**Ecological and behavioral studies of the western bean cutworm
(Lepidoptera: Noctuidae) in corn**

by

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TABLE OF CONTENTS

CHAPTER 1. General Introduction

Thesis Organization

This thesis is organized in the following manner: a general introduction including the objectives and a general literature review, a list of references cited in the general literature review, five papers written for submission to scientific journals, a general conclusion, and acknowledgments. The general literature review in chapter one contains background information on the biology and ecology of the western bean cutworm *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) in corn (*Zea mays* L.) and dry beans (*Phaseolus* spp.).

Objectives

1. To examine the effect that sex, age, mating, and Microsporidia infection level has on the flight characteristics of the western bean cutworm.

2. To evaluate two pheromone trap types at three heights within two field environments and examine the effects on adult western bean cutworm captures.

3. To evaluate the effectiveness of Herculex® and YieldGard® corn borer hybrids for controlling western bean cutworm larvae.

4. To evaluate the possible effects that genetically engineered corn (YieldGard corn borer) on the survivability of western bean cutworms during intraguild competition from European corn borer and corn earworm larvae.

5. To examine the distribution of an unidentified Microsporidia in western bean cutworms across the Corn Belt.

Literature Review

Synonymy

The western bean cutworm *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) was originally described by John B. Smith in 1887 (Smith 1887). He placed it in the genus *Agrotis* and briefly mentioned it within a larger volume describing Noctuidae of North America. In 1928 it was included in a new genus, *Loxagrotis,* created by McDunnough (1928) although he admits that *L. albicosta* (Smith) was quite atypical relative to the other members of the genus. However, he states that it was due to his lack of knowledge and material that he did not feel justified in further splitting the genus further. In 1989, Poole (1989) placed the entire genus *Loxagrotis* into the preexisting genus, *Richia*. In 2004 the western bean cutworm was moved into the new genus *Striacosta* created by Lafontaine (2004) because of "the many distinctive structural features of *albicosta*... [that] are not consistent with those species in... *Richia*."

Distribution

The western bean cutworm was first recorded in Arizona in the late 1880's (Smith 1887) and then in 1889 near Mexico City, Mexico and given the name *Agrotis manethusa* (Druce) (Lafontaine 2004). On August 13, 1896 it was collected and recorded in Colorado for the first time by an unknown person near Denver (Hoerner 1948, Hantsbarger 1969). Walkden and Whelan (1942) collected seven adults in 1935 and another one in 1936 from light traps operated in Scottsbluff, Nebraska. By 1942, Idaho was included in the western bean cutworm's distribution (Douglass et al.1955) and by 1956, it was also reported in Texas, Utah, Kansas, Iowa, and Alberta, Canada (Crumb

1956). In 1969, the western bean cutworm was reported throughout Nebraska (Keith et al. 1970) however, it did not become a serious pest in Iowa as it had in Nebraska and its occurrence was only sporadically documented in Iowa. Approximately 30 years later in 1999, it was reported for the first time in Minnesota (O'Rourke and Hutchison 2000). Two years later, larvae or their damage could be found in most western Iowa counties (Rice 2001, 2002a-d) and adult moths were being collected in blacklight traps in central Iowa (Pope and Rice, 2003, Rice 2003). In 2004, sex pheromone traps for males were placed throughout eastern Iowa including counties bordering the Mississippi River. Adult moths were collected in every trap placed in eastern Iowa and the insect was assumed to occur in every Iowa County. That year, traps were also placed for a single night in several northeast Missouri and northwest Illinois counties. Adults were trapped and reported there for the first time in Missouri (Harrison and Putnam counties) and Illinois (Warren County) (Dorhout and Rice 2004). In recent years this species has continued to move eastward and in 2006 adults were trapped for the first time in Indiana, Michigan, and Ohio (Rice and Dorhout 2006, Pope 2007).

Pest Status

Despite being native to North America, the first confirmed economic damage attributed to the western bean cutworm was in 1915 when a seed dealer in Weld County, Colorado reported that he refused pinto beans from a small area southeast of Greeley because of injury (McCampbell 1941). Western bean cutworms were confirmed as a pest of dry beans in 1942 south-central Idaho (Blackfoot and Rupert areas) by the Twin Falls Entomology Research Branch Field Station (Douglass et al. 1955). In 1950, the insect

was causing significant damage to Nebraska dry beans in the North Platte Valley (Hagen 1976).

