cutworm are the critical stage for establishment in corn. A behavior of initial larval movement toward the tassel was observed. The overall mean yield loss one western bean cutworm larva per plant was 945.52 kg/ha (15.08 bu/acre), based on 74,100 plants/ha. The ETs are expressed in percentage of plants with at least one egg mass, incorporate larval survival that was observed across three locations in Nebraska.

Dedication

To my mother Silvina and my father Waldemiro who taught me the value of the work and faith.

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Chapter 1: Introduction

Western bean cutworm

Striacosta albicosta (Smith) (Lepidoptera: Noctuidae), western bean cutworm, was first described in Arizona (Smith 1887). Several revisions of the taxonomic nomenclature of this species have been published and consequently, several scientific names can be found in the literature (Hoerner 1948; Hagen 1962; Lafontaine 2004; Rice 2006b). Dorhout (2007) describes the sequence of events of synonymy. First the species was placed in the genus *Agrotis*, and later included in the genus *Loxagrotis*. Later, all of the species of the genus *Loxagrotis* were placed in the genus *Richia*, and currently western bean cutworm is in a new genus, *Striacosta* (Lafontaine 2004; Rice 2006b).

Reports of western bean cutworm occurrence date from the beginning of the last century (Hoerner 1948). The historic distribution includes Colorado, Idaho, Kansas, Nebraska, western Iowa, Utah, Arizona, New Mexico, Texas, Alberta, and Mexico (Michel et al. 2010). In 1970, a USDA report extended the distribution of western bean cutworm to South Dakota and Wyoming, but did not include Texas (Blickenstaff and Jolley 1982). By 1999, the western bean cutworm had been documented in Minnesota sweet corn (O'Rourke and Hutchison 2000) and Iowa field corn (Rice 2000). Since then, western bean cutworm has been found in Illinois, Missouri (Dorhout and Rice 2004), Wisconsin (Cullen and Jyotika 2008), Indiana (Rice 2006a), Michigan, Ohio (DiFonzo and Hammond 2008), Pennsylvania (Tooker and Fleischer 2010) and Ontario and Quebec, Canada (Baute 2009).

Michel et al. (2010) affirm that considering the historic distribution and current reports, western bean cutworm extended its range first northward and then eastward.

Several hypotheses have been proposed to explain this expansion. Pest replacement is defended by Then (2010) as a main cause, arguing that *Bacillus thuringiensis* (Bt) corn technology has suppressed the major pests, such as *Ostrinia nubilalis* (Hübner) (corn ear borer), and eliminated intraguild competition with *Helicoverpa zea* (Boddie) (corn earworm). This hypothesis is based on the fact that previous and most current Cry proteins are not effective against western bean cutworm (Catangui & Berg, 2006), and in a lab study of interspecific competition between western bean cutworm and corn earworm (Dorhout 2007).

However, one unique factor or event is probably not enough to explain a pest that historically has undergone geographical expansion. The ecological complexity of this species should be considered. Western bean cutworm has corn (*Zea mays* L.) and dry beans (*Phaseolus vulgaris* L.) as primary hosts, which are two widely divergent crops in taxonomy and cultivation (Blickenstaff and Jolley 1982), and its survival is favored in sandy soils (Douglas et al. 1957). A review of possible factors for expansion of the western bean cutworm is presented by Hutchison et al. (2011). The reduced presence of other competitive species from the use of Bt technology and consequent reduction of insecticide application is one of the possible factors. Other factors include wide scale adoption of conservation tillage, increased synchronization between ovipositionally preferred corn stages and moth emergence, among others. Genetic analysis comparing western and eastern populations demonstrated that there is no evidence of a bottleneck event (Miller et al. 2009; Lindroth 2011), and no genetic variation was detected between populations (Lindroth 2011). This suggests the eastern movement could have been triggered by the sudden elimination of an ecological barrier, such as a change in farming practices (Miller et al. 2009). Climate changes, especially with the trend of increasing winter temperatures in the U.S. Midwest, may also favor western bean cutworm survival (Hutchison et al. 2011).

The first report of the western bean cutworm was in dry beans (Hoerner 1948), and since the 1950's there are reports of the insect in corn (Douglass et al. 1957). Other plants are reported as a host of western bean cutworm, such as tomato (*Lycopersicum esculentum* Mill), ground cherry (*Physalis subglabrata* Mackenz. and Bush), *Physalis* sp., and black nightshade (Solanum nigrum L.) (Blickenstaff and Jolley 1982). However, even though these alternative hosts may provide moth oviposition and larval feeding sites, only dry beans and corn have been demonstrated to be suitable hosts for western bean cutworm (Blickenstaff and Jolley 1982).

Western bean cutworm is a univoltine species (Antonelli 1974; Hagen 1976). The adults are strong flyers (Seymour et al. 2004; Dorhout 2007), and a fourteen year light trap summary in western Nebraska indicated that moths usually begin flying in the first week of July, with the peak of the flight occurring in the third week of July (Hagen 1976). A slight indication of six to eight years between high populations was detected, indicating a cyclical population fluctuation (Hagen 1976).

In corn, oviposition occurs on the upper surface of upper leaves with a preference for plants just prior to tassel emergence (Hagen 1962, Blickenstaff 1979, Holtzer 1983, Seymour et al. 2004, Eichenseer et al. 2008). The distribution of egg masses follows a random pattern, with the predominance of one egg mass per plant (Paula-Moraes et al.

2011). There is no oviposition preference between Bt and non-Bt corn (Paula-Moraes et al. 2011). Upon egg hatch, larvae typically move to the tassel area within the whorl to feed on developing pollen (Hagen 1962), the flag leaf, and other tissues (Seymour et al. 2004). During the fourth and fifth instar the larvae feed on corn ear tissue (Seymour et al. 2004). At the end of the fifth instar, the larva drops to the ground, burrows 12 -25 cm below the surface, and becomes a prepupa (Seymour et al. 2004, Appel et al. 1993). Reports of larval survival are variable, from approximately 40% in the laboratory (Blickenstaff and Jolley 1982), and around 4% in the field (Appel et al. 1993).

The corn ear injury and consequent grain yield loss is poorly characterized. Ear injury of 4 to 40% is reported, depending on the chemical control used (Hagen 1962). The area of injury in the ear in several Bt corn hybrids averaged 7.45 cm^2 (Catangui and Berg 2006), and the yield loss of one larva per corn ear was estimated at 3.7 bushel/acre (considering 32,000 plants/acre) (Appel 1991). Qualitative damage from mycotoxin content related with western bean cutworm infestation level is reported; however, results did not indicate a clear association between western bean cutworm larval injury and mycotoxin content of corn grain (Catangui and Berg 2006).

The current transgenic hybrids expressing the Bt protein Cry1F (Herculex I, Dow AgroSciences and Pioneer Hi-Bred International) provides an adequate level of protection against western bean cutworm; however, these hybrids are not immune to this pest and some ear feeding may occur (Eichenseer et al. 2008). The expectation is that Bt corn hybrids with pyramided genes encoding Cry1F/Cry1Ab (Optimum Intrasect Insect protection, DuPont/Pioneer) and Cry1A.105/Cry2Ab2/Cry1F (SmartStax Genuity, Monsanto) are more efficacious against western bean cutworm (DiFonzo and Cullen, 2012). However, there is still significant corn acreage that is not planted to Bt corn each year (e.g., Hutchison et al. 2010), including non-Bt sweet corn in the upper Midwest. This corn must be scouted and managed using thresholds and conventional insecticides (Seymour et al. 2004, Cullen and Jyuotika 2008).

There is a narrow treatment window for chemical control of western bean cutworm, since it is critical the insecticide application occurs before ear colonization (Seymour et al. 2004). Detection of an economic population during the egg stage is ideal, considering the behavioral characteristic of larvae, especially in early instars, to spend time concealed in the whorl or leaf axil.

The current monitoring recommendation for western bean cutworm egg masses is to sample 20 plants at each of five locations per field (100 plants total) (Rice 2006a). Three nominal action thresholds for field and sweet corn are currently used by crop consultants, depending on the corn stage and price. These action thresholds were built upon numerous field observations, and until recently the threshold for field corn was 8% of the plants infested with egg masses, with at least 95% of the plants tasseling (Seymour et al. 2004, Peairs 2006). More recent action thresholds for western bean cutworm were summarized which consider higher corn prices and suggested halving the 8% threshold to 4%, and a threshold of 5% of the plants with egg masses or small larvae on plants ranging from silking to early milk stage (R1-R3) has been suggested (Rice 2007). A threshold of 20% of the plants infested with egg masses at mid-milk stage (R3) has also been recommended (Rice 2007). A threshold of 4% of the plants with egg masses or small larvae in sweet corn, which is historically more valuable than field corn, has also been recommended (Cullen and Jyotika 2008).

The western bean cutworm sampling recommendation of 100 plants per field is based on these nominal thresholds and is not directly associated with an economic injury level. Even though action thresholds based on experience could be relatively effective for a variety of integrated pest management (IPM) programs (Nault et al. 2010), there is a need for more research-based economic injury levels (EIL) for western bean cutworm (Appel et al. 1993).

Concepts in Integrated Pest Management

Stern et al. (1959) highlighted the causes associated with the increased status of pests in agroecosystems. The authors listed: (1) manipulation of the environment, mostly with establishment of monocultures, and consequent improvement of the conditions for high pest population densities; (2) introduction of new pest species into new geographical areas; and (3) the establishment of lower economic thresholds, especially in the cases of fresh products for which cosmetic injury implies an extremely low pest tolerance (Raupp et al. 1988).

The authors were visionary in defining the prevailing problem of pest introduction into new areas. In a modern global economy, the traffic of several kinds of commodities represents pathways for introduction of invasive species which threaten ecosystems, habitats and native species (e.g. Olson 2006). The authors also defined concepts which compose the basis of what they called “The integration of chemical and biological control”, and later were defined as an “Integrated Pest Management” (IPM) (Kogan

1998; Pedigo and Rice 2009). Among several other concepts defined, they first proposed economic level injury (EIL), economic threshold (ET), and the general equilibrium position for a pest population density. However, some deficiencies were pointed out in Stern's approach, and the lack of definition of economic damage is considered most significant (Pedigo et al. 1986).

After Stern's approach, several contributions were made to the EIL concept (Stone and Pedigo 1972; Pedigo et al. 1986; Higley and Pedigo 1996). A definition of EIL is the lowest density of the pest population which causes economic damage (Stern et al. 1959). Subsequent publications introduced the concept of damage boundary, which is the point of lowest possible measured injury of the pest (Pedigo and Rice 2009). At this point the economic impact starts to be expressed in yield or quality loss (Pedigo et al. 1986). The necessary distinction between injury and damage was also made (Pedigo et al. 1986). Injury is the deleterious effect of the pest on the host crop (Pedigo and Rice 2009). Damage is the economic loss of the host crop which can be expressed in quantity or quality reduction of the host crop (Pedigo and Rice 2009). The result is a current cost-benefit equation (Pedigo and Rice 2009) which proposes a balance between the losses associated with pest damage and loss prevention from the pest management (Higley and Peterson 2009).

The EIL calculation is based on the formula (Pedigo et al. 1986)

$$EIL = C/[(ID)(V)(K)]$$

where C = cost of management per area ($/ha), V= market value per unit of produce ($/ha), ID = yield loss per insect (weight of ear in kg/western bean cutworm larvae), and K = proportionate reduction in potential injury.

The most complex aspect of the EIL is the determination of the injury caused by a pest species and quantification of the damage (Higley and Peterson 2009), and substantial data are required in order to make this characterization (Pedigo et al. 1986). In some situations the distinction between injury and damage is difficult (Pedigo and Rice 2009). However, the yield loss (damage) in host crop is related to presence of injury in vegetative tissue. This relationship has a linear response to the rate of the injury, at least in part of the injury/crop response (Pedigo and Rice 2009). Based on this linear relationship, the injury/damage is represented by the slope.

New approaches have been proposed to the EIL, such as the incorporation of environmental costs (Higley and Wintersteen 1992). The environmental cost of pesticides is ranked based on contingent analysis, and makes it possible to use a decision matrix in order to select the least environmentally damaging pesticide. However, even though the method of contingent analysis makes it possible to estimate the monetary value of avoiding levels of risk, more information is demanded about the level of environmental risk of pesticides currently in the market. The aesthetic injury level (Raupp et al. 1988) is also suggested for systems where the appearance of product has aggregated value. The importance of distinguishing between perceived damage and actual injury (e.g. in ornamental systems) has been demonstrated (e.g. Raupp et al. 1988; Coffelt and Schulthz 1990). The multiple-species EIL is considered by Higley and

Peterson (2009) as one of the most challenging and potentially significant contribution in decision management. For this it is necessary to develop an injury equivalency (Hutchins et al. 1988), considering pest species feeding on the same tissue at the same time, and group them in an injury guild (Higley and Peterson 2009). Finally, a probabilistic approach has been proposed in order to consider risk and estimate the probability of the occurrence of an EIL, called the probabilistic economic injury level (PEIL) (Peterson and Hunt 2003). This approach makes it possible to detect the most influencing variable variance, indicating the direction of future research efforts to decrease the uncertainty in EIL determination.

The EIL incorporates aspects of the injury/damage relationship of the pest and crop, and the dynamics of the cost of management and market value of the product. However, the concept of the ET is the one that has a practical application in an agroecosystem. According to Pedigo and Rice (2009), the ET is a parameter based on time, and represents the moment when the pest population must be controlled in order that it does not reach the EIL (Stern et al. 1959). The value of ET is typically set below the EIL. It should consider the pest population growth rate in cases where this information is available. The ET could also incorporate mortality factors or pest survival information (Ostile and Pedigo 1987; Barrigossi et al. 2003), and be expressed in another pest stage than that for which the EIL is defined.

The EIL and ET are appropriate in situations for which curative tactics of management are available and effective against the pest species (Pedigo et al. 1986). Criticisms exist, considering that the EIL and ET concepts are only based on a chemical approach (Higley and Pedigo 1996). However, the EIL determination, with subsequent establishment of ET, allows the discrimination of the species that have a pest status in an agroecosystem from those organisms that do not cause economic loss (Pedigo and Rice 2009), making it possible to establish a multi-component approach to manage the key pests.

Together with the EIL and ET, a sampling plan represents one of the first steps of an IPM program (Kogan 1998). Understanding insect population density is indispensable and of “paramount” importance in IPM (Pedigo and Rice 2009). The utilization of sampling techniques in an agroecosystem allows the detection of the beginning of colonization, pest population dispersion and dynamics in the crop area, pest reproduction, and damage to the plant (Southwood 1980). The correct timing of sampling makes it possible to determine the pest population density, but it should be done neither much before the pest population is established nor after the pest damage reaches the ET (Higley and Peterson 1994). Moreover, the use of curative treatments can be economically justified if a set number of samples are taken and the pest population density is adequately estimated. Ruesink (1980) also highlights the application of insect sampling to estimate insect population density in basic studies in ecology, such as measuring biodiversity. In both cases the development of sampling plans based on a probabilistic foundation has critical importance in data collection. This allows analysis and inference about the insect population under study (Young and Young, 1998).

The design of a sampling plan protocol requires a sequence of steps: (1) definition of the target and sampled pest population; (2) selection of the habitat that will be sampled, with determination of the appropriate sampling unit, and: (3) determination of the number of observations that will be necessary, considering the desired precision of the pest population density estimate. Binns and Nyrop (1992) affirm that the sampling protocols consist of at least two components: (1) a methodology for estimation of the pest population density; and (2) the economic threshold. The authors also consider that phenological forecasts can be a third component which defines the best time to estimate the population.

The appropriate level of precision in sampling and estimating the pest population density assures that information determined from sampling is close to the true pest density in the field. An important aspect in the estimation of the population density is the collection of a random sample from the population. If the population has “N” individuals, from which a sample “n” will be drawn, in one random sample every individual of the sampled population should have an equal probability to be chosen. Based on the information from the random samples, it is possible to calculate the sample mean which is an appropriate estimation of population density. However, insects are seldom randomly distributed in nature (Morris, 1960), and in many cases the demand for randomization for estimating variances may be ignored and nonrandom methods may be justified (Binns and Nyrop, 1992). This is common in sampling in IPM when the collection of samples follows predetermined patterns and the variance in the estimate will not depend on the chance of the draw, but will depend on the sampling plan and spatial distribution of the pest in the crop (Legg and Moon, 1994).

Another aspect, when designing a sampling plan is that there are cases which the habitat of the insect is not homogeneous and stratification of the habitat can be necessary in order to have a more precise estimate (Buntin and Pedigo 1981). If this is the case, the number of strata and number of samples per stratum should be chosen carefully (Binns and Nyrop 1992).

Defining the number of observations is a challenging part of developing an IPM sampling plan. The optimum sample size must be the smallest number of sample units that achieve economic viability, and has the desired precision of the population parameter estimation (Karandinos 1976). Young and Young (1998) highlight the problems in estimation of low or sparse populations, and how inadequate sample size together with lack of data validation can compromise the results of population density sampling. These aspects can be especially important in the case of pests in which a low number of insects can cause economic impact in the crop.

Knowledge of the dispersion of the pest is important in order to determine the optimal sampling pattern and sample size, and dispersion can be classified as random, uniform, or aggregated. Morris (1960) calls attention to the difference between spatial and probability distribution. Differences in spatial pattern of insects can be observed among different insect life stages, and this is especially common in species with holometabolous development (Pedigo and Zeiss 1996).

The information on the probability distribution of the pest, together with the information of its biology, ecology and behavior, are useful to determine the number of samples in pest sampling protocols. Based on the probability distribution of the pest, there are formulas which provide precise estimation of population density with a specific coefficient of variation, and other formulas that calculate the precision of required sample size expressed as a confidence interval.

The most common probability distributions to describe populations of organisms are binomial, Poisson, negative binomial and geometric (Young and Young 1998). The random distribution describes the case of habitats which have the same chance to be occupied by insects (Buntin and Pedigo 1983). The negative binomial distribution is considered by Taylor (1984) to be the most common distribution found in insect management studies. The geometric distribution is a kind of negative binomial distribution where the number of samples with an absence of insects will be large, and is a distribution that describes the movement of organism in a uniform habitat (Young and Young 1998).

An alternative resampling approach is proposed by Naranjo and Hutchison (1997), in which actual field data are resampled several times, creating an average and associated variance. In this way, the development of a sampling plan is based on the actual sampling distribution of the species, and does not use a theoretical model of distribution (Burkness et al. 1999; Hodgson et al. 2004; Galvan et al. 2007; Burkness and Hutchison 2009; Paula-Moraes et al. 2011). However, a representative and independent data set representing a range of high, medium, and low pest densities is required in order to conduct resampling analysis in Resampling for Validation of Sample Plans (RVSP).

There are two approaches for sampling populations. The first approach is a fixed sample size and the number of samples will depend on the distribution of the population and the variance. For sequential sampling, the number of samples depends on the mean density and does not give an estimate of population density, but classifies the insect population according to whether they are above or below a certain threshold. The number of samples is not fixed in advance (Hutchison 1994). This approach is also defined as decision-making sampling and informs us whether or not management is required, based on the classification of the population at an economic or non-economic density (ET) (Pedigo and Zeiss 1996).

To design a sequential sampling plan, besides requiring the population distribution, we must know the ET and probabilities of type I error and type II error. The levels of the two types of errors represent the levels of the amount of risk that can be tolerated in any sampling plan (Pedigo and Zeiss 1996). The sequential plan is characterized by a graph which has two parallel decision lines. Values of pest insect density which fall above the upper decision line indicate that management measures need to be adopted, and values below the lower decision line indicate no need for management (Pedigo and Zeiss 1996). According to Binns et al. (2000), sequential sampling may save 50% or more time when compared with fixed sample size.

However, despite the critical importance of a sampling plan, EILs and ETs, a review found that only 100 pests from 43 commodities had EILs determined (Peterson 1996). More recently, another review spanning the period of 1970 to 2009 detected 105 relevant papers published in the Journal of Economic Entomology. But, in only 60 were there studies concerning the development of EILs, ETs and sampling plans (Castle and Naranjo 2009). In half of them the thresholds developed did not come with a respective sampling plan for their effective use and adoption.

Even in cases where the statistical aspects (first barrier) of methodology to development of pest sampling plan is solved, the problem of complexity of a sampling plan can persist and cause modifications or deleterious adjustments of the protocol when it starts to be applied in the field (Naranjo et al 1997; Grieshop et al. 1988).

Another aspect of development and adoption of EILs, ETs and pest sampling plans is the actual scenario where transgenic technology, especially Bt technology, is utilized in large scale cultivation of commodities in several countries around the world. Doubts have been raised about the survival of the IPM framework with a technology which decreases the pest population density with a high level of efficiency (Kogan 1998), and is adopted as a preventive measure. These characteristics can be considered a negation of all concepts and the proposition of IPM. Hellmich et al. (2008) state that conflicts with the use of transgenic plants which express high levels of toxin, sometimes in unnecessary situations, should be discussed as the same situation as the use of resistant plants developed from conventional breeding programs. The reduction of chemical control with consequent improvement of natural biological control and area suppression of key pests are cited as benefits of the Bt technology (Naranjo 2005; Hutchison et al. 2010). However, this increased chances of the occurrence of secondary pests as a consequence of the reduction of chemical control and elimination of effective cultural practices, such as crop rotation, are cited as drawbacks of the technology (Hellmich et al. 2008). All of these consequences may impact IPM system. Thus, there are situations in which Bt technology demands modifications to the sampling protocol and the threshold applied to estimate the pest population density (Naranjo et al. 2008).

Dissertation research objectives

Several aspects of ecology and management of the western bean cutworm demand more information. Besides the historic impact of the pest in the western-central US, the recent eastward expansion in the Corn Belt requires development of IPM decision tools. A binomial sequential sampling plan presents a time-saving option for western bean cutworm management because this approach implies that plants would only be examined until one egg mass is found, and not evaluated for the number of egg masses per plant. The economic impact of western bean cutworm has scarcely been reported in the literature and development of research-based EILs and ETs is necessary.

Even with the possibility of hybrids expressing Bt toxins against western bean cutworm, information on ecology such as pest dispersal is important because the mobility of the species 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.