Reports of western bean cutworms as a significant pest of corn did not appear until approximately 40 years after they were known as a pest of dry beans. The first known report of western bean cutworms feeding on corn came on August 24, 1954 when Green Giant Company entomologist R. E. Miller brought to the Twin Falls, Idaho field station some "worms" he found in canning corn grown by Leslie Goble near Tuttle, Idaho (Douglass et al. 1957). In 1960, approximately 2,428 hectares (6,000 acres) of irrigated corn in Dundy and Chase counties, Nebraska were heavily infested (Blickenstaff 1979). It is suspected that the western bean cutworm was not causing economic damage in Nebraska prior to 1935 because Whelan (1935) did not include it in his list of serious corn pests, although he and Walkden did collect them during 1935-1936 in Scottsbluff, Nebraska (Walkden and Whelan 1942). In 1966, western bean cutworms were discovered injuring corn in Weld County, Colorado (Hanstbarger 1969). O'Rourke and Hutchison (2000) found a low level of infestation at four locations in Minnesota; but none of these infestations reached a level of economic damage in dent corn at the current nominal threshold (Apple et al. 1993). However, late-instar infestations approached the economic injury level for fresh-market and processed sweet corn. The next year in 2000, western bean cutworms were reported by Tony Weis, an Iowa State University extension specialist, as causing significant damage to corn in Holstein in Ida County, Iowa (Rice 2000). Field assessments indicated that approximately 95% of the ears were injured with as many as six larvae in an ear. This was the first report of western bean cutworms causing economic yield loss to field corn east of the Missouri River.

Description

Western bean cutworm eggs are about 0.76 mm in diameter (0.03 inch), dome

shaped and distinctly ribbed (Hoerner 1948) like a pumpkin. The eggs are pearly white

in color when first laid but darken and become bluish-black (Douglass et al. 1955) or

purple just before hatching (Hagen 1973).

Antonelli (1974) described the six larval instars, pupa, head capsule and body

measurements in detail, and gave a brief description of the adult. Oseto and Helms

(1976) described in great detail the internal and external anatomy of the adult.

Newly-hatched larvae emerging from their eggs are on average 2.49 mm long and

0.41 mm wide (0.10 by 0.02 inches) (Antonelli 1974). As the larvae mature through their

six primary instars (Antonelli 1974), their color transforms from a dark to a pinkish

brown (Hagen 1979). The sixth, and normally final, larval stage reaches an average

length of 34.5 mm and is 5.37 mm wide (1.36 by 0.21 inches) (Antonelli 1974). Older

larvae develop two short, dark stripes that run lengthwise dorsally on the prothoracic

shield capsule (Hagen 1979). These lines distinguish it from the corn earworm,

Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) which lacks these two stripes. Pupae

average 17 by 5.73 mm (0.67 by 0.23 inch) and they are orange-brown in color and

glabrous (Antonelli 1974).

The adult moth has an average wingspan of 40.3 mm and is 18.1 mm long (1.59

by 0.71 inches). There is a white costal margin on the forewing (Hoerner 1948, Hagen

1973), and generally, the forewings are colored a rich brown (Hagen 1979) with alternate

light and dark markings (Douglass et al. 1955). However Antonelli (1974) found a few

adults that exhibit a light color phase. On each of the forewings there is a white spot that is a complete circle midway along the costal margin and there is also a white outlined crescent moon-shaped spot that are both touching the costal margin and is unique to this species. The hind wings of both light and dark phases are mostly a light tan, devoid of any distinguishing characteristics, shaded to a dark rich brown along the outer margin (Douglass et al. 1955).

Biology

The western bean cutworm life cycle is univoltine, consisting of five stages: egg, larva (with typically six, or sometimes seven instars), prepupa, pupa, and adult (Antonelli 1974, Blickenstaff 1979). It is photo sensitive with the average life cycle being approximately 95 days under either continuous light or darkness (Antonelli 1974) or 150 days when reared under a long-day photoperiod (16:8 L:D). However, when reared under a short-day photoperiod (8:16 L:D) they remain in the prepupa stage for at least 220 days. Douglass et al. (1957) found that the pre-oviposition period ranged from one to five days, averaging three days. Likewise, Blickenstaff (1979) found four days to be the average pre-oviposition period. Females lay an average of 407 eggs during their lifetime (Blickenstaff 1979). Egg masses range from 21-195 and average 52 eggs (Hagen 1962). The oviposition period ranges from 2-13 days and averages 8 days (Douglass et al. 1957). Blickenstaff (1979) found a 79% natality rate with an incubation period averaging six days. Each of the first five instars takes about 5-6 days to develop, with the sixth and seventh instar averaging 7 and 10 days, respectively.