Based on these considerations, the objective of this research was to improve pest management for western bean cutworm. The specific objectives were:

1. Assess the potential for developing binomial sequential sampling plans for western bean cutworm egg masses that could be more cost-effective than the fixed size sampling currently in place.
2. Determine the feeding behavior, dispersal, and establishment of the early instars of western bean cutworm on corn.
3. Estimate the injury capacity of western bean cutworm in the lab and in the field, and define the injury that is tolerable in the corn field with development of EILs.

4. Characterize egg and larval survival and incorporate results into ETs.

References Cited

Antonelli, A. L. 1974. Resistance of *Phaseolus vulgaris* cultivars to western bean cutworm, *Loxagrotis albicosta* (Smith), with notes on the bionomics and culture of the cutworm. Ph.D. dissertation, University of Idaho, Moscow.

Appel, L. L.. 1991. Damage in field corn (*Zea mays* (L.)) caused by *Loxagrotis albiscosta* (Smith). Master Science thesis, University of Nebraska, Lincoln.

Appel, L. L., R. J. Wright, and J. B. Campbell. 1993. Economic injury levels for western bean cutworm, *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae), larvae in field corn. J. Kans. Entomol. Soc. 66: 434-438.

Baute, T. 2009. Current distribution of western bean cutworm in the Great Lakes Region. Crop pest Ontario Newsletter 14:7 http://www.omafra.gov.on.ca/english/crops/field/news/croppest/2009/18cpo09a3.pdf

Barrigossi, J.A.F., G.L. Hein, and , L.G. Higley. 2003. Economic injury levels and sequential sampling plans for Mexican bean beetle (Coleoptera: Coccinellidae) on dry beans. J. Econ. Entomol. 96: 1160-1167.

Binns M.R, J.P. Nyrop J.P., and W. van der Werf. 2000. Sampling and Monitoring in Crop Protection: The Theoretical Basis for Developing Practical Decision Guides. CABI Publishing, Wallingford, Oxon.

Binns, M.R., and J.P. Nyrop. 1992. Sampling insect populations for the purpose of IPM decision-making. Annu. Rev. Entomol. 37:427-453.

Blickenstaff, C. C. 1979. History and biology of the western bean cutworm in southern Idaho, 1942-1977. Univ. Idaho Agric. Exp. Stn. Bull. No. 592.

Blickenstaff, C. C., and P.M. Jolley. 1982. Host plants of western bean cutworm. Environ. Entomol. 11:421-425.

Buntin, G. D., and L. P. Pedigo. 1981. Dispersion and sequential sampling of green cloverworm eggs in soybeans. Environ. Entomol. 10: 980-985.

Buntin, G. D., and L. P. Pedigo. 1983. Seasonality of green cloverworm (Lepidoptera: Noctuidae) adults and an expanded hypothesis of population dynamics in Iowa. Environ. Entomol. 12: 1551-1558.

Burkness, E. C. and W. D. Hutchison. **2009.** Implementing reduced-risk integrated pest management in fresh-market cabbage: influence of sampling parameters, and validation of binomial sequential sampling plans for the cabbage looper (Lepidoptera: Noctuidae)**.** J. Econ. Entomol. 102: 1874-1883.

Burkness, E. C., R. C. Venette, P. K. O'Rourke, and W. D. Hutchison. 1999. Binomial sequential sampling for management of aster leafhopper (Homoptera: Cicadellidae) and aster yellows phytoplasma in carrot: impact of tally threshold on the accuracy of treatment decisions. Environ. Entomol. 28: 851-857.

Castle, S.J., and Naranjo, S.E. 2009. Sampling Plans, Selective Insecticides and Sustainability: The Case for IPM as Informed Pest Management. P. Manag. Science, 65: 1321-1328.

Catangui, M. A., and R. K. Berg. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry 1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environ. Entomol. 35: 1439-1452.

Coffelt, M. A., and P. B. Schultz. Development of an aesthetic injury level to decrease pesticide use against orangestriped oakworm (Lepidoptera: Saturniidae) in an urban pest management project. J. Econ. Entomol. 83: 2044-2049.

Cullen, E., and J. Jyuotika. 2008. Western bean cutworm: a pest of sweet corn. A3856, University of Wisconsin Extension, Cooperative Extension. On-line: http://learningstore.uwex.edu/pdf/A3856.pdf.

DiFonzo, C. D., and E. Cullen. 2012. Handy Bt trait table. University of Wisconsin Extension, MSU Field Crops Entomology Program. On-line: http://www.entomology.wisc.edu/cullenlab/publications/pdf.

Dorhout, D. L. 2007. Ecological and behavioral studies of the western bean cutworm (Lepidoptera: Noctuidae) in corn. Master thesis . Iowa State University, Ames.

Dorhout, D. L., and M. E. Rice. 2004. First report of western bean cutworm, *Richia albicosta* (Noctuidae) in Illinois and Missouri. Crop Mgmt. On-line: http://www.plantmanagementnetwork.org/pub/cm/brief/2004/cutworm. DOI:10.1094/CM-2004-1229-01-BR.

Douglass, J. R., J. W. Ingram, K. E. Gibson, and W. E. Peay. 1957. The western bean cutworm as a pest of corn in Idaho. J. Econ. Entomol. 50: 543-5.

Eichenseer, H., R. Strohbehn, and J. Burks. 2008. Frequency and severity of western bean cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* cry toxins. J. Econ. Entomol. 101: 555–563.

Galvan, T.L., Burkness, E.C., and W.D. Hutchison. 2007. Enumerative and binomial sequential sampling plans for the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in wine grapes. J. Econ. Entomol. 100:1000-1010.

Grieshop, J. I., F. G. Zalom, and G. Miyao. 1988. Adoption and diffusion of integrated pest management innovations in agriculture. Bull. Entomol. Soc. Am. 34: 72-78.

Hagen, A. F. 1976. A fourteen-year summary of light trap catches of the western bean cutworm in Nebraska *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae). J. Kansas Entomol. Soc. 49: 537-540.

Hagen, A. F. 1962. The biology and control of the western bean cutworm in dent corn in Nebraska. J. Econ. Entomol. 55: 628-631.

Hellmich, R. L., R. Albajes, D. Bergvinson, J. R. Prasifka, Z.Y. Wang and M. J. Weiss. 2008. The present and future role of insect-resistant genetically modified maize in IPM, pp.119-158. In J. Romeis, A. M. Shelton, and G. G. Kennedy (eds.), Integration of insect-resistant genetically modified crops within IPM programs. Springer, Netherlands.

Higley, L.G., and L.P. Pedigo. 1996. The EIL concept, pp. 9-21. In L. G. Higley and L. Pedigo (eds.), Economic Threshold for integrated pest management. University of Nebraska Press, Lincoln, NE.

Higley, L. G., and R. K. D. Peterson. 2009. Economic decision rules for IPM, pp. 25-32. In E.B. Radcliffe, W.D. Hutchison, and R.E. Cancelado (eds.), Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press, Cambridge, UK.

Higley, L. G., and R. K. D. Peterson. 1994. Initiating sampling programs, pp.119-136. In L. Pedigo and G. Buntin (eds.),Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.

Higley, L.G., and W. Wintersteen. 1992. A novel approach to environmental risk assessment of pesticides as a basis for incorporating environmental costs into economic injury levels. American Entomol. 38: 34-38.

Hodgson, E. W., E. C. Burkness, W. D. Hutchison, and D. W. Ragsdale. 2004. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. J. Econ. Entomol. 97: 2127-2136.

Hoerner, J. L. 1948. The cutworm *Loxagrotis albicosta* on beans. J. Econ. Entomol. 41: 631-635.

Holtzer, **T. O. 1983**. Distribution of western bean cutworm eggs among short-, mid, and long-season corn hybrids planted on different dates. Environ. Entomol. 12: 1375–1379.

Hutchins, S., L. G. Higley, and L. P. Pedigo. 1988. Injury equivalency as a basis for developing multiple-species economic injury levels. J. Econ. Entomol. 81: 1-8.

Hutchison, W. D., T. E. Hunt, G. L Hein, K. L. Steffey, C. D. Pilcher, and M. E Rice. 2011. Genetically Engineered Bt Corn and Range Expansion of the Western Bean Cutworm (Lepidoptera: Noctuidae) in the United States: A Response to Greenpeace Germany, J. Integ. Pest Mgmt. 2: B1-B8. DOI: 10.1603/IPM11016.

Hutchison, W. D., E. C. Burkness, P. D. Mitchell, R.D. Moon, T.W. Leslie, S.J. Fleischer, M. Abrahamson, K.L. Hamilton, K.L. Steffey, M.E. Gray, R.L. Hellmich, L.V. Kaster, T.E. Hunt, R.J. Wright, K. Pecinovsky, T.L. Rabaey, B.R. Flood and E.S. Raun. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science. 330: 222-225.

Hutchison, W. D. 1994. Sequential sampling to determine population density, pp. 207-244. In L. Pedigo and G. Buntin (eds.), Handbook of sampling methods for arthropods in agriculture. CRC, Boca Raton, FL.

Karandinos, M.G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417-421.

Kogan, M. 1998. Integrated pest management: historical perspectives and contemporary developments. Annu. Rev. Entomol. 43:243–70.

Lafontaine, J.D. 2004. Noctuoidea, Noctuidae (part): Noctuinae, Agrotini. In R.W. Hodges (ed.), The Moths of North America, fasc. 27.1. Allen Press, Inc., Lawrence, KS.

Legg, D.E., and R.D. Moon. 1994. Bias and variability in statistical estimates, pp. 99–118. In L.P. Pedigo andG.D. Buntin (eds.), Handbook of Sampling Methods for Arthropods in Agriculture, CRC Press, Boca Raton, Florida.

Lindroth, E. J. 2011. Population genetics of the western bean cutworm (*Striacosta albicosta* smith) across the United States. Ph.D. dissertation, University of Nebraska, Lincoln.

Michel, A. P., C. H. Krupke, T. S. Baute, and C. D. DiFonzo. 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. J. Integ. Pest Mgmt. 1:A1-A10. DOI: 10.1603/IPM1003.

Miller, N. J., D. L. Dorhout, M. E. Rice and T. W. Sappington. 2009. Mitochondrial DNA variation and range expansion in western bean cutworm (Lepidoptera: Noctuidae): no evidence for a recent population bottleneck. Environ. Entomol. 38: 274-280

Morris, R.F. 1960. Sampling insect populations. Annu. Rev. of Entomol. 5: 243-264.

Naranjo, S. E., J. R. Ruberson, H. C. Sharma, L. Wilson, and K. Wu 2008. The present and future role of insect-resistant genetically modified cotton in IPM, pp.159-194. In J. Romeis, A. M. Shelton, and G. G. Kennedy (eds.), Integration of insect-resistant genetically modified crops within IPM programs. Springer, Netherlands.

Naranjo, S. E. 2005. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environ. Entomol. 34: 1211-1223.

Naranjo, S. E., and W. D. Hutchison. 1997. Validation of arthropod sampling plans using a resampling approach: software and analysis. Am. Entomol. 43: 48-57.

Naranjo, S.E., J.W. Diehl, and P.C. Ellsworth. 1997. Sampling whiteflies in cotton: validation and analysis of enumerative and binomial plans. Environ. Entomol. 26: 777-788.

Nault, B.A. and A. M. Shelton. 2010. Impact of insecticide efficacy on developing action thresholds for pest management: a case study of onion thrips (Thysanoptera: Thripidae) on onion. J. Econ. Entomol. 103: 1315-1326.

O'Rourke, P. K., and W. D. Hutchison. 2000. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. J. Agric. Urban Entomol. 17: 213-217.

Olson, L.J. 2006. The economics of terrestrial invasive species: a review of the literature. Agr. Res. Econ. Rev. 35: 178–94.

Ostlie, K. R. and L. P. Pedigo. 1987. Incorporating pest survivorship into economic thresholds. Bull. Entomol. Soc. Am. 33:98–102.

Paula-Moraes, S.V., E. C. Burkness, T. E. Hunt, R. J. Wright, G. L. Hein, and W. D. Hutchison. 2011. Cost-effective binomial sequential sampling of western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), egg masses in corn. J. Econ. Entomol. 104: 1900-1908.

Peairs, F. B. 2006. Western bean cutworm: characteristics and management in corn and dry beans. Colorado State University Extesion. 3/02.n. 5.5538. On-line: http://www.ext.colostate.edu/pubs/insect/05538.html

Pedigo, L. P. and M. E. Rice. 2009. Entomology and pest management. New Jersey: Prentice Hall.

Pedigo, L.P., S.H. Hutchins, and L.G. Higley. 1986. Economic injury levels in theory and practice. Annu. Rev. Entomol. 31:341–368.

Pedigo, L. R. and M.R. Zeiss. 1996. Analyses in insect ecology and management. Iowa State University Press, Ames, Iowa.

Peterson, R.K.D., and T.E. Hunt. 2003. The probabilistic economic injury level: incorporating uncertainty into pest management decision-making. J. Econ. Entomol. 96:536-542.

Peterson, R.K.D. **1996.**The status of economic-decision level development, pp.151-178. L.G. Higley, L and L.P. Pedigo, (eds.). Economic thresholds for integrated pest management. University of Nebraska Press, Lincoln, NE.

Raupp, M., A. Davidson, C. S. Koehler, C. S. Sadof, and K. Reichelderfer. 1988. Decision-making considerations for aesthetic damage caused by pests. Bull. Entomol. Soc. Am. 34: 27-32.

Rice, M. E. 2007. Economic thresholds for western bean cutworms. Iowa State. University Extension, Ames, IA.On-line: (http://www.ipm.iastate.edu/ipm/icm/2007/7-2/wbcth.html).

Rice, M. E. 2006a. Eastern movement of the western bean cutworm into Indiana and Ohio. Proceedings: Indiana CCA Conference, Indianapolis, IN.

Rice, M.E. 2006b. Name change for western bean cutworm. Integrated Crop Mgt. IC-496 (6): 79. Iowa State University Extension, Ames, IA. http://www.ipm.iastate.edu/ipm/icm/2006/4-10/wbcw.html).

Rice, M. E. 2000. Western bean cutworm hits northwest Iowa. Integrated Crop Mgt. IC-484 (22): 163. Iowa State University Extension, Ames, IA. (http://www.ipm.iastate.edu/ipm/icm/2000/9-18-2000/wbcw.html).

Ruesink, W.G. 1980. Introduction to Sampling Theory, pp. 61-78. In. M. Kogan and D.C. Herzog (eds.), Sampling methods in soybean entomology. Springer-Verlag, New York, EUA.

Seymour, R. C., G. L. Hein, R. J. Wright, and J. B. Campbell. 2004. Western bean cutworm in corn and dry beans. Neb. Coop. Ext. Guide G1359. Lincoln, NE.

Southwood, T.R.E. 1980. Ecological Methods: with particular reference to the study of insect populations. Chapman and Hall, London, England.

Stern, M., **R.F. Smith, R. van den Bosch, and K.S. Hagen. 1959.** The integrated control concept. Hilgardia, 29: 330-350.

Stone, J.D. and L.P. Pedigo. 1972. Development and economic-injury level of the green cloverworm on soybean in Iowa. J. Econ. Entomol. 65:197-201.

Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. Annu. Rev. Entomol. 29:321–357.

Then, C. 2010. New plant pest caused by genetically engineered corn. The spread of the western bean cutworm causes massive damage in the US, a Testbiotech Report. http://www.testbiotech.de/sites/default/files/WBC en_25_3_2010.pdf

Tooker, J. F and S. J. Fleischer. 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. Crop Mgmt. DOI: 10.1094/CM-2010-0616-01-RS.

Young, L.J., and J.H. Young. 1998. Statistical Ecology*:* a population perspective. Kluwer Academic, Boston, EUA.

Chapter 2: Cost-effective binomial sequential sampling of western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), egg masses in corn

Introduction

Striacosta albicosta (Smith) was first described in Arizona (Smith 1887) as a native pest of dry beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) in the west-central United States (Hoerner 1948; Douglass et al. 1957). Prior to 1999, it was reported in Idaho, Kansas, Nebraska, Iowa, Utah, Colorado, Arizona, New Mexico, Texas, South Dakota, Wyoming, Oklahoma (Appel et al. 1993). Beginning in 1999, however, *S. albicosta* larval infestations were documented in Minnesota sweet corn (O'Rourke and Hutchison 2000), and Iowa field corn (Rice 2000). Since then, *S. albicosta* has been found in Illinois, Missouri (Dorhout and Rice 2004), Wisconsin (Cullen and Jyotika 2008), Indiana (Rice 2006), Michigan, Ohio (DiFonzo and Hammond 2008), Pennsylvania (Tooker and Fleischer 2010) and Ontario and Quebec, Canada (Michel et al. 2010). Reasons for the apparent range expansion of *S. albicosta* remain unknown (Miller et al. 2009).

Striacosta albicosta is univoltine (Hagen 1962) and adults oviposit egg masses on the upper surface of corn leaves on the top half of the plant and in the whorl just prior to tassel emergence (Hagen 1962). Larvae feed for about 31 days, initially feeding on the anthers of the tassel, pollen, and then silks until they reach the fourth instar, when they move inside the ear and feed on kernels (Appel et al. 1993; Seymour et al. 2004). For each late instar per ear that survives to the dent stage, yield loss is estimated at 232.13 kg/ha (Appel et al. 1993). Transgenic hybrids expressing the *Bacillus thuringiensis* (Bt) protein Cry1F (Herculex I, Dow AgroSciences and Pioneer Hi-Bred International) provides an adequate level of protection against S. *albicosta* feeding damage. However, these hybrids are not immune to *S. albicosta* and some ear feeding damage may occur

(Eichenseer et al. 2008). In 2010, Bt corn hybrids with pyramided genes encoding Cry1F/Cry1Ab (Optimum Intrasect Insect protection, DuPont/Pioneer) and Cry1A.105/Cry2Ab2/Cry1F (SmartStax Genuity, Monsanto) were also commercially available. These hybrids are expected to be more efficacious against *S. albicosta* (e.g., DiFonzo and Cullen, 2010).

Despite the availability of the transgenic technology, many Lepidopteran resistant transgenic hybrids are not efficacious against *S. albicosta* and there is still significant corn acreage that is not planted to Bt corn each year (e.g., Hutchison et al. 2010), including non-Bt sweet corn in the upper Midwest. This corn must be scouted and managed using thresholds and conventional insecticides (Seymour et al. 2004, Cullen and Jyuotika 2008). Traditionally, Nebraska Integrated Pest Management (IPM) guidelines have indicated that 100 plants should be sampled, on average, for *S. albicosta* egg masses or larvae for making treat, no-treat decisions in corn. Although likely effective, this guideline is time-consuming and has presented obstacles to management of this insect. In addition, field experience and preliminary data suggest that a smaller sample size might be possible. Practical, cost-effective arthropod monitoring and sampling plans are critical elements of an IPM program that provide a foundation for determining when control measures should be applied.

Several economic, or action thresholds have been proposed for *S. albicosta* in corn. A common threshold used for field corn is 8% of the plants infested with egg masses on corn that is at least 95% tasseled (Seymour et al. 2004, Peairs 2006). Rice (2007) summarized more recent thresholds that consider higher corn prices and suggested halving the 8% threshold to 4%; a threshold of 5% of the plants with egg masses or small larvae on plants ranging from silking to early milk stage (R1-R3) has also been suggested. Finally, a threshold of 20% of the plants infested with egg masses at mid-milk stage (R3) has also been recommended (Rice 2007). Cullen and Jyotika (2008) recommend a threshold of 4% of the plants with egg masses or small larvae in sweet corn, which is historically more valuable than field corn. The sampling plan associated with these nominal thresholds could be viewed as an action threshold, as it is not directly associated with an economic injury level. However, an action threshold based on experience, and built upon numerous field observations can be very effective for a variety of IPM programs (e.g., Nault et al. 2010). For *S. albicosta*, and regardless of the desired threshold, the recommendations have required a fixed sample size of 100 plants per field (Rice 2006).

A binomial sequential sampling plan represents one time-saving option (Kuno 1991, Jones 1994) that allows one to determine only the presence or absence of the pest. For *S. albicosta*, this approach implies that plants would only be examined until one egg mass is found (tally threshold of one), and not evaluate the number of eggs masses per plant; samplers can then move on to the next plant (e.g., Binns et al. 2000). Moreover, the number of samples is not fixed in advance, and the pest population is classified as an economic or non-economic density after a cumulative number of the samples are examined in the field (Hutchison 1994).

The objective of this study was to assess the potential for developing binomial sequential sampling plans for *S. albicosta* egg masses that could be more cost-effective than the fixed-sample size monitoring currently in place. Three sampling plans were developed that consider the action thresholds currently used in the U.S. Corn Belt (4, 8, and 20% of plants infested with one or more egg masses) for both field and sweet corn.

Materials and Methods

Egg masses of *S. albicosta* were sampled in a total of 70 commercial corn fields; 34 fields were sampled in 2005, 20 fields in 2006 and 16 fields in 2007 in southwest Nebraska and northeast Colorado. Fields were planted with Bt transgenic corn (Herculex™) expressing the Cry1F toxin and non-Bt commercial field corn. The two types of hybrids (Bt and non- Bt), were planted in alternating strips of 4 to 12 rows each (typically 6 or 8) in the center of the fields. The remainder of the field were planted to a herculex or non-herculex variety. All fields were under center-pivot irrigation and conventional agronomic practices were followed for the region. In each field, at least three and at most 10 sites were sampled in a transect across the field and the number of egg masses was counted on each of 20 consecutive plants per sampling site.

Spatial distribution of egg masses. The relationship between the mean and variance was tested using Taylor's power law (TPL; Taylor 1961) to describe the dispersion pattern of *S. albicosta* egg masses in the corn. The effect of Bt and non-Bt corn on *S. albicosta* oviposition preference was tested by comparing the proportion of plants infested with egg masses between the two hybrids in each field (Proc Glimmix, SAS Institute 2009).

Binomial sampling. The binomial sampling plan was developed using Wald's sequential probability ratio test (Wald 1947), and validated using Resampling for Validation of Sampling Plans (RVSP) software (Naranjo and Hutchison 1997). As discussed by Young and Young (1998), for designing Wald's sequential sampling plan the following information are necessary: (a) economic threshold; (b) safety level (the lower and upper bounds around economic threshold); (c) probabilities of type I and II errors (α and β); and (d) and the distribution of the pest to be sampled. In presence/absence sampling, the binomial distribution is the parametric form of the distribution. The binomial classification is used to create the most cost efficient sampling plan for making pest management decisions for *S. albicosta*.

After removing data sets with a mean *S. albicosta* egg mass density of zero, 55 of the 70 field data sets were used in the analysis. Several parameters were established in order to conduct Wald's binomial sequential probability ratio test. The number of resampling iterations for each field's data set was set at 500, the sampling was done with replacement, and the minimum sample size was defined as 20 sample units (plants) for each simulation (Naranjo and Hutchison 1997). The tally threshold was set at one *S. albicosta* egg mass, which is the number of egg masses required for the sample to be defined as infested.

Three different *S. albicosta* sampling plans were developed using thresholds of 4% of plants infested with egg masses for silking/blister/early milk-stage corn (Cullen and Jyotika 2008); 8% of plants infested with *S. albicosta* egg masses on corn at least 95% tasseled (Seymour et al. 2004; Peairs 2006); and 20% of plants infested with *S. albicosta* egg masses for mid-milk-stage corn (Rice 2007). For each threshold at least two different upper and lower boundaries were tested that represented the proportion of plants infested with *S. albicosta* egg masses (Jones 1994). For the 4% threshold, three different ranges of variation were simulated (Burkness and Hutchison 2009). Two ranges of variation were similar and symmetric in lower and upper bounds (0.01-0.07 and 0.02-0.06). Since the 0.02 - 0.06 range of variation for the 4% threshold resulted in an ASN of

100 plants (current recommendation) a third sampling plan simulation was run. The boundaries of the third plan were asymmetric; with the lower bound at 0.01 and upper bound at 0.09. For the 8% and 20% thresholds, the ranges of variation were symmetric. The lower and upper bounds were 0.04 and 0.05 from each threshold, respectively. These boundaries were necessary for the calculation for the sequential model and final stop lines (treat or no-treat decision lines) for the Wald's binomial sequential probability ratio test. The α value (type I error) was set at 0.10 (Binns 1994). The β value (type II error) was set at 0.05.

The RVSP software used a uniform random number generator to select samples from the *S. albicosta* egg mass data set until the sequential rule was satisfied (treat or no-treat decision was reached). When the sequential rule was satisfied, the software calculated the average proportion of infested samples (PI), and the average sample number (ASN), which represents the required sample size over all sampling interactions. The operating characteristic (OC) function was also calculated and the data sets were classified as above or below for each action threshold. The OC expresses the probability of not taking action relative to the true mean of infested plants with *S. albicosta* egg masses, and ASN expresses the mean sample size required in order to reach this decision (Naranjo and Hutchison 1997). The impact of the different action thresholds was analyzed using ASN. The OC and ASN were also plotted against the proportion of infested plants from the field data, using TableCurve2D software (Systat Software, Inc. 2002).

Sequential sampling simulations were validated using independent field data for each action threshold and a decision matrix was used as an extension of the validation process (Calvin 1986; Burkness et al. 1999; Burkness and Hutchison 2009). Our decision matrix calculates the probability of making the correct decision (treat or no-treat). First, the observed PI (proportion of plants infested with *S. albicosta* egg masses) calculated from field data was compared to the action threshold to determine which decision should be made. If PI is greater than the action threshold the decision is to treat. On the other hand, if PI is less than the action threshold the decision is to no-treat. Second, the average PI was calculated as an average of infested plants. This number was generated by averaging the resampling results over the 500 resampling bouts. Again, the average PI was compared with the action threshold following the same evaluation as for the observed PI. The third step was the comparison between values of observed PI and average PI and the categorization of the results as follows: 1) observed PI and average PI are larger than the action threshold and the correct decision is to treat; 2) observed PI and average PI are smaller than the action threshold and the correct decision is no-treat; 3) observed PI is smaller than the action threshold but average PI is larger resulting in an incorrect decision to treat; or 4) observed PI is larger than the action threshold but the average PI is smaller resulting in an incorrect decision to no-treat.

Sampling cost and final sampling plan selection criteria. The cost estimates of sampling, considering the time spent to inspect *S. albicosta* egg masses on corn plants, were obtained by sampling egg masses from five commercial corn fields in Nebraska during 2005. Sampling was conducted by a researcher with previous experience with *S.*

albicosta egg sampling. Considering the practical recommendation of sampling 100 plants per field, the time spent to sample 10 sites with 10 plants each was measured. The mean sampling time, including the time to walk between each sample, is presented in Table 2.2. Cost estimates are also provided based on current value of hourly labor for crop consultants.

Binomial sampling plan benefits were analyzed using a benefit-cost ratio formula (Burkness et al. 1999; Hodgson et al. 2004; Galvan et al. 2007) as follows:

$$\text{benefit-cost ratio} = [\sum P_c / (n \times c)] \times 100,$$

where P_c is the sum of the overall proportion of correct decisions (A+D column in Table 2.1), n is the average sample size required to make a decision, and c is the cost of collecting the sample. The cost includes both the time of sampling each plant for egg masses and the time to travel between each sample (Table 2.2).

For each action threshold, one sampling plan was selected as most appropriate considering the balance among OC, ASN, α, β (Table 2.1 and Fig. 2.1). The benefit-cost ratio was also considered as a final criterion of sampling plan selection (Table 2.3).

Results

Spatial distribution of egg masses. Taylor's Power Law (TPL) regression of the log-mean and log-variance of *S. albicosta* egg masses densities was highly significant ($P = 0.0001$) with a coefficient of determination (R^2) of 0.99. The y-intercept was 1.13 (SE 0.02) and slope was 1.03 (SE 0.01). From the Student t-test, the slope of the regression was not significantly different from 1.0 ($P > 0.05$), suggesting the egg mass distribution in corn follows a random pattern. No differences were detected between percentage of egg masses found in Bt corn (8.4 SD 12.37) and non-Bt corn (5.20 SD 8.15) ($P = 0.20$). Of the total number of plants sampled (5640), 540 were infested with egg masses. Of the infested plants, 85.7% had one egg mass, 12.2% had 2 egg masses, 2.0% had 3 egg masses, and 0.7% had 4 egg masses.

Binomial Sampling. Variation in upper and lower boundaries for each action threshold affected the OC and ASN (Table 2.1, Fig. 2.1). Results from Wald's binomial analysis are reported for three action thresholds (4%, 8%, and 20%; Table 2.1). The probability of making correct decisions includes correct decisions to treat (A column) and correct decisions to no-treat (D column). The probability of making incorrect decisions includes incorrect decisions to no-treat (B column) and incorrect decisions to treat (C column). These probabilities were calculated from the estimated proportion of plants infested with *S. albicosta* egg masses, and OC values. The overall proportion of correct decisions are presented in the A+D columns for each simulation. For the 4% threshold, the probabilities to make a correct decision (A+D) ranged from 0.805 to 0.874. For the 8% threshold, the probabilities to make a correct decision (A+D) ranged from 0.895 to

0.939. The probabilities of making a correct decision for the 20% thresholds were quite similar, at 0.957 and 0.961. The average number of plants required to reach either a treat or no-treat decision (ASN) ranged from 37 to 100 plants. For most simulations the ASN ranged from 40 to 55 plants. Only one simulation for the 4% threshold resulted in an ASN of 100 plants.

The value of α was pre-set at 0.10 and β was pre-set at 0.05 for all simulations. Estimated α and β values were also calculated (Table 2.1). The estimated values of α and β are variable as a function of the different threshold ranges, inherent variation in the data, and the resampling process (Naranjo and Hutchison 1997). For all thresholds, and all simulations, the estimated α values were lower than the value set in the software (0.10). The estimated values of β varied above and below the value set in the software (0.05).

For the 4% threshold, the symmetric range of lower and upper bounds of 0.01 to 0.07 resulted in the lowest estimated β (0.03) which is below the set value (0.05). In the case of asymmetric range of boundaries around the threshold (0.01 to 0.09), the estimated β value (0.057) is greater than the set value; however, the slight difference between the estimated and set β value is compensated for by a low ASN (40.9 plants). For the 8% threshold, both simulations resulted in an estimated β that is greater than the set value. But, again the greater estimated β is compensated for by ASNs that are less than 50% of the current suggested sample size of 100 plants. For the 20% threshold, both simulations resulted in an estimated β less than the set value and the ASN values were much less than 100 plants.

For the 4% threshold, the simulation with bounds set at 0.01 to 0.09 was selected as the most appropriate sampling plan (Table 2.1). The criterion for this selection considered the average of 40.9 plants required to be sampled in order to make a correct decision with an acceptable value of A+D (0.83). Even though the value of incorrect decisions to no-treat is comparatively larger than the other simulations for the 4% threshold, this simulation results in an ASN that is 40% of the current suggested sample size of 100 plants. For the 8% threshold, the ASN values for both simulations decreased the number of plants to be sampled (38 and 49 plants) (Table 2.1). If the decision criterion was only based on the large value of the total correct decision (A+D) (Burkness et al. 1999), the simulation with a range of variation of 0.04 to 0.12 seems to be the most appropriate (Table 2.1). For the 20% threshold, the simulation with a range of variation of 0.15 to 0.25 was selected as the most appropriate (Table 2.1). The correct decision values were only slightly different, but the ASN was reduced by 13 plants.

Sampling cost and final sampling plan selection criteria. The average sample time required to evaluate one plant (Table 2.2) was 0.49 min. (30 seconds/plant). This average time to sample one corn plant was used in the benefit-cost ratio formula and summarized for each sampling plan simulation (Table 2.3). The final simulation selected, for the 4% threshold sampling plan, reduced sampling cost by 60% (ASN = 40.9 plants), compared to the current 100 plant sampling recommendation (Table 2.1). This plan also had the larger benefit-cost ratio (4.09) (Table 2.3). In the same way, the selected simulation for the 20% threshold sampling plan resulted in a reduced number of plants required to be sampled (ASN = 41.4 plants) and a high benefit-cost ratio (4.67) (Table

2.3). In the simulations for the 8% threshold there was a trade-off between precision and efficiency (Galvan et al. 2007). As presented previously, if the criteria of sampling plan selection is only based on the total probability of correct decision (A+D), the most appropriate sampling plan is the simulation with a range of variation from 0.04 to 0.12 (0.939), with an increase in ASN (48.9 plants) (Table 2.3). In contrast, the higher benefit-cost ratio (4.82) indicated that the sampling plan for the 8% threshold with the lower number of the sampled plants (ASN = 38 plants) is most favorable (Table 2.3). The final decision considered the practical aspects related with the cost-efficiency.

The OC and ASN functions are plotted against observed proportion of infested plants with *S. albicosta* egg masses for each selected simulation which represents a better balance between all parameters previously discussed (Fig. 2.1).

Stop lines were calculated for each of the sampling validated plans (Fig. 2.2). For each plan the decision to treat should be adopted if the cumulative number of plants with one or more *S. albicosta* egg masses is above the upper line. Conversely, control measures should not be implemented if the cumulative number of plants with one or more *S. albicosta* egg masses is below the lower line. In situations where the cumulative number of plants with one or more *S. albicosta* egg masses is between the two stop lines, additional samples are required before reaching a final treatment decision (Pedigo and Zeiss 1996). For each threshold the intercept of the lower stop line on the x-axis indicates the minimum number of plants that must be sampled to allow both treat and no-treat decisions to be made (Fig. 2.2). At least 35 plants are necessary to be sampled in order to make a decision when the threshold is 4%. For the 8% and 20% threshold, at least 27 and 23 plants are required to be sampled, respectively.

Discussion

In this study, sampling plans for three different *S. albicosta* action thresholds were developed and validated using resampling software based on Wald's binomial sampling plan (Naranjo and Hutchison 1997). The egg mass data represented a range of high, medium, and low egg densities, conducive for resampling analysis (Burkness and Hutchison 2009).

In all simulations, the probability of making a correct decision was always at least 80%. The ASN in all but one case produced a reduction of the current practical recommendation of 100 corn plants. Only in one simulation for the 4% threshold did the ASN equal the current recommendation. Moreover, the benefit-cost ratio demonstrated that the reduction of the number of the sampled plants is followed by an increase of cost-efficiency. Only in the case of the 8% threshold there was a trade-off between precision and efficiency (Galvan et al. 2007) and the final decision in selection of the sampling plan was considering the lower number of plants required to be sampled.

The selection of the most appropriate simulation for each threshold should consider the balance among OC, ASN, α, β and the benefit-cost ratio. However, in general it is possible to conclude that 40 represented an appropriate average number of plants required in order to reach either a treat or no-treat decision.

The estimated α value (Table 2.1) is lower than the pre-set value of 0.10 in all simulations and represents a reduction in probability of a type I error (adopting a control action when it is not necessary). In the case of β values, most binomial sampling plan studies set this variable at 0.10 (Jones 1994; Naranjo and Hutchison 1997); however, for simulations in this study the β value was set at 0.05. The reduced β value promoted an overall increase of the ASN in all simulations, but this also decreased the probability of a type II error (control actions are not taken when necessary). This risk is critical in the adoption of sampling plans for any crop because it represents the risk of potential economic losses. In the present study, the thresholds for *S. albicosta* egg masses are action thresholds (based primarily on experience, not threshold-specific research) and were based on considerable field experience from consultants and extension personnel (Seymour et al. 2004; Peairs 2006; Rice 2007; Cullen and Jyotika, 2008). However, since these thresholds are action thresholds, and used over broad regions without significant consideration of market values (Pedigo and Rice 2009) and validation from research data, a conservative β is appropriate. The estimated β value in any case was always below 0.10 with the highest value at 0.075 (Table 2.1).

In all graphical representations of ASN versus the proportion of infested plants (Fig. 2.1D, 2.1E, and 2.1F), the greatest ASN values are near the action thresholds. These results exemplify one of the most interesting characteristics of a binominal sampling plan, which is that the number of samples will be related to the density of the pest population (Jones 1994). In other words, at low and high pest densities, either no-treat or treat decisions will be indicated after a relatively low number of samples. In addition to the increase in efficacy of sampling programs, another advantage of binomial sampling plans is that the categorization of the pest as presence/absence reduces the effect of unusual values in a sample when compared with enumerative analysis, where measures of population density are made (Jones 1994). Evaluation of *S. albicosta* egg masses based on presence/absence is an appropriate approach, since it saves time and increases the efficacy of detection, especially considering that majority of the infested corn plants in the 70 fields sampled in this study were infested with one egg mass per plant.

The OC function was calculated for each sampling plan considering each different action threshold and its selected range of variation. Besides being used in a decision matrix to calculate correct and incorrect decisions, it is also plotted against the observed proportion of infested plants (Fig. 2.1A, 2.1B, and 2.1C). According to Naranjo and Hutchison (1997), when α and β are equal, the OC value would be ideally equal to 0.5 when at the action threshold value. The OC for the sampling plan for the 4% threshold simulated with the asymmetric range of variation was 0.46, which is near 0.5 (Fig. 1A). For the sampling plan with the 8% threshold with a 0.04 variation range, the OC was 0.34 (Fig. 1B), and the OC for the sampling plan for the 20% threshold with a 0.04 variation range was 0.4 (Fig. 1C). Therefore, in all three sampling plans the probability of a no-treat decision was lower than 50%. This means that the sampling plans for the 8% and 20% thresholds could be considered conservative and when at the threshold it is more likely that you will make a treat decision than a no- treat decision (Galvan et al. 2007).

In Fig. 2.2, the stop lines indicate that there are three different decision choices for each action threshold and the cumulative number of samples required to make a decision will depend on the action threshold for each sampling plan. For the 4% threshold, sampling at least 35 plants will be necessary before reaching a decision (intersection of lower bound) (Fig. 2.2A). For the 8% and 20% thresholds the minimum numbers of samples are 27 and 23, respectively (Fig. 2.2B and 2.2C). However, it is important to consider the time required to sample when the infestation level of the pest is in between the upper and lower stoplines. Binns (1994) highlights the necessity to set a time limit in this zone. In the case of soybean aphid it is not recommended to take more than 31 samples to make a control decision (Hodgson et al. 2004). This resampling period should be based on the reproductive potential of the pest species. The current *S. albicosta* egg mass sampling recommendation of 100 plants can serve as a sampling limit, when necessary, if egg mass counts fall within the stoplines.

Correct timing of sampling is critical and should not be initiated before the pest population is active or after the pest damage has become unacceptable (Higley and Peterson, 1994); for *S. albicosta*, management intervention must occur before larvae enter the ear. Because *S. albicosta* has one generation per year (Antonelli 1974), it would be opportune to associate the sequential binomial sampling plan proposed in this work with other research on the degree days required for adult emergence and adult field trapping techniques (Douhout and Rice 2008). For example, Holtzer (1983) found the relationship between *S. albicosta* light trap catches and the presence of egg masses was consistent in timing and magnitude. Therefore, by using moth counts from light trap the efficiency of *S. albicosta* egg mass sampling can be further improved.

For *S. albicosta*, there is a narrow treatment window between egg hatch and ear colonization, so detection of a possible economic population during the egg stage is critical in order to apply control measures before larvae enter the ear. Moreover, considering the behavioral characteristics of larvae (e.g., early instars spend time concealed in the whorl or leaf axil), sampling plans that evaluate egg mass density are more appropriate than sampling the larvae (Seymour et al. 2004).

Knowledge of the dispersion of a pest is important in order to determine the optimal sampling pattern in the field (e.g., Hutchison 1994). The TPL regression analysis of *S. albicosta* indicated the egg mass distribution in the field is random, with the slope of the linear equation equal to 1. This indicates that the probability is equal throughout the cornfield for a plant to receive a *S. albicosta* egg mass. In this way, the spatial sampling pattern should be random across a cornfield, and every sampling site should have the same chance to be selected. Establishing one sampling transect through a cornfield (Pedigo and Rice 2009) would be an appropriate option, with periodical evaluations of corn plants along the route. However, planting date, hybrid maturity and even differences in corn development in the same field should be considered in the division of the sampling sites, since these factors may influence the attractiveness of the plants and deposition of the egg masses.

One other variable that could influence the spatial distribution of the egg masses is the effect of Bt hybrids. However, our data indicated that there were no differences in oviposition by *S. albicosta* females on Bt or no-Bt corn, even though Cry1F has efficacy on *S. albicosta* larvae (e.g., Eichenseer et al. 2008).

The binomial sequential sampling plans for *S. albicosta* developed here will significantly reduce the time and effort required for effective management of this pest; however, because they are based on action thresholds, not thoroughly researched economic thresholds, and because much is unknown about the pest's biology and behavior across ecoregions, there is the potential for improvement. Future research describing the yield-loss relationships for *S. albicosta* in corn would provide more accurate thresholds for sampling plan development, and future studies investigating the role of larval survival (Ostlie and Pedigo 1986) across ecoregions could provide information that could be incorporated into the management decision matrix, such as that used to make control decisions for second generation *Ostrinia nubilalis* (e.g., Wright 2007).

References Cited

Antonelli, A. L. 1974. Resistance of *Phaseolus vulgaris* cultivars to western bean cutworm, *Loxagrotis albicosta* (Smith), with notes on the bionomics and culture of the cutworm. Ph.D. dissertation, University of Idaho, Moscow.

Appel, L. L., R. J. Wright, and J. B. Campbell. 1993. Economic injury levels for western bean cutworm, *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae), larvae in field corn. J. Kans. Entomol. Soc. 66: 434-438.

Binns, M. R. 1994. Sequential sampling for classifying pest status, pp. 137-174. In L. Pedigo and G. Buntin (eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.

Binns, M. R., J. P. Nyrop and W. van der Werf. 2000. Sampling and monitoring in crop protection – the theoretical basis for developing practical decision guides. CABI publishing, Wallingford, UK.

Burkness, E. C. and W. D. Hutchison. 2009. Implementing reduced-risk integrated pest management in fresh-market cabbage: influence of sampling parameters, and validation of binomial sequential sampling plans for the cabbage looper (Lepidoptera: Noctuidae)**.** J. Econ. Entomol., 102: 1874-1883.

Burkness, E. C., R. C. Venette, P. K. O'Rourke, and W. D. Hutchison. 1999. Binomial sequential sampling for management of aster leafhopper (Homoptera: Cicadellidae) and aster yellows phytoplasma in carrot: impact of tally threshold on the accuracy of treatment decisions. Environ. Entomol. 28: 851-857.

Calvin, D. D., M. C. Knapp, K. Xingquan, F. L. Poston and S. M. Welch. 1986. Using a decision model to optimize European corn borer (Lepidoptera: Pyralidae) egg-mass sampling. Environ. Entomol. 15: 1212-1219.

Cullen, E., and J. Jyotika. 2008. Western bean cutworm: a pest of sweet corn. A3856, University of Wisconsin Extension, Cooperative Extension. On-line: http://learningstore.uwex.edu/pdf/A3856.pdf.

DiFonzo, C. D., E. Cullen. 2010. Handy Bt trait table. University of Wisconsin Extension, Field Crops Entomology MSU Program. On-line: http://www.entomology.wisc.edu/cullenlab/publications/pdf.

DiFonzo, C. D., and R. Hammond. 2008. Range expansion of western bean cutworm, S*triacosta albicosta* (Noctuidae), into Michigan and Ohio. Crop Mgt. doi:10.1094/CM-2008-0519-01-B. Online: http://msuent.com/assets/DiFonzoHammondWBC.pdf.

Dorhout, D. L., and M. E. Rice. 2004. First report of western bean cutworm, *Richia albicosta* (Noctuidae) in Illinois and Missouri. Crop Mgmt. On-line:

http://www.plantmanagementnetwork.org/pub/cm/brief/2004/cutworm. DOI:10.1094/CM-2004-1229-01-BR.

Dorhout, D. L., and M. E. Rice. 2008. An evaluation of western bean cutworm pheromone trapping techniques (Lepidoptera: Noctuidae) in a corn and soybean agroecosystem. J. Econ. Entomol. 101: 404-408.