The recorded larval mortality rates for each of the instars is 20% (1st instar), 12% (2nd instar), 6% (3rd instar), 3% (4th instar), 2% (both 5th and 6th instars), and 40% (7th instar). Blickenstaff (1979) was unable to reconcile the large mortality during the last instar relative to the penultimate instar, however, both Blickenstaff and Antonelli (1974) found that only a few larvae ever reached the seventh stage; most larvae ceased feeding and entered the soil during the sixth instar. Upon entering the soil, the larva constructs a pupal cell typically at a depth of 7.6-15.2 cm (3-6 inches) (Blickenstaff 1979) to 20.3 cm (8 inches) (Douglass et al. 1957), where it overwinters as a prepupa. Overwintering mortality of the prepupa ranged from 72% in the laboratory to 90% under field conditions (Blickenstaff 1979).

Fields with a sandy soil tend to have greater populations of western bean cutworm, particularly sandy fields with overhead irrigation, than clay or loam fields (Hoerner 1948, Hanstbarger 1969, Douglass et al. 1955). Hoerner (1948) compared the emergence of adults in sandy and clay soils and found that very few adults emerged from the clay soil while the adults emerged easily from the sandy soil and mentioned that rain or irrigation appeared to facilitate this emergence.

In Nebraska, larvae pupate in late May (Hagen 1962) while pupae were not found until June 26 in Colorado (Hoerner 1948). During the first 5-6 days, most of the adults that emerge are females (Blickenstaff 1979). In laboratory conditions there was a 1:1 male to female ratio, but in field studies conducted using a standard light trap there was a 2.8:1 male to female ratio. However, in both field and laboratory studies Blickenstaff (1979) found that females predominated until 4-9 days before peak flight, after which males started to dominate trap captures.

The female sex pheromone blend contains a mixture of (Z)-5-dodecenyl acetate, (Z)-7-dodecenyl acetate, 11-dodencenyl acetate, and dodecyl acetate in 5:1:5:5 ratio (Klun et al. 1983). During a field test, the authors discovered that only a mixture of the three olefinic acetates is required for maximal capture of western bean cutworm males. Klun et al. (1983) speculated that dodecyl acetate might be involved in modulating male behavior such as short range stimulus, landing stimulus, extending length of time spent at the stimulus, or sustaining upwind flight.

Biology on Corn and Dry Beans

Eggs are typically laid on the upper surface of the topmost corn leaf near the ligule and on the bottom side in dry beans where the canopy is the thickest (Douglass et al. 1955, Blickenstaff 1979). Holtzer (1983) found that corn just beginning to tassel received more eggs than plants that were either in an earlier or later growth stage. Once larvae hatched, they remained near the egg mass for about 10 hours while feeding on the egg chorions (Seymour et al. 1998).

The distance that larvae moved from plant to plant appears to be related to the host plant and in particular to the density of its canopy (Blickenstaff 1983). In corn, Douglass et al. (1957) found that larvae may disperse 3 m (10 ft) from the egg mass. Hagen (1962) found a similar dispersal range, 1.8-3 m (6-10 ft), but also noted that there was no correlation between the number of eggs in the mass and the size of infested area. Measuring dispersion in dry beans, Blickenstaff (1983) affirmed Hagen's (1962) observation that the larvae migrate more within rows than across rows. Interestingly, Blickenstaff (1983) also found that larvae dispersal was almost twice the distance, 3.05-

3.66 m (10-12 ft) in the release row and 2.45-3.05 m (8-10 ft) across the rows when compared to Douglass (1957) and Hagen's (1962) corn dispersal ranges. He further observed that at higher egg densities, dispersal across rows may be increased up to 3.66 m, (12 ft) but that 72% of the damage occurred within an area 1.83 m (6 ft, three rows wide) by 3.66 m (12 ft) long. Blickenstaff (1983) suggested that the differences in dispersal ranges within the two host plants might be related to the more closed canopy of dry beans as compared to corn. He notes that canopy density and larval migration distances might explain why western bean cutworm damage is sporadic in fields with a low level of infestation, emphasizing the importance of scouting numerous, small, widely scattered areas as opposed to a few large, adjoining areas.

Movement on a host plant is determined by the plant and larval growth stage (Douglass et al. 1957). Larvae that emerge from eggs laid on corn and dry beans first feed on their egg chorions before moving elsewhere on the plant. It is during this time in the life cycle that pathogens present in the mother might be passed on to the eggs (Su 1976). Larvae that hatch on pretassel corn proceed to the whorl where they feed on the developing flag leaf, tassel flowers, and other yellow tissue (Seymour et al. 1998). The feeding may produce "shot holes" that can be mistaken for European corn borer damage. Once tasseling begins the larvae move to the developing silks of the ear. If the corn has already tasseled when the larvae hatch they proceed directly to the ear and feed on the developing silks. The larvae continue to feed on green silks until they reach the fourth instar when they begin feeding on the developing ear tip. If at this time the ear tip is crowded, the larvae may move down the ear, chew a hole through the husk and begin feeding on the developing kernels. The larvae are not considered to be cannibalistic.