Douglass, J. R., J. W. Ingram, K. E. Gibson, and W. E. Peay. 1957. The western bean cutworm as a pest of corn in Idaho. J. Econ. Entomol. 50: 543-5.

Eichenseer, H., R. Strohbehn, and J. Burks. 2008. Frequency and severity of western bean cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* cry toxins. J. Econ. Entomol. 101: 555–563.

Galvan, T.L., Burkness, E.C., and W.D. Hutchison. 2007. Enumerative and binomial sequential sampling plans for the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in wine grapes. J. Econ. Entomol. 100:1000-1010.

Hagen, A. F. 1962. The biology and control of the western bean cutworm in dent corn in Nebraska. J. Econ. Entomol. 55: 628-631.

Higley, L. G., and R. K. D. Peterson. 1994. Initiating sampling programs, pp.119-136. In L. Pedigo and G. Buntin (eds.),Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.

Hodgson, E. W., E. C. Burkness, W. D. Hutchison, and D. W. Ragsdale. 2004. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. J. Econ. Entomol. 97: 2127-2136.

Hoerner, J. L. 1948. The cutworm *Loxagrotis albicosta* on beans. J. Econ. Entomol. 41: 631-635.

Holtzer, **T. O. 1983**. Distribution of western bean cutworm eggs among short-, mid-, and long-season corn hybrids planted on different dates. Environ. Entomol. 12:1983. 1375–1379.

Hutchison, W. D. 1994. Sequential sampling to determine population density, pp. 207-244. In L. Pedigo and G. Buntin (eds.), Handbook of sampling methods for arthropods in agriculture. CRC, Boca Raton, FL.

Hutchison, W.D., E.C. Burkness, P.D. Mitchell, R.D. Moon, T.W. Leslie, S.J. Fleischer, M. Abrahamson, K.L. Hamilton, K.L. Steffey, M.E. Gray, R.L. Hellmich, L.V. Kaster, T.E. Hunt, R.J. Wright, K. Pecinovsky, T.L. Rabaey, B.R. Flood and E.S. Raun. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science. 330: 222-225.

Jones, V. P. 1994. Sequential estimation and classification procedures for binomial counts, pp. 175-205. In L. Pedigo and G. Buntin (eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC, Boca Raton, FL.

Kuno, E. 1991. Sampling and analysis of insect populations. Annual Review Entomology, 36: 285-304.

Michel, A. P., C. H. Krupke, T. S. Baute, and C. D. DiFonzo. 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. J. Integ. Pest Mgmt. 1:A1-A10. DOI: 10.1603/IPM1003.

Miller, N. J., D. L. Dorhout, M. E. Rice and T. W. Sappington. 2009. Mitochondrial DNA variation and range expansion in western bean cutworm (Lepidoptera: Noctuidae): no evidence for a recent population bottleneck. Environ. Entomol. 38: 274-280

Naranjo, S. E., and W. D. Hutchison. 1997. Validation of arthropod sampling plans using a resampling approach: software and analysis. Am. Entomol. 43: 48-57.

Nault, B.A. and A. M. Shelton. 2010. Impact of insecticide efficacy on developing action thresholds for pest management: a case study of onion thrips (Thysanoptera: Thripidae) on onion. J. Econ. Entomol. 103: 1315-1326.

O'Rourke, P. K., and W. D. Hutchison. 2000. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. J. Agric. Urban Entomol. 17: 213-217.

Ostlie, K. R. and L. P. Pedigo. 1987. Incorporating pest survivorship into economic thresholds. Bull. Entomol. Soc. Am 1987. 33:98–102.

Peairs, F. B. 2006. Western bean cutworm: characteristics and management in corn and dry beans. Colorado State University Extesion. 3/02.n. 5.5538. On-line: http://www.ext.colostate.edu/pubs/insect/05538.html

Pedigo, L. P. and M. E. Rice. 2009. Entomology and pest management. New Jersey: Prentice Hall .

Pedigo, L. R. and M. R. Zeiss. 1996. Analyses in insect ecology and management. Iowa State University Press: Ames.

Rice, M. E. 2007. Economic thresholds for western bean cutworms. Iowa State University Extension, Ames, IA.On-line: (http://www.ipm.iastate.edu/ipm/icm/2007/7-2/wbcth.html).

Rice, M. E. 2006. Eastern movement of the western bean cutworm into Indiana and Ohio. Proceedings: Indiana CCA Conference, Indianapolis, IN.

Rice, M. E. 2000. Western bean cutworm hits northwest Iowa. Integrated Crop Mgt. IC-484 (22): 163. Iowa State University Extension, Ames, IA. (http://www.ipm.iastate.edu/ipm/icm/2000/9-18-2000/wbcw.html).

SAS Institute. 2009. SAS user's guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Seymour, R. C., G. L. Hein, R. J. Wright, and J. B. Campbell. 2004. Western bean cutworm in corn and dry beans. Neb. Coop. Ext. Guide G1359. Lincoln, NE.

Systat Software, Inc. 2002. TableCurve 2D v5.01 for Windows, 2002. Systat Software, Inc., San Jose, CA.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732-735.

Tooker, J. F and S. J. Fleischer. 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. Crop Mgmt. DOI: 10.1094/CM-2010-0616-01-RS.

Young, L.J. and J.H. Young. 1998. Statistical Ecology*:* a population perspective. Kluwer Academic, Boston, EUA.

Wald, A. 1947. Sequential analysis. Wiley, New York.

Wright, R. J. 2007. Second generation European corn borer scouting and treatments decisions. University of Nebraska Ext. Neb Guide G1783. Lincoln, NE.

Table 2.1. Probability of correct and incorrect treatment decisions for S. albicosta egg mass sampling, with three action thresholds (4, 8, 20% of plants with one or more egg masses each), the estimated proportion of plants infested and operating characteristic (OC). Final sampling plans selected for each threshold are bolded (*see also Table 2.3*).

Range of variation		Estimated		Correct decision		Incorrect decision		Total correct (A+D) [e]	Average sample number (ASN)
Lower bound	Upper bound	α	β	A[a]	D[b]	B[c]	C[d]		
				Action threshold					
				4%					
0.01	0.07	0.03	0.03	0.400	0.405	0.018	0.176	0.805	53.2
0.01	**0.09**	**0.043**	**0.057**	**0.384**	**0.444**	**0.034**	**0.139**	**0.830**	**40.9**
0.02	0.06	0.05	0.07	0.406	0.469	0.012	0.113	0.874	100.1
				8%					
0.03	**0.13**	**0.05**	**0.06**	**0.235**	**0.660**	**0.001**	**0.085**	**0.895**	**37.5**
0.04	0.12	0.062	0.075	0.254	0.685	0.0008	0.061	0.939	48.9
				20%					
0.15	**0.25**	**0.08**	**0.02**	**0.126**	**0.831**	**0.019**	**0.023**	**0.957**	**41.4**
0.16	0.24	0.088	0.007	0.118	0.843	0.009	0.030	0.961	54.5

[a] A, probability of correct decision to treat.

[b] B, probability of incorrect decision to no-treat.

[c] C, probability of incorrect decision to treat.

[d] D, probability of correct decision to no-treat.

[e] Total probability of correct decision.

Table 2.2. Sampling time necessary to search and record *S. albicosta* egg masses in tasseling corn, and estimated costs for a commercial corn field, western Nebraska, 2005.

Field (NE-2005)	N. of sites/field[a]	Time spent sampling (min)/site[b]	Hourly cost of labor (consultants)[c]
1	10	48	20.00
2	10	41.5	17.29
3	10	50	20.83
4	10	58	24.17
5	10	50	20.83

[a] Conducted to provide a total of 100 plants per field (10 sites with 10 plants each).

[b] Mean of sampling time for one sampler with previous experience with *S. albicosta* egg sampling. This includes time to walk between sample site and the time to inspect 10 consecutive plants for egg masses.

[c] Considering a U.S. crop consultant cost ($25.00/hr).

Table 2.3. Comparison of efficiency of the binomial sampling plans for *S. albicosta* egg masses based on the benefit-cost ratio criterion. Final sampling plans selected, parameters and sampling effort for each threshold are bolded.

Sampling plan lower and upper bound		Average sample number (ASN)	Total correct decisions (proportion) (A+D)[a]	Average sample time/plant (minutes)[b]	Benefit-cost ratio[c]
			Action threshold		
			4%		
0.01	0.07	53.2	0.805	0.495	3.06
0.01	**0.09**	**40.9**	**0.830**	**0.495**	**4.09**
0.02	0.06	100	0.874	0.495	0.01
			8%		
0.03	**0.13**	**37.5**	**0.895**	**0.495**	**4.82**
0.04	0.12	48.9	0.939	0.495	3.88
			20%		
0.15	**0.25**	**41.4**	**0.957**	**0.495**	**4.67**
0.16	0.24	54.5	0.961	0.495	3.56

[a] Probability of correct decisions (*see Table 2.1*).

[b] Sampling time of one plant, including the mean time to walk between plants and among sites (*see Table 2.2*).

[c] Benefit-cost ratio = $[\sum P_c / (n \text{ x } c)]$ x 100, where P is the sum of proportional correct decisions, *n* is the average number of samples required to make a decision, and *c* is the cost (expressed in time) of sampling the egg masses in corn plants (e.g., Galvan et al. 2007).

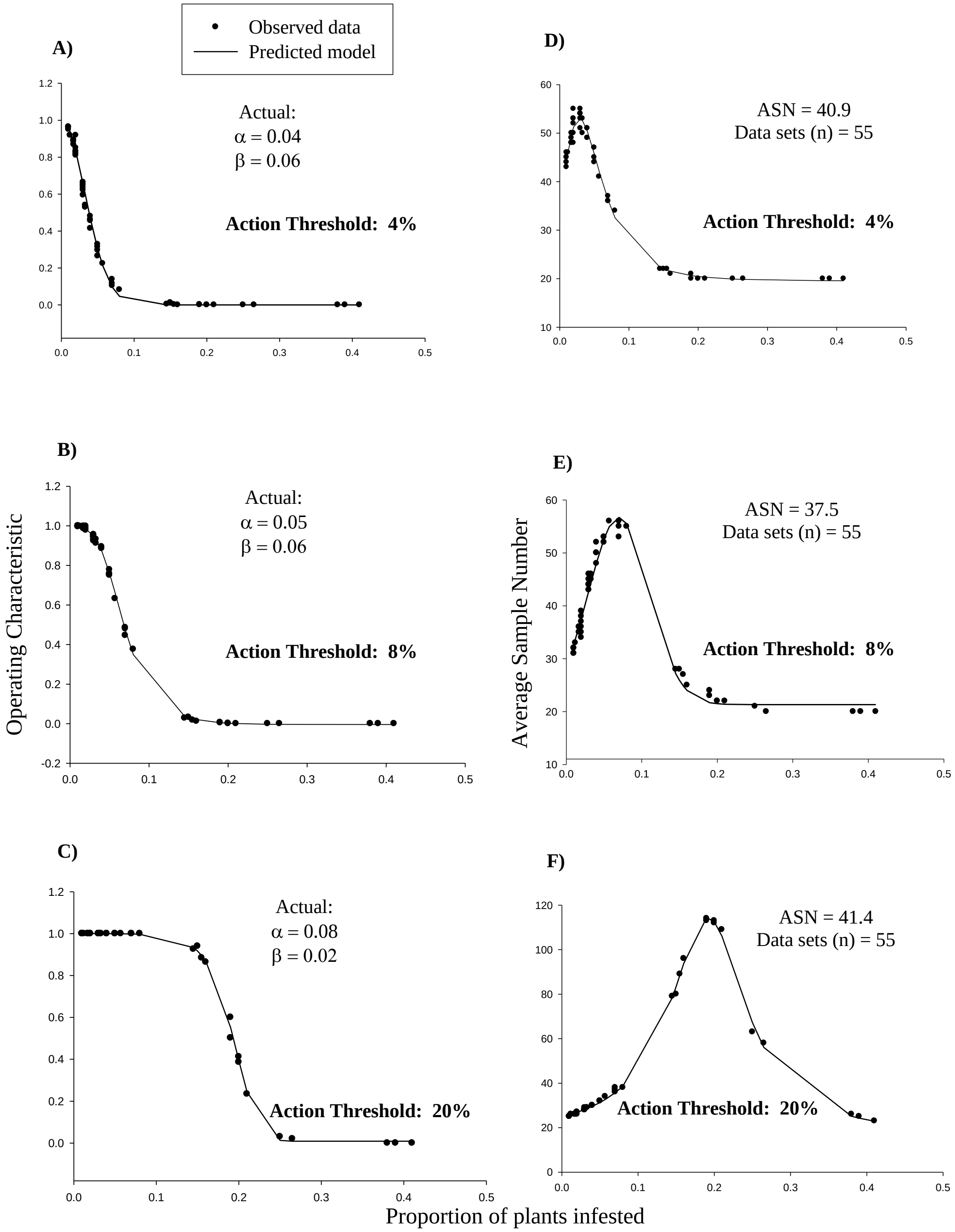

Fig. 2.1. Operating characteristic (OC) and average sample number (ASN) functions for Wald's sequential probability ratio sampling plan, considering 4, 8 and 20% *S. albicosta* action thresholds (i.e., percentage of plants with one or more egg masses each). The OC

and ASN were plotted against the observed proportion infested based on resampling validation (RVSP software) using field data. Predicted models were fitted using equation 8189 from Table Curve for A, B, C, and D. Predicted models in E and F used equation 8003 and 8004, respectively. The actual α and β values are presented on each graph (A-C).

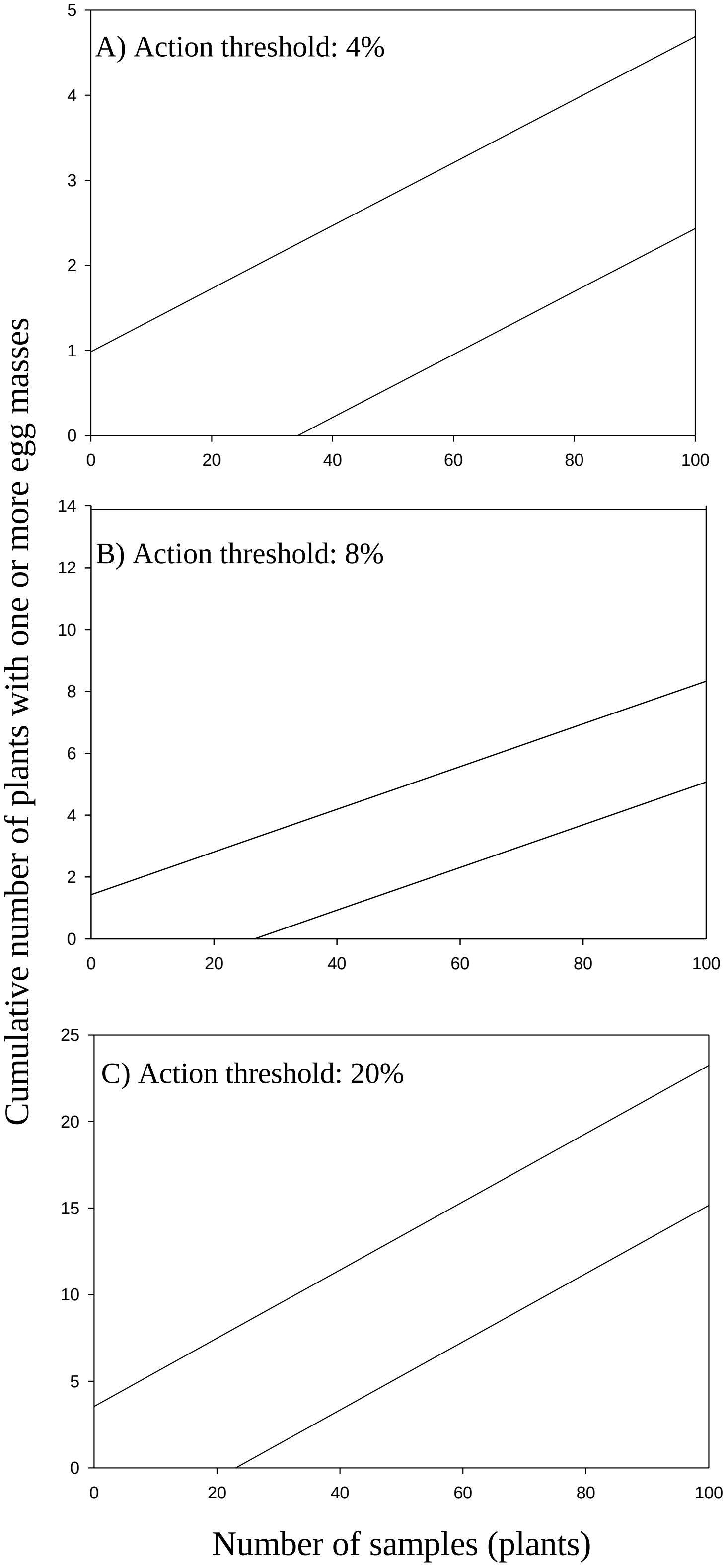
A) Action threshold: 4%
0
1
2
3
4
5
0
20
40
60
80
100
B) Action threshold: 8%
0
2
4
6
8
10
12
14
0
20
40
60
80
100
C) Action threshold: 20%
0
5
10
15
20
25
0
20
40
60
80
100
Cumulative number of plants with one or more egg masses
Number of samples (plants)

Fig. 2.2. Stop-lines for binomial sequential sampling plans considering 4, 8 and 20% action thresholds for *S. albicosta* egg masses in corn. Equations for 4% action threshold: upper line is $y = 0.99 + 0.04\ x$ and lower line is $y = -1.27 + 0.04\ x$; Equations for 8% action threshold: upper line is $y = 1.43 + 0.4\ x$ and lower line is $y = -1.83 + 0.07\ x$; Equations for 20% action threshold: upper line is $y = 3.54 + 0.2\ x$ and lower line is $y = -4.54 + 0.2\ x$.

Chapter 3: On-plant movement and feeding behavior of western bean cutworm early instars on corn

Introduction

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

Since previously it was a pest only in the west-central United States (Hoerner 1948; Douglass et al. 1957), little is known about its ecology and behavior, particularly across ecoregions. In part because of its recent range expansion, there is an increased demand for the development of fundamental integrated pest management 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), Douglas et al. (1957) indicate that western bean cutworm larva can feed on all parts of the corn plant, including leaves, stems, tassels, 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 (overwintering stage) (Douglas et al. 1957; Michel et al. 2010). The next year the insect pupates, and adults typically emerge in July, mate, and oviposit on the top surface of leaves on the upper part of the corn plant (Seymour et al. 2004; Douglass et al. 1957).

One of the obscure aspects of western bean cutworm ecology is related to insect dispersal behavior, defined as any adult movement away from the initial population habitat or in the case of neonate larva, 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 in order 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 technology of plants expressing 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; Direi 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; Caprio 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) in order to deposit eggs on the upper leaves of the plant (Douglass et al. 1957). The distribution of egg masses 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 instars 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, and dispersal and establishment of early instar western bean cutworm larvae on the corn plant.

Materials and Methods

On-plant larval movement. The survival, development, and distribution of the larvae on the corn plant were characterized in the field. 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 (Catangui and Berg 2006) was used to minimize the confounding effect of European corn borer (*Ostrinia nubilalis)* (Hübner).

The treatment design was a 3 x 5 factorial. There were three corn stages, pre-tassel, tassel, and post-tassel, 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 (RCBD). The larval movement on the corn plant was evaluated based on the number of the larvae of western bean cutworm in each plant zone. In 2009 and 2010 there were six blocks, and in 2011 four blocks. Each experimental plot was 3 rows by 3 m, with approximately 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" in order 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 4 to 6 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. Western bean cutworm moths were collected in cages positioned under ultra-violet light traps. One male and two females were confined on the top part of the corn plant with a large flat mesh pollination bags (46 x 51 cm). Two days after infestation, the presence of egg masses was determined and thinned so only one egg mass was left per plant. Egg masses were photographed in order to count the number of the eggs. Plants on which the western bean cutworm females did not lay egg masses were artificially infested following the same methodology of 2010. Plots were inspected for natural western bean cutworm egg masses, but in all years no natural infestation was detected.

In 2009 plant sampling was done 4 d and 6 d after infestation (eggs hatched within 24 hours of infestation). In 2010 plant sampling was done 3 d 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 d and 9 d after infestation. One plant was evaluated in each plot, on each sampling day. Plant inspection took 15-20 minutes per plant. In 2010, the number of the hatched eggs per egg mass was recorded in order 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 in order to access different parts of 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 hour recovery period).

The percentage of egg hatch and larvae survival were calculated. Larval survival was based on recovered larvae. In 2010 and 2011, the 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 constant variance assumption was violated, and a separate variance was estimated for each plant zone. This was implemented via the GROUP option in PROC GLIMMIX (SAS Institute 2009) (Appendix A). The 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).

Larval feeding. Larval survival and development was evaluated 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 (YieldGard, Monsanto, St Louis, MO).

The larval feeding treatments were based on the availability of corn tissue when corn was at pre-tassel (whorl leaf with developing tassel), tassel (pollen and pollen + silk), and post-tassel stage (silk). The different plant tissues were removed from corn plants in the field when needed and cleaned with alcohol (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 25ºC 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 0.05 m x 0.05 m x 0.05 m cages.

The study was conducted as a RCBD with three replications (growth chambers). In 2009, three different corn food sources were evaluated: silk, pollen, and leaves from whorl. One hundred neonate larvae were placed in a 10 cm diameter container and held at 25ºC/24h light 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 was provided.

In 2011, four different corn food sources were evaluated: leaves from the whorl + tassel, silk, pollen, and silk + pollen. Five neonate larvae were placed in a 10 cm diameter container and held at 25ºC/24h light for 7 d. The reduced number of neonates per container allowed for better manipulation of the insects, more accurate evaluation, and avoided intraspecific competition. 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 evaluated by comparing the percentages of remaining live larvae. Development was evaluated 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 (1973).