On dry beans, the larvae consume the egg chorions and move to the top of the plants to feed on the young leaves and buds (Hoerner 1948, Douglass et al. 1955). Once they are about 12.7 mm long (0.5 inche) they begin eating holes into the bean pods and seeds. Late-stage larvae typically feed during the night and on cloudy days. During the day, most larvae cease feeding and burrow about 25 mm (1 inch) into the soil at the base of the plant when they are not feeding. If the beans are harvested before the larvae have completed development they congregate under the windrows and feed on the green and immature seeds (Douglass et al. 1955). Western bean cutworms starve if only mature, dried, dry beans are available. Once they have finished feeding the mature larvae burrow into the soil and overwinter.

Other than corn and dry beans, only tomato has been confirmed as a crop host capable of supporting the western bean cutworm's entire life cycle (Blickenstaff 1979). Hoerner (1948) stated that immature larvae would feed on ground cherry fruits if dry beans were harvested before the larvae had fully developed. Douglass et al. (1955) mentioned that "before changing its habits to attack beans, the western bean cutworm fed upon the fruits of ground cherry and nightshade." Hoerner (1948) mentioned that "no native host plant has been found." Blickenstaff et al. (1975) repeated that western bean cutworms fed and completed their life cycle on ground cherry and nightshade but, when he tested hosts he found that western bean cutworms were only able to complete their life cycle on corn, dry beans, and tomatoes, although he reported that tomatoes were a poor host (Blickenstaff 1979). Soybeans also were found to be unsuitable hosts for western bean cutworms, even when presented with glabrous varieties (Blickenstaff and Jolley 1982). The idea that western bean cutworms changed their habits to attack dry beans also

was disputed by Blickenstaff (1979) and again by Blickenstaff and Jolley (1982) where they drew on other published material that showed how corn and dry beans had been planted together in an intercropping system for hundreds of years. This provides a potential explanation for why western bean cutworms would have two widely divergent species as primary hosts, (Francis et al. 1976) and suggest that their distribution expansion is related to the introduction of corn and dry beans to an area.

Scouting and Economic Injury Levels in Corn and Dry Beans

Because western bean cutworm larvae are inaccessible to foliar-applied insecticides during a large portion of their development, correct timing of an application is crucial for successful management in corn or dry beans (Blickenstaff 1979). Degree days, light traps, and pheromone traps can be useful tools in understanding when to scout for this insect.

Adult emergence can be determined using degree days starting May 1 with a base developmental temperature of 10°C (50°F). For 25, 50 and 75% emergence, degree days are 1319, 1422, and 1536, respectively (Ahmad 1979, Seymour et al. 1998). These degree days can be used to predict emergence in Iowa (Vagts 2002). Blacklight and pheromone trapping originally were used to determine the presence of populations (Hagen 1976), but Blickenstaff (1979) attempted to relate trap counts with action thresholds. In Idaho, Blickenstaff et al. (1975) found that if 700 moths were cumulatively captured in a single blacklight trap by July 25, treatment should be applied for dry beans. Mahrt et al. (1987) correlated blacklight catches with pheromone catches in an effort to capitalize on light trap archive data to develop a method for alerting dry

bean growers of damaging western bean cutworm populations. They found that while trap counts from either blacklight or pheromone traps were useful as a general warning for growers on an area basis, the traps could not accurately predict the severity of western bean cutworm injury for a specific location or field.

There are two methods for relating the number of eggs and larvae found on a certain number of corn plants to action thresholds. Appel (1991) and Appel et al. (1993) found that 32.7 eggs or 5.0 larvae per plant would justify treatment, assuming control costs of $8 per acre and corn value of $2 per bushel. However, the nominal threshold currently recommended is 8% infested plants for field corn and 5 % for seed and popcorn plants (Hagen 1979, Wright and Seymour 1996, Seymour et al. 1998, Bontrager 2002, Rice 2002, Vagts 2002, Peairs 2002, Butzen and Eichenseer 2003, and Peairs 2004). Infestation levels may be determined by checking 10-20 consecutive plants in 5 locations spread evenly throughout a field. Because field infestations may be spotty, scouting more than 5 locations might be necessary when populations are close to the action threshold which emphasizes the need for small samples throughout the entire field. It is recommended that application be timed such that 90-95% of the tassels have emerged or if they have emerged already then application should be timed when 70-90% of the egg masses have hatched (Seymour et al. 1998).