Results

On-plant larval movement. In 2009 and 2010, egg hatch was over 85%. In 2011, visual evaluations after infestation detected low egg hatch (approximately 5%), which is explained by 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 the number of the eggs infested and hatched. A decrease in the larval recovery was also observed from the first to the second sampling evaluation 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 date, respectively. In 2011, besides the low egg survival mentioned before, the larval survival ranged from 7.7% (50 recovered larvae) to 2% (9 recovered larvae) on first and second sampling date, respectively. Following Antonelli (1973), all larvae recovered on the first sampling date were classified as first instar, and at the second sampling date all larvae were second instar.

In 2009, the effect of corn stage on larval recovery was significant for the first sampling date (Table 3.1). Larval recovery was not significantly different between corn in tassel and pre-tassel stages (Fig. 3.1), but larval recovery for both corn stages was significantly higher than for the post-tassel stage (Fig. 3.1).

In 2009 the effect of plant zone on larval recovery was also significant (Table 3.1). The comparison of larval recovery in the first ear zone (where egg masses were placed in 2009) with other zones indicated a significantly lower number of larvae in the zone below the ear (Fig. 3.2). No interaction between corn stage and plant zone was detected (Table 3.1). On the second sampling date, the effect of corn stage was significant, but there was no plant zone or interaction effect (Table 3.2).

In 2010, only a plant zone effect was detected (Table 3.1). The distribution of the western bean cutworm larvae was not influenced by corn stage on the first sampling date or the second sampling date. Larval recovery was significantly different between plant zones on the first (Fig. 3.3) and second sampling date (Fig. 3.4). The larval distribution was concentrated in the upper part of the plant in both cases. No interaction was detected between corn stage and plant zone on the first (Table 3.1) or second sampling date (Table 3.2).

In 2011, the corn stage effect was neither detected on the first sampling date (P = 0.98) or second sampling date (P = 0.73). Larval recovery was also not significantly different between plant zones on the first (P = 0.37), and second sampling date (P = 0.44). Interaction between effects was not detected for both sampling dates (P = 0.87 and P = 0.98). Because larval survival was extremely low, an alternative analysis was done combining zones in: below ear (same as 2009 and 2010); ear (primary + secondary ears); above ear + tassel (Table 3.1). Under this classification, there was a significantly higher larval recovery from the above ear + tassel zone (Fig. 3.3).

Larval feeding. In 2009, there were no significant differences in larval survival between western bean cutworm larvae reared for 13 d on pollen or silk (Table 3.3). There was a significant difference in larval weight between larvae reared on the two diets. Larvae reared on silk had a significantly higher weight than those reared on pollen (Table 3.3). For both corn tissues, most of the larvae were classified as fourth instar (Antonelli

1973) (Table 3.4). Survival of larvae reared on leaves from the whorl was significantly lower than on the other two corn tissues. Only four larvae were recovered, and 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 behavior. 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 3.3). There were no significant differences in weight or survival between larvae reared on the other diets. Head capsule width was similar for all corn tissues, with most of the larvae classified as first instar (Table 3.4).

Discussion

In this study, the on-plant movement of early instars of western bean cutworm was characterized in the field, based on the larval distribution among corn plant zones in different growth stages. The effect of different corn tissues on larval development and survival was tested in the lab, based on a feeding study.

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. The low egg hatch in 2011 was likely the result of plant stress due to a non-functional irrigation system coupled with uncommonly high temperatures. In the period between infestation and neonate emergence in 2011, the average temperature was approximately 26ºC in Concord, NE, with a high of 35ºC (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 western bean cutworm larval survival is low (Seymour et al. 2004; Eichenseer et al. 2008), and few neonates survive to maturity. Even in field cages, the average survival is low. In Nebraska 3.3% survival was reported in experimental field cages (Appel et al. 1993). The results from the present study support low larval survival of this species, and indicate that the early instars of western bean cutworm are the critical stages for establishment in corn. Low larval survival in

Lepidoptera has been previously reported, especially in early instars (Zalucki et al. 2002). In the case of western bean cutworm this low larval survival in the early instars is probably related to the complex interaction of larvae with internal and external plant factors, environmental effects, and an array of potential natural enemies.

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). The early larvae 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). This early instar movement of western bean cutworm has been reported to differ according to corn plant stage (Hagen 1962). Until tassels emerge from the whorl, the western bean cutworm movement would be toward the tassel. In corn fields where plants are tasseled or are in the silking stage, the expectation is that the larva would migrate to the silk rather than to the tassel zone (Hagen1962; Seymour et al. 2004). However, the results from this study indicate significant on-plant larval movement, with a concentration of larvae in the upper part of the plant (Fig. 3.3 to 3.5), regardless of plant stage.

The results in 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 3.1). For the early instars, the greatest larval concentration was on the upper part of the plant, independent of the pre-tassel, tassel, or post-tassel stage (Tables 3.1 and 3.2.). In 2009, a corn stage effect was observed (Table 3.2) with a significantly lower number of larvae on the below ear zone (Fig. 3.2). However, considering that the infestation of the egg mass was done close to the primary ear, the 2009 data support the results of 2010 and

2011. Even though the egg mass was infested at the primary ear zone, no differences were detected in larval recovery between the primary ear, above ear, and tassel zones (Fig. 3.2), which is indicative of larvae moving toward the upper part of corn plant. In addition, some larvae may have been missed during the inspection of the tassel in 2009 (hence the use of Berlese funnels in 2010 and 2011).

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, in 2009, with the significant corn stage effect, the greatest larval survival was when the corn was in the pre-tassel and tassel stages (Fig. 3.1). These results may help to explain the benefits of this upward movement of western bean cutworm during the in 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 about how feeding requirements affect larval development. The selection of the corn tissues was based on the possible feeding scenarios for western bean cutworm larvae in the field, considering different corn stages.

The first study in 2009 tested the corn tissue scenarios of silk, pollen, and leaf from whorl. The larval weight (Table 3.3) and instar classification (Table 3.4) demonstrate that only leaf tissue from the whorl was an unsatisfactory source of food for young western bean cutworm larvae. After two days of infestation, approximately 50% mortality occurred. The head capsules from larvae that remained were not measured, but visual observation of the small size indicated the larvae were first instar. Larvae reared on corn silk were heavier than those reared on pollen. However, in this initial study the larvae were kept on the diets for 13 d (Table 3.3), with 2 d evaluation intervals until larvae reached the fourth instar. However, within a 13 d period in the field, western bean cutworm 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 realities. In this way, the feeding study focused only on the initial larval feeding behavior.

The results from 2011 confirmed the late whorl + tassel tissue as the best food source for early instar western bean cutworm. It is possible that late whorl + tassel tissue is beneficial to early instars because the tassel, with the associated developing pollen, satisfies the early instar nutritional requirements. Mature pollen alone did not provide the same benefit for the development of the larva (Table 3.3). In addition, the late whorl stage probably plays a role in providing shelter and a favorable microclimate for the small larvae, resulting in better larval survival and development.

The results from the field together with the lab studies demonstrated that the behavior of the western bean cutworm neonate 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, beginning tassel 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 larvae. The female moth is responsible for the selection of the most suitable corn stage (Renwick and Chew 1994), and larvae would have the predetermined behavior to move to the tassel. This adaptive behavior was investigated in European corn borer and it was determined that the oviposition site selection by females 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 the mixed feeding behavior, which follows a sequence of feeding, instead of being a function of corn stage. This mixed feeding behavior of western bean cutworm can also explain the high intrinsic rate of larval mortality. The mixed feeding behavior demands 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 early instar of western bean cutworm, when the larva is small, hiding inside the tassel, and before colonization of the corn ear. Management recommendations for larvae, such as insecticide application, should be done before larva colonize the ear. This is the period that the larvae move on the corn plant and have the higher level of exposure and efficacy of control measures could be maximized.

Moreover, larval on-plant movement behavior and feeding can be useful for modeling Bt resistance development and developing western bean cutworm resistance management strategies. In the present study, the corn hybrid (DKC 61-72 RR) expressing Cry1Ab was used, which does not affect western bean cutworm larvae (Catangui and Berg 2006; Eichenseer et al. 2008). However, others hybrids are commercially available expressing Cry1F (Herculex I, Dow AgroSciences and Pioneer Hi-Bred International), which is toxic to western bean cutworm (Eichenseer et al. 2008), and more recently, Bt corn hybrids have been introduced with pyramided genes encoding Cry1F/Cry1Ab (Optimum Intrasect Insect protection, DuPont/Pioneer) and Cry1A.105/Cry2Ab2/Cry1F (SmartStax Genuity, Monsanto) (DiFonzo and Cullen, 2010). Even in the case Cry 1F-only hybrids, which provide adequate levels of protection, some larval feeding was detected and the plant is not immune to western bean cutworm (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 of western bean cutworm is an aspect that should be the focus of future studies of the evolution of resistance of western bean cutworm to Bt toxins.

References Cited

Antonelli, A. L. 1974. Resistance of *Phaseolus vulgaris* cultivars to western bean cutworm,*Loxagrotis albicosta* (Smith), with notes on the bionomics and culture of the cutworm. Ph.D. dissertation, University of Idaho, Moscow.

Appel, L. L., R. J. Wright, and J. B. Campbell. 1993. Economic injury levels for western bean cutworm, *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae), larvae in field corn. J. Kans. Entomol. Soc. 66: 434-438.

Blickenstaff, C. C. 1979. History and biology of the western bean cutworm in southern Idaho, 1942-1977. Univ. Idaho Agric. Exp. Stn. Bull. No. 592.

Blickenstaff, C. C., and P.M. Jolley. 1982. Host plants of western bean cutworm. Environ. Entomol., 11:421-425.

Caprio, M. A. 1998. Evaluating resistance management strategies for multiple toxins in the presence of external refuges. J. Econ. Entomol. 91: 1021-1031.

Caprio, M. A. 2001. Source-sink dynamics between transgenic and nontransgenic habitats and their role in the evolution of resistance. J. Econ. Entomol. 94: 698-705.

Catangui, M. A. and R. K. Berg. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry 1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environ. Entomol. 35: 1439-1452.

Cullen, E., and J. Jyuotika. 2008. Western bean cutworm: a pest of sweet corn. A3856, University of Wisconsin Extension, Cooperative Extension. On-line: http://learningstore.uwex.edu/pdf/A3856.pdf.

DiFonzo, C. D., E. Cullen. 2010. Handy Bt trait table. University of Wisconsin Extension, Field Crops Entomology MSU Program. On-line: http://www.entomology.wisc.edu/cullenlab/publications/pdf.

DiFonzo, C. D., and R. Hammond. 2008. Range expansion of western bean cutworm, S*triacosta albicosta* (Noctuidae), into Michigan and Ohio. Crop Mgt. doi:10.1094/CM-2008-0519-01-B. Online: http://msuent.com/assets/DiFonzoHammondWBC.pdf.

Dirie, A.M, M.B. Cohen, and F. Gould. 2000. Larval dispersal and survival of *Scirpophaga incertulas* (Lepidoptera: Pyralidae) and *Chilo suppressalis* (Lepidoptera: Crambidae) on cry1Ab-transformed and Non-transgenic rice. Environ. Entomol. 29: 972-978.

Dorhout, D. L. 2007. Ecological and behavioral studies of the western bean cutworm (Lepidoptera: Noctuidae) in corn. Master dissertation. Iowa State University, Ames.

Douglass, J. R., J. W. Ingram, K. E. Gibson, and W. E. Peay. 1957. The western bean cutworm as a pest of corn in Idaho. J. Econ. Entomol. 50: 543-5.

Eichenseer, H., R. Strohbehn, and J. Burks. 2008. Frequency and severity of western bean cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* cry toxins. J. Econ. Entomol. 101: 555–563.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Hagen A. F. 1963. Evaluation of populations and control of the western bean cutworm in field beans in Nebraska. J. Econ. Entomol. 56: 222-224.

Hagen, A. F. 1962. The biology and control of the western bean cutworm in dent corn in Nebraska. J. Econ. Entomol. 55: 628-631.

Hellmich, R. L., B. Siegfried, M. K., Sears, D. E. Stanley-Horn, H. R. Matilla, T. Spencer, and K. C. Lewis. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis* purified proteins and pollen. Proc. Natl. Acad. Sci. U.S.A. 98: 11925-11930.

High Plains Region Climate Center. 2011. University of Nebraska – Lincoln. www.hpcc.unl.edu.

Hoerner, J. L. 1948. The cutworm *Loxagrotis albicosta* on beans. J. Econ. Entomol. 41: 631-635.

Holtzer, **T. O. 1983**. Distribution of western bean cutworm eggs among short-, mid, and long-season corn hybrids planted on different dates. Environ. Entomol. 12: 1375–1379.

International Life Sciences Institute. 1998. An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and and risk management. Health and Environmental Sciences Ins., USA. 78 pp.

Michel, A. P., C. H. Krupke, T. S. Baute, and C. D. DiFonzo. 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. J. Integ. Pest Mgmt. 1:A1-A10. DOI: 10.1603/IPM1003.

Nguyen, H. T. and J. A. Jehle. 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. J. Plant. Disease Protec. 114: 82-87.

O'Rourke, P. K., and W. D. Hutchison. 2000. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. J. Agric. Urban Entomol. 17: 213-217.

Paula-Moraes, S.V., E. C. Burkness, T. E. Hunt, R. J. Wright, G. L. Hein, and W. D. Hutchison. 2011. Cost-effective binomial sequential sampling of western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), egg masses in corn. Journal Economic Entomology, 104: 1900-1908.

Renwick, J. A. A. and F. S. Chew. 1994. Oviposition behavior in lepidoptera. Annu. Rev. Entomol. 1994. 39:377-400.

Rice, M. E. 2000. Western bean cutworm hits northwest Iowa. Integrated Crop Mgt. IC-484 (22): 163. Iowa State University Extension, Ames, IA. (http://www.ipm.iastate.edu/ipm/icm/2000/9-18-2000/wbcw.html).

Rice, M. E. 2006. Eastern movement of the western bean cutworm into Indiana and Ohio. Proceedings: Indiana CCA Conference, Indianapolis, IN.

Ritchie, S.E., J.J. Hanway, and G.O. Benson. 1993. How a corn plant develops. Iowa Coop. Ext. Serv. Spec. Rep. 48.

Ross, S. E., and K. R. Ostlie. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. J. Econ.Entomol. 83: 831-836.

SAS Institute. 2009. SAS user’s guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Seymour, R. C., G. L. Hein, R. J. Wright, and J. B. Campbell. 2004. Western bean cutworm in corn and dry beans. Neb. Coop. Ext. Guide G1359. Lincoln, NE.

Shelton, A. M., J. Z. Zhao, and R. T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Ann. Rev. Entomol. 47:845-881.

Southwood, T.R.E. 1980. Ecological Methods: with particular reference to the study of insect populations. Chapman and Hall, London.

Spangler, S. M., and D. D. Calvin. 1990. Vertical distribution of european corn borer (Lepidoptera: Crambidae) egg masses on sweet corn. Environ. Entomol. 30:274-279.

Stinner, R. E., C. S. Barfield, J. L. Stimac, and L. Dohse. 1983. Dispersal and movement of insect pests. Ann. Rev. Entomol. 28:319-335.

Székács, A., E. Lauber, J. Juracsek, and B. Darvas. 2010. Cry1Ab toxin production of Mon810 transgenic maize. Environ. Tox. Chem. 29:182-190.

Tooker, J. F., and S. J. Fleischer. 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. Crop Mgmt. DOI: 10.1094/CM-2010-0616-01-RS.

Zalucki, M. P., A. R. Clarke, and S. B. Malcom. 2002. Ecology and behavior of first instar larval lepidoptera. Ann. Rev. Entomol. 47:361-393.

Table 3.1. Corn stage and plant zone effects on western bean cutworm recovery on the first sampling date.

Effect	DF	P-value
2009[a]		
Corn stage	2	0.002
Plant zone	4	0.009
Corn stage x plant zone interaction	8	0.112
2010		
Corn stage	4	0.100
Plant zone	2	<0.0001
Corn stage x plant zone interaction	8	0.624
2011[b]		
Corn stage	2	0.169
Plant zone	2	0.054
Corn stage x plant zone interaction	4	0.554

[a] Infestation of egg mass on leaf close to primary ear.

[b] Analysis considering larval recovery in tassel zone + above ear zone, and primary ear + secondary ear zone.

Table 3.2. Corn stage and plant zone effects on western bean cutworm recovery on the second sampling date.

Effect	DF	P-value
2009[a]		
Corn stage	2	0.030
Plant zone	4	0.732
Corn stage x plant zone interaction	8	0.681
2010		
Corn stage	4	0.589
Plant zone	2	0.004
Corn stage x plant zone interaction	8	0.618
2011[b]		
Corn stage	2	0.540
Plant zone	2	0.221
Corn stage x plant zone interaction	4	0.851

[a] Infestation of egg mass on leaf close to primary ear.

[b] Analysis considering larval recovery in tassel zone + above ear zone, and primary ear + secondary ear

Table 3.3. Effect of different corn tissues on weight and survival of western bean cutworm larva.

Corn tissue	Larval weight (mg)[a]	Larval survival (%)[b]
	2009 (13 d)[c]	
Leaf from whorl	.	4 (± 5.51) a
Pollen	22.39 (± 14.99) a	61 (± 5.51) b
Silk	26.39 (±14.51) b	66 (± 4.36) b
	2011(7 d)[d]	
Pollen	0.1 (± 0.16) a	31 (± 7.50) a
Silk + pollen	1.87 (± 2.35) a	56 (± 10.26) a
Silk	1.06 (± 1.39) a	45 (± 10.01) a
Late whorl (tassel + leaf)	3.78 (± 3.42) b	74 (± 16.65) b

[a] Final larval weight. Mean ± SEM. The means with the same letter are not significantly different, P≤ 0.05. Larvae reared on leaf of whorl, in 2009 were too small to be weighted.

[b] Percentage larval survival. Mean ± SEM. The means with the same letter are not significantly different, P≤ 0.05.

[c] 100 neonate larvae per container for 13 d. Total number of observations N = 377.

[d] Five larvae per container for 7 d. Total number of observations N = 130 in 2011.

Table 3.4. Effect of different corn tissues on development of western bean cutworm larvae.

Corn tissue	Mode of head capsule width (mm)	Range of head capsule width (mm)
	2009 (13 d)[b]	
Pollen	1.70	(0.6 to 2.0)
Silk	1.70	(0.8 to 1.9)
	2011(7 d)[c]	
Pollen	0.40	(0.3 to 1.2)
Silk + pollen	0.40	(0.3 to 1.0)
Silk	0.30	(0.2 to 0.40)
Late whorl (tassel + leaf)	0.40	(0.30 to 1.2)

[a] Most frequently occurring value of head capsule in each corn tissue. Classification of the larval instar according to Antonelli (1973).

[b] 100 neonate larvae per container for 13 d.

[c] Five larvae per container for 7 d.

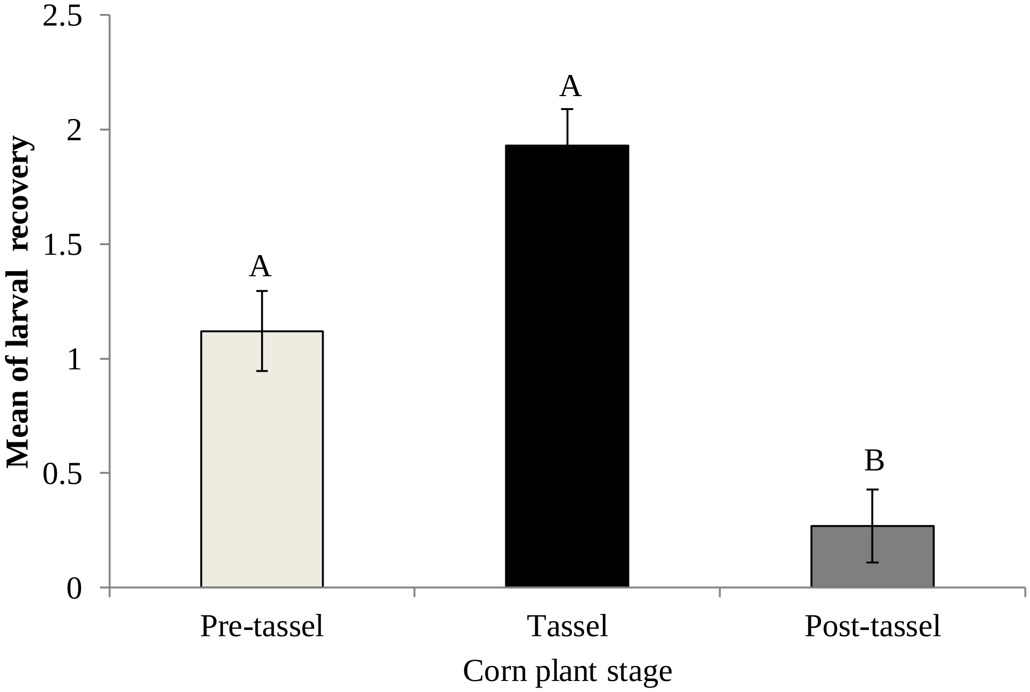

Fig. 3.1. First instar larval recovery (1st sampling date) of western bean cutworm (mean ±SE) on different plant stages in 2009. Infestation of egg mass near primary corn ear. Error bars represent S.E.M. Bars sharing the same letter are not significantly different ($P \leq 0.05$).

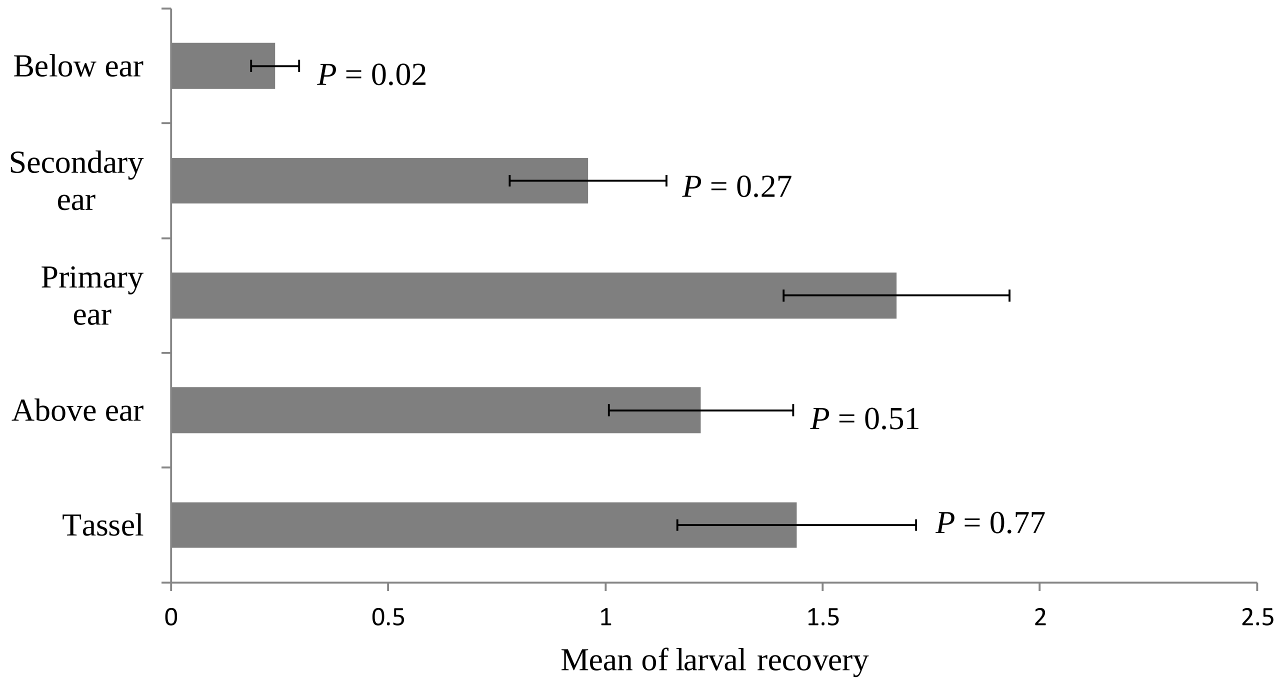

Fig. 3.2. First instar larval position (1st sample date) of western bean cutworm in different plant zones infested near primary ear 2009. Error bars represent S.E.M. Mean comparison based on ear zone as a reference (Dunnett test).

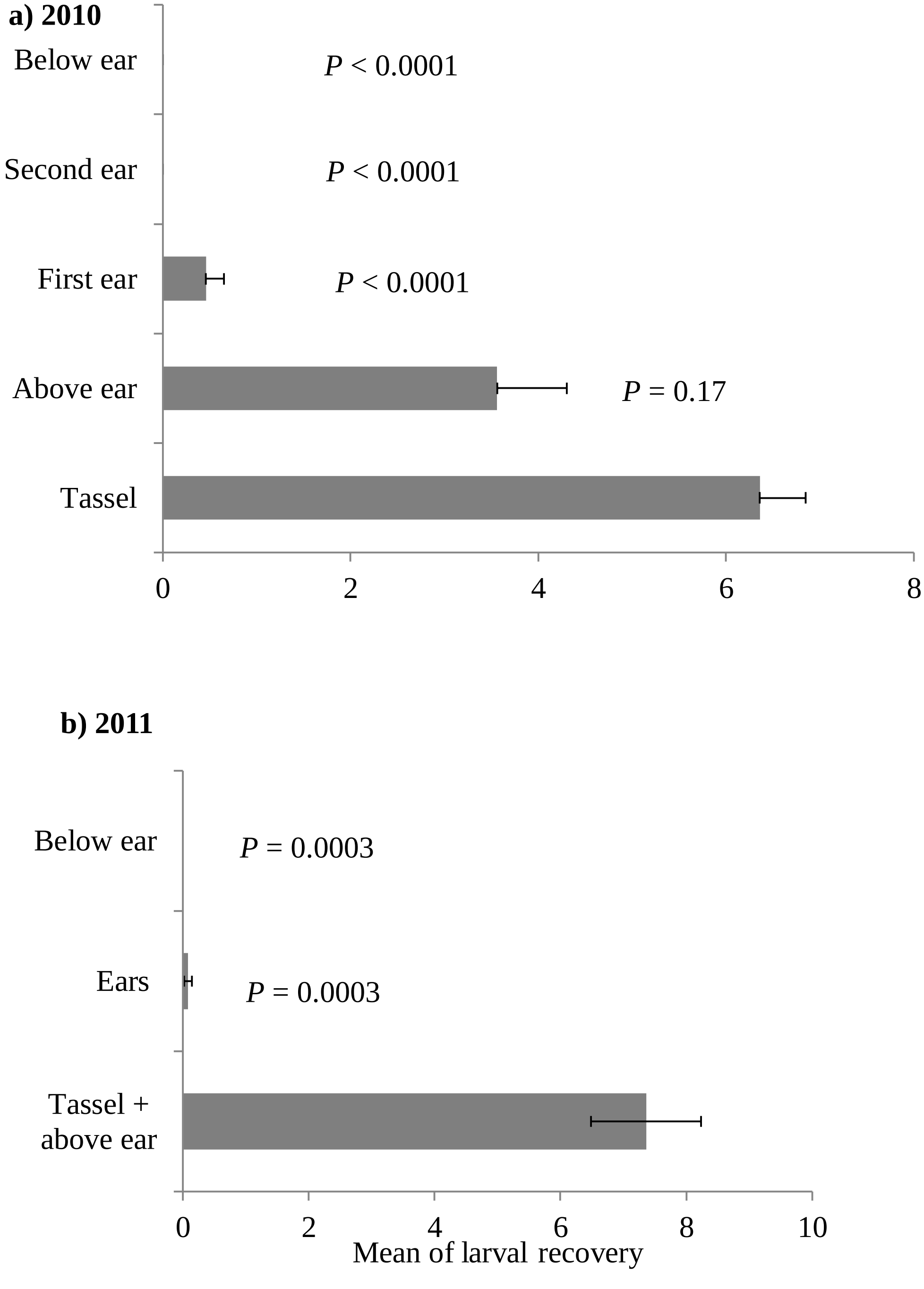

Fig. 3.3. First instar larval position (1st sample date) of western bean cutworm on different plant zones infested in the upper part of the plant. Error bars represent S.E.M. Mean comparison based on tassel zone as a reference (Dunnett test).

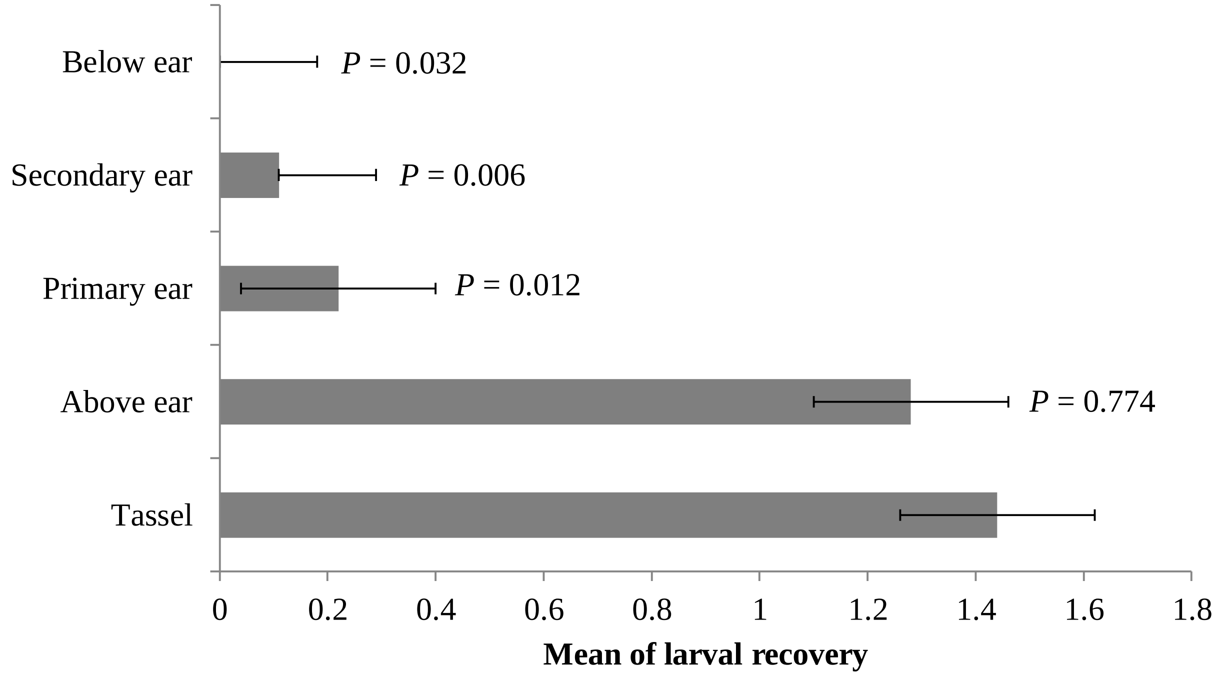

Fig. 3.4. Second instar larval position (2nd sample date) of western bean cutworm on different plant zones infested in the upper part of the plant 2010. Error bars represent S.E.M. Mean comparison based on tassel zone as a reference (Dunnett test).

Chapter 4: Western bean cutworm egg and larval survival and the development of economic injury levels and economic thresholds in field corn

Introduction

Western bean cutworm *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) is a pest of beans and corn (Blickenstaff and Jolley 1982), and historically distributed in the western United States (Douglas et al. 1957, Hagen 1976, Blickenstaff 1979). The insect's expansion eastward is documented beginning in 1999 (O'Rourke and Hutchison 2000, Dorhout and Rice 2004, Cullen and Jyotika 2008, Rice 2006, DiFonzo and Hammond 2008, Tooker and Fleischer 2010, Michel et al. 2010), and it has been reported in 11 states and provinces (Michel et al. 2010).

Western bean cutworm is univoltine (Hagen 1962, Seymour et al. 2004). The adults typically emerge in the middle of July (Hagen 1976) and lay eggs on corn leaves, with preference for plants prior to tassel emergence (Hagen 1962, Blickenstaff 1979, Holtzer 1983, Seymour et al. 2004, Eichenseer et al. 2008). When the eggs hatch, the larvae feed on different corn tissues, and by the of fourth instar, they colonize the ear where they feed on kernels (Seymour et al. 2004, Appel et al. 1993).

Information of western bean cutworm biology, ecology, and economic impact in the literature is relatively limited. Hagen (1962) reported ear injury from western bean cutworm ranging from 4 to 40%. Catangui and Berg (2006) reported 7.45 cm^2 ear injury per larva in several Bt corn hybrids. Appel (1991) estimated yield loss by western bean cutworm based on the number and weight of injured kernels, and several nominal action thresholds for field and sweet corn have been proposed (4%, 8%, and 20% of plants infested with an egg mass) roughly based on corn stage and price of the corn (Seymour et al. 2004, Peairs 2006; Rice 2007; Cullen and Jyuotika 2008). Because current thresholds are rudimentary, it is necessary to develop economic injury levels (EIL) and economic thresholds (ETs) for western bean cutworm based on more current understandings of pest injury and damage, dynamic costs of control and product market values, and pest biology (Pedigo and Rice 2009). The economic injury level (EIL) is the lowest density of the pest population which causes economic damage (Stern et al. 1959). The economic threshold (ET) is the parameter with practical application and represents the moment at which the pest population needs to be managed in order to prevent from reaching the EIL (Stern et al. 1959). This parameter is usually set below the EIL, and can be expressed in previous life stages of the pest from which the EIL was determined.

Foliar insecticides are typically used to control western bean cutworm, and their efficacies are compromised after the larvae enter the ear. Thus, ETs expressing the percentage of plants infested with egg masses (Appel et al. 1993, Seymour et al. 2004, Paula-Moraes et al. 2011), which also incorporate larval survival (Ostile and Pedigo 1987, Barrigossi et al. 2003), would present an improvement in management decision-making. The incorporation of the variability of larval survival under different environmental conditions would make it possible to more accurately estimate the resultant pest density.

Hence, the objective of this study was to estimate the injury capacity of western bean cutworm and develop EILs and ETs that incorporate dynamic corn prices and management costs, egg survival, and variable larval survival as measured across major ecoregions (Level III) (Chapman et al. 2001) in Nebraska.

Materials and Methods

Studies of western bean cutworm egg survival, larval survival, and larval corn consumption were conducted at University of Nebraska facilities across three ecoregions (Chapman et al. 2001): Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord (Western Corn Belt Plains); South Central Agricultural Laboratory, Clay Center (Central Great Plains); Panhandle Research and Extension Center, Scottsbluff (Western High Plains). 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 (Catangui and Berg 2006) was used to minimize the confounding effect of European corn borer in Clay Center and Concord. In Scottsbluff , due to absence of a European corn borer effect, the corn hybrid was non-Bt (2008 – DKC51-45, 2009 – Pionner 35F37, 2010 – Mycogen 2R416).

Field experiments were conducted during 2008, 2009, 2010 and 2011. Experiments evaluated egg and larval survivorship, larval consumption, and yield impact. The studies are highlighted and presented as separate topics for better comprehension of the results.

Based on the studies listed above, economic injury levels (EIL) were calculated and expressed in larval density. Because western bean cutworm sampling typically considers the presence of egg masses (Paula-Moraes et al. 2011), the economic thresholds (ET) incorporated variable larval survival and were expressed as percent of plants infested with egg masses.

Egg survivorship and development time (laboratory). Western bean cutworm egg developmental time and survival were evaluated at 16ºC, 25ºC and 30ºC. One day-old egg masses were collected from oviposition cages, placed in cotton lined Petri dishes, covered to maintain humidity and incubated in growth chambers. Three replications at each temperature were used. Temperatures were randomly assigned to growth chambers in a complete randomized design (CRD) (total of nine chambers). Eggs totaled 955 at 16ºC, 437 at 25ºC and 462 at 30ºC. Eggs were monitored every day for 14 d and the day of hatch was recorded. Egg survival in different temperatures and the linear relationship between the temperature and time of development was estimated (PROC GLIMMIX, SAS Institute 2009).

Egg survivorship (field). Western bean cutworm egg hatch was evaluated at the Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE during 2008 and at the South Central Agricultural Laboratory, Clay Center, NE during 2008 and 2011.

At both locations in 2008, the experimental design was a randomized complete block design (RCBD) with four replications. Each plot was 6 rows by 12 feet long, with a total of 100 plants per plot. Plots were infested with western bean cutworm egg masses and the treatment consisted of egg mass infestation rates of 0, 1, 3, 5 and 10%. The infestation was artificial, using western bean cutworm egg masses collected from commercial fields. A small square of screen was stapled loosely behind the egg mass forming a “sandwich” in order to keep the leaf from curling and dislodging egg masses. The infestation was done by stapling the leaf sandwich to the upper surface of a corn leaf above the primary ear. The infested plants were identified by tying flagging tape at the base of the plant. To minimize injury to the eggs, the number of eggs/egg mass was determined by photographing each egg mass and counting the eggs in the laboratory prior to plant infestation. The total number of eggs infested at Concord was 4,037, and at Clay Center was 3,927. At two weeks following infestation, the sandwiches were removed from the field and examined with microscopy in order to record the number of unhatched eggs. The percentage of eggs hatched was calculated. Temperatures during egg incubation at Concord (07/24/2008 - 07/31/2008), and at Clay Center (07/28/08 - 08/04/2008) were obtained from the High Plains Region Climate Center, (http://hprcc.unl.edu).

At Clay Center during 2011, western bean cutworm egg hatch was evaluated in two corn fields at different corn development stages: VT and silking (Ritchie et al. 1993). In each corn field the infestation was one egg mass per plot, and plots were 15 feet long with 25 plants per row (100 plants per plot). The infestation was artificial, but utilized natural oviposition. Western bean cutworm moths were captured in cages positioned under ultra-violet light traps. One male and two female moths were confined on the top part of a corn plant with a large flat mesh pollination bag (46 x 51 cm). Two days after infestation, the presence of egg masses was determined and thinned, when necessary, so only one egg mass was left per plant. Again, the number of eggs in each egg mass was determined by photographing the egg masses for later counting. Plants on which the western bean cutworm females did not lay egg masses were artificially infested following the same egg mass sandwich method of 2008. The percentage of eggs hatching was calculated for each corn stage. Temperatures during egg incubation in Clay Center (07/21/11 - 07/28/11) were obtained from the High Plains Region Climate Center (http://hprcc.unl.edu). Egg infestation methods (egg mass sandwich vs. moths in pollination bags) were compared with data from Clay Center (2011) for silking and VT stages.

An overall mean number of eggs per egg mass was calculated based on the 714 egg mass pictures. These egg masses were from confined moths inside pollination bags and large cages, but predominantly from commercial fields, collected from several ecoregions of Nebraska and eastern Colorado, during the four years of study.

Larval survivorship (field). Evaluations were conducted during 2008, 2009, and 2010 at three locations across Nebraska: Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord; South Central Agricultural Laboratory, Clay Center; Panhandle Research and Extension Center, Scottsbluff. During 2008, 2009 and 2010 a RCBD was used with 4 replications. Details are presented in Table 4.1. Natural infestation of western bean cutworm in experimental plots was monitored. In Clay Center and Concord, plants were inspected for the presence of western bean cutworm egg masses prior to artificial infestation. The natural infestation was extremely low during 2008 to 2009 at both locations. At Scottsbluff, inspections of plants were done after the large plot cages were installed in the experimental plots. Egg masses from natural infestation were eliminated from all experimental plots.

In 2008, the larval survival at Clay Center and Concord was evaluated in the same experimental plots which had the egg hatch evaluated. Approximately 20 d after western bean cutworm egg infestation, the number of the larvae was estimated based on the evaluation of 30 randomly selected plants per plot (Table 4.1). In 2009 (Clay Center and Concord), larval survival were evaluated following the same methodology of 2008. The plots were also infested by egg mass sandwich method at rates of 0, 1, 3, 5 and 10% (Table 4.1).

In 2010, larval survival was evaluated in Clay Center and Concord approximately 20 days after western bean cutworm egg infestation. The infestation was done using the pollination bag method and sandwich method at a rate of 0, 1, 3, 5 and 10%. Three plots per block were infested with 3% egg mass infestation level. One plot was infested using egg mass sandwich method, other two were infested with wild moths confined in pollination bags. Two plots were infested at 10%, both by using pollination bag method. Larval survival was evaluated at Concord in three plots per block, and all 100 plants per plot were inspected for the presence of western bean cutworm larvae. The evaluated plots were infested at a level of 3% with a pollination bag, another infested at a level of 3% with the sandwich method, and the last one infested at a level of 10% with a pollination bag. In Clay Center, larval survival was done in all plots, inspecting 50 plants per plot, and the number of larvae per plot was estimated based on this sample (Table 4.1).

During the three years of the study in Scottsbluff, the artificial infestation was done by placing a 1.83 m X 1.83 m X 2.45 m cage over a central part of the plot and introducing moths for oviposition. A variable number of moths was introduced into each cage based on the target egg mass infestation, and later egg mass density checked. Egg masses were thinned if necessary. One extra plot was artificially infested at 3%, using the sandwich method. Twenty days after infestation, the number of western bean cutworm larvae was evaluated on 30 plants in each plot (Table 4.1).

The number of recovered larvae was correlated with the number of infested eggs, and the percentage of larval survival was calculated. The effect of method of infestation (egg mass sandwich versus wild moths in big cages) for larval survival was tested in Scottsbluff using ANOVA (2008 and 2009 data) (PROC GLIMMIX, SAS Institute 2009).

Larval corn consumption and yield impact (field). Larval consumption and the relationship between western bean cutworm infestation, corn ear injury and yield was also characterized in the same three locations and experimental plots previously described. From 2008 to 2010 (Table 4.1), at harvest, the amount of injury (square cm of grain surface with western bean cutworm injury/ear) was measured using a transparent plastic sheet with grid lines. The total injury was measured considering tip and side ear injury. The grain moisture and yield were also recorded.

The number of evaluated corn ears was variable depending of the year (Table 4.1). In 2008 and 2009, approximately 70 plants remained after the larval survival evaluation. At Clay Center in 2010, the presence of the western bean cutworm was recorded without destruction of the ear in the two central rows of the plot (50 plants) and the plant was marked with flagging tape. At harvest, the injury was evaluated in the same corn ears. At Concord in 2010, the larval survival was evaluated with destructive sampling in three plots per block. The other remaining six plots (100 plants per plot) were hand harvested and injury was evaluated. At Scottsbluff, during 2008 and 2009, 70 corn ears per plot were evaluated. In 2010, due to the attack of grasshoppers in plants outside large cages, approximately 15 corn ears per plot inside the cages were harvested and had injury evaluated (Table 4.1). For all location in 2008, 2009 and 2010, the total weight of the grain was determined in each plot, corrected to 15.5% moisture content, and data converted to one corn ear.

In 2011, at Concord and Clay Center, direct corn ear infestation was done with fourth instar larva. Larvae were reared on a meridic diet specifically developed for western bean cutworm, and obtained from USDA-ARS, Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA. Ear infestation was done at two fields per location. For each location, one field was infested when the corn was at blister stage, and the other infested when the corn was at milk stage (Ritchie et al. 1993). Fields had been planted on different dates, and the larval infestation was done on the same date. Each experimental area consisted of eight rows, and the study was laid out as a RCBD. Twenty blocks were marked, evenly spaced from each other, in the central four rows of experimental area. In each block, the experimental unit was two corn plants per row which were infested with the same randomly assigned level of 0, 1, 2 or 3 western bean cutworm larvae per ear.

The larvae were confined in the corn ear with flat mesh pollination bags (25 x 30 cm). Inspection for larval survival was done approximately one month after larval infestation. The presence of the western bean cutworm pre-pupa and injury were examined in order to confirm the level of infestation per ear. At harvest, corn ears were hand harvested, the injury area measured, ear grain shelled and weighed, and moisture corrected to 15.5%.

The linear relationship between injury area and number of western bean cutworm larvae, and corn grain yield and number of western bean cutworm larvae were determined (PROC GLIMMIX, SAS Institute 2009). An overall mean of larval injury area was also calculated (PROC GLIMMIX, SAS Institute 2009) (Appendix B).

Larval corn consumption (laboratory). Western bean cutworm larval consumption was also evaluated in the laboratory at the Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE. The experiment was performed in 2009 and 2011, and the corn was infested when in blister and milk stage. Injury area (square cm of grain surface) caused from the beginning of fourth instar to end of fifth instar was recorded.

In 2009, the study was conducted as a RCBD with three replications (replication by growth chamber) set at 25ºC. Five small circular containers (10 cm diameter and 4 cm height) per corn stage (blister and milk) were placed in each growth chamber, each container with one half a corn ear without the husk. The corn ear was placed on a layer of sand. Another methodology was also tested using a larger circular container (30cm diameter and 7.6 cm height) which received a whole corn ear in milk stage. Western bean cutworm larvae were reared on pollen and silk of corn, and one larva was placed on the ear at the beginning of the fourth instar. After approximately 20 d, the injury area was measured in square cm of grain consumed per corn ear.

In 2011, the study was similarly conducted, except each growth chamber received 10 large circular containers with one ear at blister stage, and after 20 days, the injury area was measured. The mean injury area was calculated for each corn stage, and the effect of corn stage on area injured was compared. The methods were also compared using ANOVA (PROC GLIMMIX, SAS Institute 2009) (Appendix C).

Economic injury level and economic threshold. Economic injury levels (EIL) and economic thresholds (ET) were calculated based on the results from experiments previously described.

The EIL was calculated, based on the formula

$$\mathrm{EIL} = \mathrm{C}/[\,(\mathrm{ID})(\mathrm{V})(\mathrm{K})]$$

where **C** = cost of management per area (e.g. $/ha), **V=** market value per unit of produce (e.g. $/ha), **ID** = yield loss per insect (weight of grain loss in kg/western bean cutworm larvae), and **K** = proportionate reduction in potential injury (Pedigo et al. 1986, Pedigo and Rice 2009).

The cost of management was calculated based on the total expense of chemical control of western bean cutworm (Klein and Wilson 2012), and included the range of control cost variation considering aerial, ground application, and insecticide expense.

The crop value of corn was calculated based on the price and yield. The price was related to corn, grain – monthly average of the price received, measured in $/bu by the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, and Nebraska

(United States Department of Agriculture - http://quickstats.nass.usda.gov/, 2012). The period was from January 2000 to December 2011. These nominal prices were converted to real prices using PPI - PRODUCER PRICE INDEX (FRED - Federal Reserve Economic Data, base December 2011), and reflect scenarios of low, medium and high corn price. The crop values range was calculated by multiplying the selected higher, lower and real average of corn prices times the average yield. The yield refers to an average yield of 13629.26 kg/ha (Klein and Wilson 2012). The yield was calculated based on a conversion of 220 bushel/acre, one acre with 30,000 plants/acre (74,100 plants/ ha).

The yield loss/insect (**DI**) was obtained from the relationship between corn yield and the number of western bean cutworm larvae (Pedigo and Rice 2009, Bode and Calvin 1990) considering the linear regression equations from 2011 (Table 4.5). The calculation of grain reduction (grams) per corn ear was based on one larva/plant, and was converted to kg. The worst-case scenario of yield loss was selected and used in the EIL formula. The **K** was set at 95%, and represents the expected proportion of the population of western bean cutworm larvae killed by a chemical control (Peairs et al. 2011).

The ET was calculated as an operational level for management decision-making. The mean numbers of eggs/egg mass and larval survival were incorporated in the ET calculations (Wright 2007, Ostlie and Pedigo 1987) (Table 4.3). The ET was expressed as percentage of infested plants with at least one western bean cutworm egg mass (Paula-Moraes et al. 2011), considering the number of eggs/egg masses determined previously.

Results

Egg survivorship (laboratory and field). In the lab, the time of egg development of western bean cutworm decreased with the increase of temperature (*P* = 0.001) (Fig. 4.1). Egg hatch was 87.96% at 16°C, 93.36% at 25°C, and 51.31% at 30°C (Fig. 4.1), and the overall mean egg hatch was 77% (Table 4.2).

At Concord (2008), under field condition, the egg hatch mean was 86.58% (Table 4.2). During the period of the study, the lowest temperature mean was 19.48°C, and the highest temperature mean was 30.67°C. At Clay Center, the egg hatch mean was 78% (2008) and 76% (2011) (Table 4.2). The highest and lowest temperature means at Clay Center in 2008 were 19.56°C and 30.88°C. In 2011, the highest and lowest temperature means were 32.30°C and 20.15°C.

Considering 714 egg masses used in this and other studies described previously, the overall mean of number of eggs/egg mass was 85.49 eggs (range = 2 and 345 eggs/egg mass). The comparison between the egg mass sandwich infestation method and wild moths confined in pollination bags at Clay Center (2011) did not indicate significant differences in egg hatch. The two methods of infestation were compared in corn fields in silking (*P* = 0.83), and VT (*P* = 0.54) stages. The overall mean egg hatch was 80% (Table 4.2).

Larval survival (field). The percentage of western bean cutworm larval survival (approximately 20 d after egg infestation) is presented for each year and location in Table 4.3. For 2009 and 2010 the percentage of larval survival was calculated based on the total number of eggs infested. In 2008 the percentage of larval survival at Clay Center and

Concord was also corrected considering the number of eggs hatched (Table 4.3). There was no significant effect of method of infestation (sandwich versus wild moths in large cages) in larval survival at Scottsbluff, considering data from 2008 and 2009 ($P = 0.19$).

Larval consumption in corn field and yield impact. The relationship between corn ear injury area (square cm) and level of western bean cutworm larval infestation, in three ecoregions of Nebraska, is presented on Table 4.4. In most years, there was a significant linear relationship between corn injury and the level of western bean cutworm larval infestation. The overall mean of western bean cutworm injury/ear across all sites was 15.17 cm^2 (SE 4.96 cm^2; N = 11,966 corn ears).

A significant linear relationship between corn yield (grams) and number of western bean cutworm/ear was only detected at Scottsbluff in 2008 ($\hat{y} = 186.48(\text{SE } 1.76) – 3.46 (\text{SE } 1.46)\ x$) ($P \leq 0.05$). In other years and locations, there was significant relationship between the number of the western bean cutworm number larvae and corn ear injury (Table 4.4); however, no relationship was detected between levels of western bean cutworm larvae with consequent corn yield loss. This was presumably due to experimental problems (e.g. birds, Scottsbluff 2009; grasshoppers, Scottsbluff 2010; hail damage, Concord 2009), but also because of a general low larval survival across location/years, resulting in low actual infestation levels.

In 2011, the direct infestation of corn with fourth instar western bean cutworm eliminated the problems of early instar mortality (Table 4.3). There was a significant linear relationship between western bean cutworm larval infestation and yield loss ($P \leq 0.05$) when corn was infested in blister and milk stages at Clay Center, and when infested in blister stage at Concord (Table 4.5). The overall means of larval injury at Clay Center was 12.46 cm^2 (SE 1.40) for corn infested in blister stage and 13.52 cm^2 (SE 0.91) for corn infested in milk stage. At Concord, the overall means of larval injury were 9.94 cm^2 (SE 0.64) and 7.98 cm^2 (SE 0.54) for corn in blister and milk stage, respectively

Larval consumption (laboratory). In 2009, differences in injury area from western bean cutworm larvae were not detected ($P = 0.30$) when the corn ear was infested in blister or milk stage (small container method). The observed injury means were 13.25cm^2 (SE 1.60) and 11.10cm^2 (SE 1.10), when the corn ear was in blister and milk stage, respectively. There were no significant differences between injury of milk stage corn ear in the small container and big container ($P = 0.09$), and the mean of injury area with whole corn ear (large container method) was 15.13cm^2 (SE 0.98). In 2011, the mean injury area with whole corn ear infested in blister stage (large container method) was 16.87 cm^2 (SE 1.41).

Economic injury level and economic threshold. Based on each regression equation (Table 4.5), the yield losses per one western bean cutworm larva in Clay Center (2011) were 9.21 and 12.76 grams per corn ear when the corn was infested in blister and milk stages, respectively (Table 4.5). In Concord (2011), the yield loss per one larva was 9.64 grams when corn was infested with fourth instar larvae in blister stage, and 3.54 when corn was infested in milk stage.

The slope of the linear regression equation from Clay Center with corn infested when in milk stage was selected as the worst-case scenario of yield loss/insect (Table

4.5). The relationship between observed and predicted values from this linear regression is presented in Fig. 4.2.

A range of values of crop and costs of management were used to calculate EILs. EILs are expressed in number of western bean cutworm larvae/ear, and are presented in Table 4.6. EILs presented were calculated based on insecticide application that gives 95% control (Pedigo et al. 1986). Cost of management reflects a range of actual price scenarios in Nebraska. Crop value considers a range of crop market price for the period of 2000-2011, including low, average and high prices of corn. Besides the metric unit of dollar/hectare, the values are also converted in dollars/acre. The crop values are also converted in dollars/ bushel. Based on 12.76 grams of yield loss/ear infested with one western bean cutworm larva, conversion was done to kilograms to hectare considering 74,100 plants/ha (30,000 plants/acre), and resulted in a yield loss mean of 945.52 kg/ha (15.07 bushel/acre).

The ETs were developed from EILs, expressed as the percentage of infested plants with at least one egg mass (Table 4.7). The ET was calculated based on the mean of 85 eggs/egg mass, and again considering a plant population of 74,100 plants/hectare (30,000 plants/acre). Larval survival was incorporated in the ET calculation considering the range of larval survival observed during three years across ecoregions of Nebraska (Table 4.3) and reports from consultants working in northeast Colorado and northwestern Nebraska.

Discussion

The study of western bean cutworm egg survivorship in the laboratory indicated that increasing temperature decreases the time of the egg development. However, at 30ºC there is a significant decrease in egg hatch (Fig. 4.1). The pattern of high heat negatively affecting egg hatch was not observed in the field study when egg hatch was evaluated in 2008 and 2011. The average temperatures during the summers of 2009 and 2011 surpassed 30ºC during some parts of the day. However, this temperature was not constant all day as it was inside the growth chamber, and it was probably variable in parts of the plant canopy (Schoonkoven et al. 2005). The water vapor diffusion in transpiration process on corn leaf surface in addition to leaf rotation, with consequent decreasing of sun exposure, probably created a more favorable microclimate for the eggs (Smith 1954, Mani 1968). All of these factors probably contributed to decreased temperatures surrounding western bean cutworm eggs, and consequently higher egg survival in corn field.

The factors related with temperature and affecting *Ostrinia nubilalis* (European corn borer) mortality were investigated (Showers et al. 1978). High temperature in association with moisture-stress during early larval instars resulted in high egg hatch mortality (81%). However, the survivorship of European corn borer larvae was independent of egg laying and related to available feeding sites on host plant. In the same way, in the present study, egg mortality was demonstrated not to be a critical factor in survival and establishment of the western bean cutworm on the host plant, and the egg hatch was approximately 80% across ecoregions of Nebraska.

Western bean cutworm infestation methods were tested in 2008 and 2009 in Scottsbluff. Larval recovery was compared between plots infested with large cages and plots that received egg mass sandwiches (both at the same 3% of level of infestation) (Table 4.1). At Clay Center in 2011, the infestation by deposition of the eggs from wild moths confined in pollination bags was compared with the egg mass sandwich method (Table 4.1). There were no significant differences observed in any comparison, so it can be concluded that that the artificial infestation methods used did not affect the results.

Larval survival of western bean cutworm was evaluated in the field across three ecoregions of Nebraska, during three years (Table 4.3). The results indicated variability of larval survival in different locations, and also in different years (Table 4.3). In Concord (2009) the very low larval survival observed (1.51%) is possibly explained by the exceptionally low temperatures in the summer of 2009 (http://snr.unl.edu/lincolnweather/records/July-Records). The daily low average temperature after egg infestation reached 8°C at night (http://hprcc.unl.edu). On the other hand, the higher larval survival of western bean cutworm registered at Scottsbluff), in 2008 (Table 4.3), is possibly related with the method of infestation. The large cages remained approximately 2 weeks in the field which moderated the environmental and provided protection against biocontrol agents. The final decision in ET calculation (Table 4.7) was to incorporate the western bean cutworm larval survival range observed during 3 years of study, across 3 ecoregions in Nebraska (Table 4.3). The 20% larval survival rate was also incorporated based on reports of high larval survival near the Missouri River and further east. In this ecoregion the moisture is higher because of more prevalent rainfall, and the association of high temperature, high moisture and overcast conditions could favor western bean cutworm larval survival (G. Hein, personal communication).

Variable low larval survival of western bean cutworm was previously reported in the laboratory (Blickenstaff and Jolley 1982) when fresh corn tissue was used to feed larvae for 24 d, and survival ranged from 19% to 40%. Under field cage conditions, larval survival of western bean cutworm was estimated at 3.3% in Perkins County, NE (southwestern Nebraska) (Appel et al. 1993). Variation of larval survival at the same location was also detected. In an Iowa study investigating ear damage from western bean cutworm in transgenic corn hybrids, estimated larval survival was 13% on susceptible hybrids, and 1.3% in a corn hybrid expressing *Bacillus thuringiensis* (Bt) protein Cry1F (Herculex I, Dow AgroSciences and Pioneer Hi-Bred International) (Eichenseer et al. 2008).

In the Chapter 2 and the present study, it was demonstrated that early larval stage is the critical for the establishment of western bean cutworm on the corn plant. Mortality in larval stages of Lepidoptera has been reported to be high, especially in early instars (Zalucki et al. 2002). However, the range of mortality is also reported to be quite variable, depending on the species. Larval survival of European corn borer ranges from approximately 40% (Lee 1988) to approximately 15% for second generation (Wright 2007).

The mode of egg laying and larval feeding behavior (Zalucki et al. 2002) are factors that can help to explain the higher larval mortality. In the case of western bean cutworm, egg laying is characterized by deposition of higher numbers of small size eggs (average of 85 eggs/egg mass). This is an example of "r strategy", which is characterized by a low expectation in a unique individual of the species, but with an investment that at least some will survive (MacArthur and Wilson 1967). Although the egg stage is not the critical moment for survival of western bean cutworm (Table 4.2), the small size of the egg with few provisions represents a constraint in the establishment of the early larva which needs to quickly find a suitable feeding niche before starvation, desiccation, or biocontrol. Moreover, the western bean cutworm larva has a mixed diet, feeding on different corn tissues (Hagen 1962; Seymour et al. 2004; Eichenseer et al. 2008). This mixed larval feeding behavior requires movement and exposure to several places and in the environment of the corn canopy that probably contributes to a higher level of larval mortality (Zalucky et al. 2002). The mortality of western bean cutworm is possibly greatest before the larva becomes protected inside the ear, at the beginning of the fourth instar (Seymour et al. 2004).

In summary, the corn stage probably plays a role in the establishment of western bean cutworm (pre-tassel stages providing the best nutrition and protection for early larval instar), and this is probably why females select for specific plant stage for oviposition (Blickenstaff 1979, Holtzer 1983, Eichenseer et al. 2008). However, in 2010 the infestation at Clay Center and Concord was near the V18 (pre-tassel) corn stage, and in Scottsbluff (2008) the infestation was also near V18 stage. In both cases the overall larval survival can be considered low (around 4% at east and 13% at western). Based on these results, it is possible to conclude that corn stages did not totally explain the intrinsic low larval survival of the species. Aspects related with the behavior of the early larval instars also play a role in mortality (Chapter 2), besides other biotic factors of mortality, that was partially eliminated with big cage effect in Scottsbluff (2008), but need to be better investigated (Michel et al. 2010).

During three years of egg mass infestation (Table 4.1), it was possible to detect significant linear relationships between the number of the western bean cutworm larva and ear injury (Table 4.4). However, the intrinsic low larval survival of the species (Table 4.2), and the levels of egg infestation adopted (Table 4.1) did not make it possible to detect a yield loss in experimental areas at Clay Center and Concord (eastern locations in Nebraska). A previous studies had the same problem (Appel 1991), and emphasized the need to improve the methodology of infestation and to determine egg and larval survival under field conditions. The egg and larval survival were characterized in the present study (Table 4.2 and 4.3) based on several attempts during the years to improve the field methodology (Table 4.1), but usable data related to actual corn yield loss/insect, under field conditions at Clay Center and Concord, were only obtained when corn ears were directly infested in 2011 with fourth instar larvae, eliminating early instar mortality factors.

The type of larval injury is another complexity in describing the relationship between larval ear injury and yield loss of western bean cutworm. This insect is a direct pest (Pedigo et al. 1986) on the corn ear, but predominantly with an ear tip feeding behavior. Previously studies reported the same type of injury (Appel 1991, Eichenseer et al. 2008). Indirect estimation of yield loss has been done by weighing a corresponding number of the kernels injured (Appel 1991; Dorhout 2007, Eichenseer et al. 2008), but this does not account for indirect effects of development of uninjured ear kernels.

Reports of western bean cutworm damage are quite variable in the literature, and also in this research, where yield loss per larvae varied from 3.46 – 12.76 grams. There are anecdotal reports of late instar movement in and out of the ear, possibly influenced by environment, but this is not well documented in the literature. This behavior, coupled with feeding on different corn tissues, may be in part responsible for this variability. In any case, because of this variability, the EIL calculations were based on the worst-case scenario of corn ear yield loss per western bean cutworm larva (12.76 grams per insect), selected from 2011 experiments (Table 4.5 and Fig. 4.2). Several EILs were calculated based on this yield loss/insect, and also the dynamics of the current market crop value and costs of management in U.S Corn Belt (Table 4.6).

In the present study, corn injury from one western bean cutworm larva was measured (cm^2) in the laboratory and in the field. The overall mean injury area in the field during 2008 to 2010 was approximately at 15.17 cm^2 (calculated from 11,966 corn ears). This injury is close to the overall mean observed in the 2009 (13.43 cm^2, SE = 0.74), and the 2011 laboratory studies (16.87 cm^2). However, the yield loss used for EIL calculation was based on the linear relationship between weight of corn yield loss of the ear (kg/ear) in four levels of larval infestation (0, 1, 2 and 3 larvae/ear) in 2011. The decision was based on the difficulty to be accurate when estimating the amount of injured kernels in the ear tip, and how much these injured kernels contribute to the yield.

The overall mean of yield loss per western bean cutworm larva observed from the 2011 study is 936.62 kg/ha (14.93 bu/acre), based on 74,100 plants/ha. This result is different than what was previously reported for the relationship between larval density and dent stage corn (Appel et al. 1993). The authors detected a yield reduction of 3.7 bu/acre (considering 32,000 plants/acre) at an infestation of 1 larvae/ear. However, due to the method used for larval infestation and measuring injury and damage in 2011, the yield loss linear regression considered the actual larval injury and resultant yield loss. In this way, all problems associated with estimation of the number of injured kernels in the ear tip were eliminated.

The results of EIL calculation (Table 4.6), considering the actual injury of one larva/ear, express the damage of western bean cutworm in field corn, and characterize western bean cutworm impact on corn production. The ET calculation incorporated the average number of the eggs/egg mass and larval survival (Table 4.7). The average of 85 eggs of western bean cutworm/egg mass estimated in this study was higher than 64 eggs previously reported (Blickenstaff 1979); however, the average of eggs estimated in this study was based on 714 egg masses which include egg mass sampling from three ecoregions across Nebraska and eastern Colorado, over the four years. These egg masses were from confined moths inside pollination bags and large cages, but predominantly from free moths.

The incorporation of larval survival has a practical application of EIL for management decisions of western bean cutworm, and represents a refinement of the ET (Higley and Peterson 2009). First, it allows savings in management, since it accounts for the actual population of the injurious life stage of the species, and the effect of mortality factors (Ostlie and Pedigo 1987). Secondly, using the percentage of plants infested with at least one egg mass allows for the timely implementation tactics of management (Bode and Calvin 1990; Appel et al.1993; Wright 2007; Michel et al. 2010; Paula-Moraes et al. 2011).

More local information about larval survival and late instar behavior will make possible adjustments for ET of western bean cutworm (Michel et al 2010) across the Corn Belt. The characterization of larval survival and behavior will also be important when considering Bt corn hybrids expressing Bt toxins for western bean cutworm, but do not have complete control and may require additional management, as observed for cotton bollworm (Naranjo et al. 2008).

References Cited

Antonelli, A. L. 1974. Resistance of *Phaseolus vulgaris* cultivars to western bean cutworm, *Loxagrotis albicosta* (Smith), with notes on the bionomics and culture of the cutworm. Ph.D. dissertation, University of Idaho, Moscow.

Appel, L. L.. 1991. Damage in field corn (*Zea mays* (L.)) caused by *Loxagrotis albiscosta* (Smith). Master Science thesis, University of Nebraska, Lincoln.

Appel, L. L., R. J. Wright, and J. B. Campbell. 1993. Economic injury levels for western bean cutworm, *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae), larvae in field corn. J. Kans. Entomol. Soc. 66: 434-438.

Barrigossi, J.A.F., G.L. Hein, and L. G. Higley. 2003. Economic injury levels and sequential sampling plans for Mexican bean beetle (Coleoptera: Coccinellidae) on dry beans. J. Econ. Entomol. 96: 1160-1167.

Blickenstaff, C. C. 1979. History and biology of the western bean cutworm in southern Idaho, 1942-1977. Univ. Idaho Agric. Exp. Stn. Bull. No. 592.,

Blickenstaff, C. C., P.M. Jolley. 1982. Host plants of western bean cutworm. Environ. Entomol., 11:421-425.

Bode, W.M. and D.D. Calvin. 1990. Yield-loss relationship and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn). J. Econ. Entomol. 83:1595–1603.

Catangui, M. A. and R. K. Berg. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry 1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environ. Entomol. 35: 1439-1452.

Chapman, S. S., J.M. Omernik, J. A. Freeouf, D. G. Huggins, J. R. McCauley, C. C. Freeman, G. Steinauer, R. T. Angelo, and R. L. Schlepp. 2001. Ecoregions of Nebraska and Kansas (color poster with map, descriptive text, summary tables and photographs): Reston, Virginia, U.S Geological Survey (map scale 1:1,950,000).

Cullen, E., and J. Jyuotika. 2008. Western bean cutworm: a pest of sweet corn. A3856, University of Wisconsin Extension, Cooperative Extension. On-line: http://learningstore.uwex.edu/pdf/A3856.pdf.

DiFonzo, C. D., E. Cullen. 2010. Handy Bt trait table. University of Wisconsin Extension, Field Crops Entomology MSU Program. On-line: http://www.entomology.wisc.edu/cullenlab/publications/pdf.

Dorhout, D. L. 2007. Ecological and behavioral studies of the western bean cutworm (Lepidoptera: Noctuidae) in corn. Masters thesis. Iowa State University, Ames.

Dorhout, D. L. and M. E. Rice. 2004. First report of western bean cutworm, *Richia albicosta* (Noctuidae) in Illinois and Missouri. Crop Mgmt. On-line: http://www.plantmanagementnetwork.org/pub/cm/brief/2004/cutworm. DOI:10.1094/CM-2004-1229-01-BR.

Douglass, J. R., J. W. Ingram, K. E. Gibson, and W. E. Peay. 1957. The western bean cutworm as a pest of corn in Idaho. J. Econ. Entomol. 50: 543-5.

Eichenseer, H., R. Strohbehn, and J. Burks. 2008. Frequency and severity of western bean cutworm (Lepidoptera: Noctuidae) ear damage in transgenic corn hybrids expressing different *Bacillus thuringiensis* cry toxins. J. Econ. Entomol. 101: 555–563.

Hagen, A. F. 1976. A fourteen-year summary of light trap catches of the western bean cutworm in Nebraska *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae). J. Kansas Entomol. Soc. 49: 537-540.

Hagen, A. F. 1962. The biology and control of the western bean cutworm in dent corn in Nebraska. J. Econ. Entomol. 55: 628-631.

Higley, L.G. and R. K. D. Peterson. 2009. Economic decision rules for IPM. In: E.B. Radcliffe et al. (eds.), Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press, Cambridge, UK.

Holtzer, **T. O. 1983**. Distribution of western bean cutworm eggs among short-, mid, and long-season corn hybrids planted on different dates. Environ. Entomol. 12: 1375–1379.

Klein, R. N. and R. K. Wilson. 2012. Nebraska crop budgets 2011. Extension Circular 972, University of Nebraska Extension, http://www.ianrpubs.unl.edu/epublic/live/ec872/build/ec872.pdf

Lee, D. A. 1988. Factors affecting mortality of the European corn borer, Ostrinia nubilalis (Hübner), in Alberta. Can. Entomol. 120: 841-853.

Lewis, L.C., D. J. Bruck, J. R. Prasifka, and E. S. Raun. 2009. *Nosema pyrausta*: its biology, history, and potential role in a landscape of transgenic insecticidal crops. Biological Control, 48: 223-231.

MacArthur, R. H. and E. O. Wilson. 1967. The theory of island biogeography. Princeton Univ. Press, Princeton. 203 pp.

Mani, M.S. 1968. Ecology and biogeography of high altitude insects. New York: Springer. 456p.

Michel, A. P., C. H. Krupke, T. S. Baute, and C. D. DiFonzo. 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. J. Integ. Pest Mgmt. 1:A1-A10. DOI: 10.1603/IPM1003.

Naranjo, S. E., J. R. Ruberson, H. C. Sharma, L. Wilson, and K. Wu 2008. The present and future role of insect-resistant genetically modified cotton in IPM, pp.159-194. In J. Romeis, A. M. Shelton and G. G. Kennedy (eds), Integration of insect-resistant genetically modified crops within IPM programs. Springer, Netherlands.

O'Rourke, P. K. and W. D. Hutchison. 2000. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. J. Agric. & Urban Entomol. 17: 213-217.

Ostlie, K. R. and L. P. Pedigo. 1987. Incorporating pest survivorship into economic thresholds. Bull. Entomol. Soc. Am 1987. 33:98–102.

Paula-Moraes, S.V., E. C. Burkness, T. E. Hunt, R. J. Wright, G. L. Hein, and W. D. Hutchison. 2011. Cost-effective binomial sequential sampling of western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), egg masses in corn. J. Econ. Entomol., 104: 1900-1908.

Peairs, F. B., J. B. Rudolph, and T. L. Randolph. 2011. 2010 Colorado field crop insect management research and demonstration trials. Colorado State Univ. Agric. Exp. Sta. Tech. Rep. TR11-03, 31pp.

Peairs, F. B. 2006. Western bean cutworm: characteristics and management in corn and dry beans. Colorado State University Extesion. 3/02.n. 5.5538. On-line: http://www.ext.colostate.edu/pubs/insect/05538.html

Pedigo, L. P. and M. E. Rice. 2009. Entomology and pest management. New Jersey: Prentice Hall.

Pedigo, L. P., S. H. Hutchins, and L. G. Higley. 1986. Economic injury levels in theory and practice. Annu. Rev. Entomol. 31:341–368.

Rice, M. E. 2007. Economic thresholds for western bean cutworms. Iowa State University Extension, Ames, IA.On-line: (http://www.ipm.iastate.edu/ipm/icm/2007/7-2/wbcth.html).

Rice, M. E. 2006. Eastern movement of the western bean cutworm into Indiana and Ohio. Proceedings: Indiana CCA Conference, Indianapolis, IN.

Ritchie, S. E., J. J. Hanway, and G. O. Benson. 1993. How a corn plant develops. Iowa Coop. Ext. Serv. Spec. Rep. 48.

SAS Institute. 2009. SAS user's guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Schoonkoven, L. M, J. J. A. Van Loon, and M. Dicke-Smith. 2005. Insect-plant biology. Oxford: University of Oxford. 412p.

Seymour, R. C., G. L. Hein, R. J. Wright, and J. B. Campbell. 2004. Western bean cutworm in corn and dry beans. Neb. Coop. Ext. NebGuide G1359. Lincoln, NE.

Showers, W. B., M. B. de Rozari, G. L. Reed, and R. H. Shaw. 1978. Temperature-related climatic effects on survivorship of the European corn borer. Environm. Entomol. 7: 717-723.

Smith, R. F. 1954. The importance of the microenvironmentin insect ecology. J. Econ. Entomol. 47, 205-10.

Stern, M., **R.F. Smith, R. van den Bosch, and K.S. Hagen. 1959.** The integratedcontrol concept. Hilgardia, 29: 330-350.

Tooker, J. F and S. J. Fleischer. 2010. First report of western bean cutworm. (*Striacosta albicosta*) in Pennsylvania. Crop Mgmt. DOI: 10.1094/CM-2010-0616-01-RS.

Wright, R. J. 2007. Second generation European corn borer scouting and treatments decisions. University of Nebraska Ext. Neb Guide G1783. Lincoln, NE.

Zalucki, M. P., A. R. Clarke, and S. B. Malcom. 2002. Ecology and behavior of first instar larval lepidoptera. Ann. Rev. Entomol. 47:361-393.

Table 4.1. Field experimental design of larval survival, consumption and yield impact at three locations of Nebraska**.**

Location	Egg mass infestation levels (%)	Infestation methodology	Corn stage at infestation	Plant position of egg mass	Larval survival evaluation (plants/plot) [a]	Ear injury and yield evaluation (plants/plot)
			2008			
Clay Center	0, 1, 3, 5, 10	Egg mass sandwich	Blister	Above primary ear	30	70
Concord	0, 1, 3, 5, 10	Egg mass sandwich	Silk	Above primary ear	30	70
Scottsbluff	0, 1, 3, 5, 10	Large cages, egg mass sandwich	V18	Natural deposition and top of the plant	30	70
			2009			
Clay Center	0, 1, 3, 5, 7, 10	Egg mass sandwich	Silk	Above primary ear	30	70
Concord	0, 1, 3, 5, 7, 10	Egg mass sandwich	Blister	Above primary ear	30	70
Scottsbluff	0, 1, 3, 5, 10	Large cages, egg mass sandwich	V18	Natural deposition and top of the plant	30	70
			2010			
Clay Center	0, 1,3, 5, 7, 10	Pollination bag, egg mass sandwich	V18	Top of the plant	30	50
Concord	0, 1,3, 5, 7, 10	Pollination bag, egg mass sandwich	V18	Top of the plant	30	100
Scottsbluff	0, 1, 3, 5, 10	large cages, egg mass sandwich	V18	Natural deposition and top of the plant	6	15

[a] Larval survival evaluation approximately 20 days after egg infestation on plants randomly selected per plot.

Table 4.2. Egg survival of western bean cutworm in laboratory and corn field, Nebraska, NE.

Year	Total of eggs [a]	Egg hatch mean (%) [b]
	Laboratory	
2009	1176 (34 egg masses)	77.19 (SD ± 22.24)
	Concord, NE	
2008	3927 (76 egg masses)	86.58 (SD ± 14.46)
	Clay Center, NE	
2008	4037 (73 egg masses)	77.69 (SD ± 25.50)
2011	1828 (24 egg masses)	76.86 (SD ± 24.46)

[a] Artificial infestation of egg mass sandwich method on field corn, Clay Center and Concord, 2008. Artificial infestation of western bean cutworm females inside pollination bags on field corn, at three corn stages, Clay Center 2011.

[b] Laboratory egg hatch mean is an overall estimation for 16°C, 25°C and 30°C. At Clay Center (2011) overall egg hatch mean in the corn field at three corn stages (V18;Vt; and silking).

Table 4.3. Western bean cutworm larval survival on corn field at three locations in Nebraska.

Year	Larval survival [a,(b)] %	Number of evaluated larvae
Clay Center, NE		
2008	2.91(2.43)	42
2009	4.34	110
2010	4.28	282
Concord, NE		
2008	4.23(4.83)	71
2009	1.51	42
2010	5.40	240
Scottsbluff, NE		
2008	12.82	480
2009	4.47	152.
2010	0.33	.

[a] Larval survival percentage based on total number of eggs infested.

[b] Larval survival percentage based on total number of eggs hatched (Clay Center and Concord, 2008 only).

[c] Larval sampling around 20 days after infestation. Mode, minimum, and maximum values of larval head capsule (millimeters).

Table 4.4. Linear regression equations for the relationship between corn ear injury area (square cm) and number of western bean larvae at three locations in Nebraska.

Year	Regression equation $\hat{y}$ = corn ear injury area x = n. of larvae/ear	SE Slope	 Intercept	P value	N [a]
		Clay Center, NE			
2008	$\hat{y} = 0.89 + 3.67x$	2.79	0.27	0.2055	1362
2009	$\hat{y} = 1.24 + 8.28x$	0.38	1.83	0.0002	1642
2010	$\hat{y} = 0.68 + 5.22x$	1.43	0.37	0.0015	1093
		Concord, NE			
2008	$\hat{y} = - 0.04 + 9.93x$	2.40	0.37	0.0006	1401
2009	$\hat{y} = 0.35 + 4.13x$	1.53	0.17	0.0130	1604
2010	$\hat{y} = 2.74 + 8.60x$	2.46	0.70	0.0025	1769
		Scottsbluff, NE [b]			
2008	$\hat{y} = 0.61 + 2.15x$	0.17	0.20	<0.0001	1330
2009	$\hat{y} = 0.17 + 1.72x$	0.08	0.24	<0.0001	1406

[a] Number of evaluated corn ears (injury area measuring in square cm).

[b] Linear regression not tested in 2010, at Scottsbluff , due problems in experimental area, and limited number of replications.

Table 4.5. Linear regression equations for the relationship between corn yield (grams per ear) and number of the western bean cutworm larvae by plant growth stage in two locations of Nebraska.

Plant Stage	Regression equation $\hat{y}$ = corn ear weight x = number of larvae [a]	Standard error Slope	 Intercept	P value
Clay Center, NE				
Blister	$\hat{y} = 244.29 - 9.21x$	2.18	4.93	0.0003
Milk	$\hat{y} = 226.69 - 12.76x$	2.90	5.23	0.0004
Concord, NE				
Blister	$\hat{y} = 217.16 - 9.64x$	1.43	2.44	<0.0001
Milk	$\hat{y} = 191.55 - 3.54x$	1.88	2.95	0.0754

[a] Weight of corn ear in grams Regression based on levels of western bean cutworm larval infestation: 0,1,2 and 3.

Table 4.6. Economic injury levels values for western bean cutworm larval population per corn ear, with variable cost of management and crop value.

Crop value [a]			Management cost, $/acre [a]						
			4	6	8	10	12	14	16
			Management cost, $/ha						
$/bu	$/acre	$/ha	10	15	20	25	30	35	40
3	660	1,630	0.50	0.75	1.00	1.25	1.50	1.75	2.00
4	880	2,174	0.37	0.56	0.75	0.94	1.12	1.31	1.50
5	1,100	2,717	0.30	0.45	0.60	0.75	0.90	1.05	1.20
6	1,320	3,260	0.25	0.37	0.50	0.62	0.75	0.87	1.00
7	1,540	3,804	0.21	0.32	0.43	0.54	0.64	0.75	0.86

[a] Conversions considering yield of 220 bushel/acre, 30,000 plants/acre, 74,100 plants/ha, and range of corrected real market price of corn in December of 2011.

Table 4.7. Economic threshold values for percentage of infested plants with at least one egg mass of western bean cutworm, considering mature larval survival.

Crop value [a]			Management cost, $/acre [a]						
			4	6	8	10	12	14	16
			Management cost, $/ha						
$/bu	$/acre	$/ha	10	15	20	25	30	35	40
			2% larval survival						
3	660	1,630	29	44	59	74	88	100	100
4	880	2,174	22	33	44	55	66	77	88
5	1,100	2,717	18	26	35	44	53	62	71
6	1,320	3,260	15	22	29	37	44	51	59
7	1,540	3,804	13	19	25	32	38	44	50
			4% larval survival						
3	660	1,630	15	22	29	37	44	51	59
4	880	2,174	11	17	22	28	33	39	44
5	1,100	2,717	9	13	18	22	26	31	35
6	1,320	3,260	7	11	15	18	22	26	29
7	1,540	3,804	6	9	13	16	19	22	25
			8% larval survival						
3	660	1,630	7	11	15	18	22	26	29
4	880	2,174	6	8	11	14	17	19	22
5	1,100	2,717	4	7	9	11	13	15	18
6	1,320	3,260	4	6	7	9	11	13	15
7	1,540	3,804	3	5	6	8	9	11	13
			12% larval survival						
3	660	1,630	5	7	10	12	15	17	20
4	880	2,174	4	6	7	9	11	13	15
5	1,100	2,717	3	4	6	7	9	10	12
6	1,320	3,260	2	4	5	6	7	9	10
7	1,540	3,804	2	3	4	5	6	7	8
			20% larval survival						
3	660	1,630	3	4	6	7	9	10	12
4	880	2,174	2	3	4	6	7	8	9
5	1,100	2,717	2	3	4	4	5	6	7
6	1,320	3,260	1	2	3	4	4	5	6
7	1,540	3,804	1	2	3	3	4	4	5

[a] Crop value calculated considering yield of 13629.26 kg/ha (220 bushel/acre), 30,000 plants/acre, 74,100 plants/ha, and range of corrected real market price of corn in December of 2011. Mean of 85 western bean cutworm eggs/egg mass.

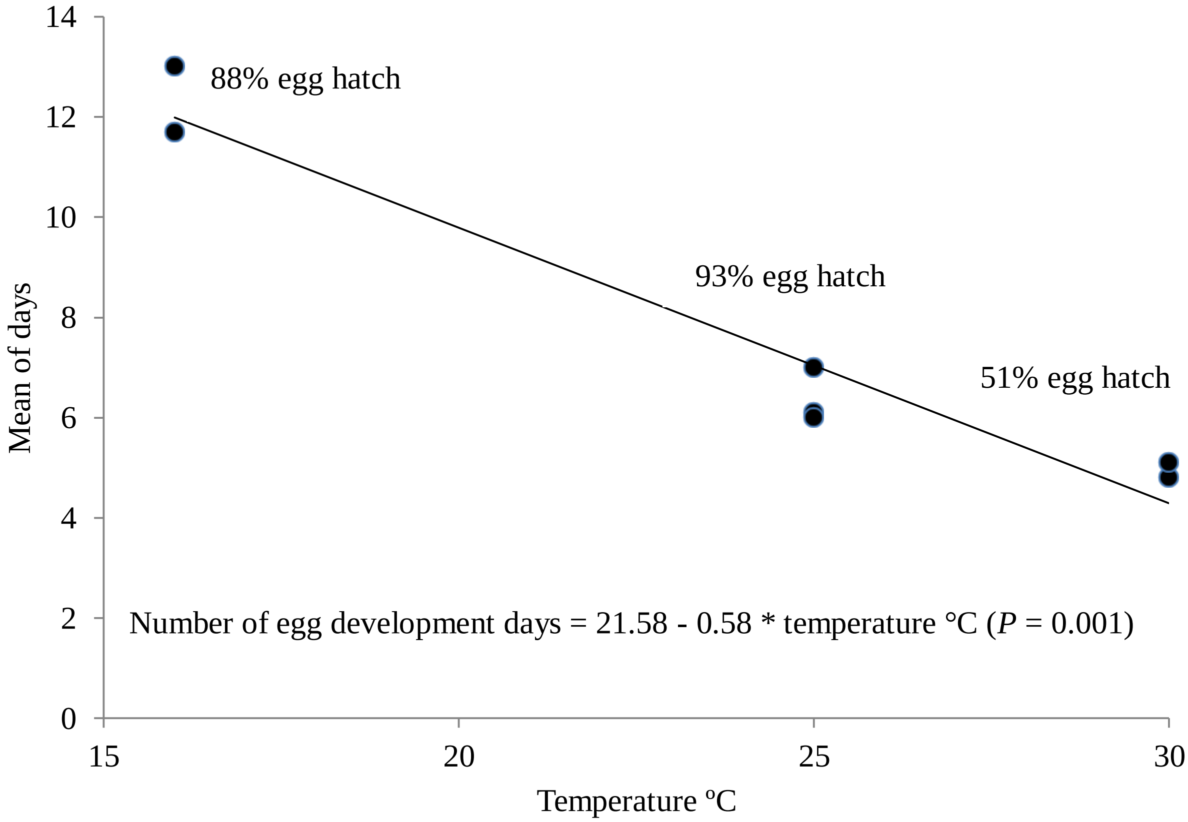

Fig. 4.1. Western bean cutworm egg development time and egg hatch mean at different temperatures.

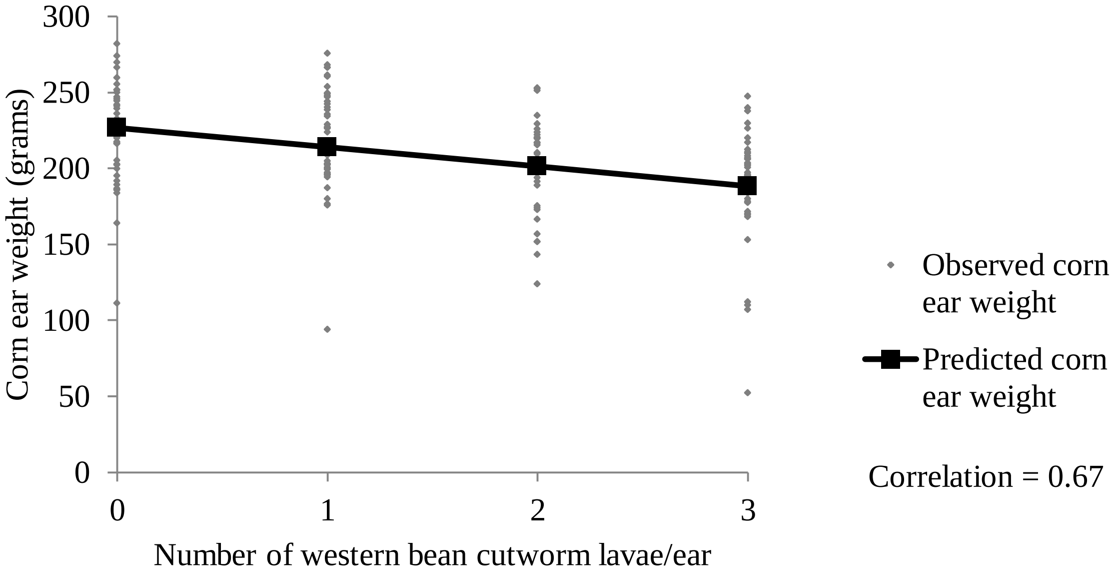

Fig. 4.2. Relationship between western bean cutworm larvae and corn ear weight (grams). Observed values from corn at Clay Center, infested at milk stage, 2011. Predicted values from linear regression: $\hat{y} = 226.69 - 12.76x$ (P = 0.0004) (*see also Table 4.5*).

Appendices

Appendix A: SAS protocol - on-plant larval movement analysis

```
Data Disperion2010Firstdate;*real data considering first date;
input date plot $ cornstage $ egghatch location $ totallarvaeplant
totallarvaelocation;
  datalines;


ods html;
ods graphics on;
Proc glimmix plot=residualpanel;
class date plot cornstage egghatch location;
model totallarvaelocation=cornstage|location/ddfm=kr;*testing effect of
zone in the plant and cornstage;
random plot /group=location;*group means separation of the variance in
each zone;
lsmeans location/pdiff=control('tasselZo') cl adjust=dunnett;
run;
ods graphics off;
ods html close;
run;
```

Appendix B: SAS protocol - linear regression: corn grain weight and number of western bean cutworm larvae.

```
Data ClayCenterMilk2011;
input location $ year plot earnumber numberoflarvae mold $ tipinjury $
sizeinjury $ grainsize $ allsize injuryarea WT moisture correctedWT;
datalines;

*regression and correlation;
proc glimmix nobound; *nobound asks SAS to ignore negative variance;
class plot numberoflarvae;
model correctedWT=numberoflarvae/ solution;
random plot*numberoflarvae;*telling SAS to consider that the
experimental unit are two infested plants with same number of larvae;
title CC2011 Milk earWT vs larvaeear;
nloptions maxiter=10000;*number or iterations run to converge data;
output out=ClayCenterMilk2011 pred(ilink)=p ;
run;
proc print data=ClayCenterMilk2011; run; *determines success of
predicted values;
proc corr data=ClayCenterMilk2011;
 var correctedWT p;*p = predictor of correctedWT;
run;
```