In dry beans, producers consider 2% damaged beans as an economic level (Blickenstaff 1979, Mahrt 1987). Seymour et al. (1998) suggests that there is a low probability of significant damage if light and pheromone traps capture less than 700 moths per trap cumulatively, but there is a moderate risk of significant damage if the

individual traps capture between 700-1,000 moths cumulatively. Treatment should be applied between 7-18 days after peak moth flight (Blickenstaff 1979).

Management

Management tactics for western bean cutworms can be broken into five categories: chemical, cultural, biological, mechanical, and host plant resistance. Once a field's infestation level is determined then the best management technique may be selected based on seasonal and long-term objectives.

Chemical control has been the most routinely employed method of managing western bean cutworms with the majority of research addressing this technique (Hoerner 1948, Hagen 1962, Hagen 1963, Hanstbarger 1969, Hirnyck 1983). Blickenstaff and Peckenpaugh (1981) summarized the results of insecticide tests in southern Idaho on corn and dry beans from 1950-1980 and made recommendations and application suggestions. They also established that delaying the application past the peak flight reduces its efficacy and that applications do not work once the larvae have entered the ear.

Working with dry beans Hagen (1963), found that a soil application of carbaryl (Sevin WP) provided some control, but it failed to prevent 2% or more of the beans being damaged during the one year that differences between treatments were observed. He also reported that a combination of soil and foliage applications did not produce significantly different results when compared to a foliage only application which provided good control and less than 2% injury. Rice (2004) lists the insecticides that are currently labeled for western bean cutworms in corn and their rates as of 2004.

Attempts to control western bean cutworms through host plant resistance have been attempted with dry beans but have met with poor success partly due to the difficulty of the mass rearing needed for artificial infestations (Antonelli 1974, Antonelli and O'Keeffe 1981). Antonelli and O'Keefe (1981) found significant differences in the percent of seeds injured among the 16 varieties that they tested with 'Michigan Improved Cranberry' having the highest percent of seeds injured (4.06 and 2.63%; 1971 and 1973, respectively) and the non-commercial N203 (R-275) having the lowest percent of seeds injured (0.27 and 0.28%; 1971 and 1973, respectively). However, they did not find any significant differences between the varieties as measured by oviposition preference or pod damage. There is no published information on breeding resistance in corn for the western bean cutworm.

Genetically modified corn may suppress western bean cutworms, but it depends on the trait. YieldGard® Corn Borer (event MON810) (Monsanto Company, St. Louis, MO), expresses the *Bacillus thuringiensis* sp. *kurstaki* (Berliner) endotoxin Cry1Ab but it does not control western bean cutworm larvae (O'Rourke and Hutchison 2000, Catangui and Berg 2006). However Herculex® (event TC1507) expresses the *B. thuringiensis* sp. *aizawai* (Berliner) endotoxin Cry1F, which does suppress the western bean cutworm larvae (Catangui and Berg 2006).

Planting date as a method of managing western bean cutworms has not been seriously considered because of the duration of the moth flights and because there would not be enough time in the growing season left for the plants to properly mature before the typical killing frost in Idaho (Douglass et al. 1957).

Mechanical control has had some success in controlling western bean cutworms. Blickenstaff (1979) reported that almost 100% mortality of western bean cutworms was achieved for overwintering prepupae in a field after it was plowed or disked in the fall. He did not elaborate or mention if that helped reduce western bean cutworm populations in the field the following year.

Biological control may provide some population suppression of western bean cutworm, but little research has been done in this area. Blickenstaff (1979) listed four groups of predatory insects and listed how many and what stage western bean cutworm they ate.

One disease, *Nosema loxagrotidis* sp. N. (Microsporida: Nosematidae), is mentioned in the literature (Su 1976) and is suggested by Seymour et al. (1998) to naturally reduce western bean cutworm populations. However, when Helms and Wedberg (1976) fed *Nosema* infected larvae *Bacillus thuringiensis* sp. *kurstaki,* they reported that the toxin "apparently caused the midgut epithelial cells to 'shed' the Microsporidian spores and vegetative cells into the midgut lumen. Regenerated epithelium exhibited only a slight infection in contrast to the original heavy infections… thus the treatments were beneficial not debilitative."

Rearing

Antonelli (1974) worked on rearing techniques for western bean cutworms from 1970-1973 and had between 0.8-19.6% successful first generation adult emergence. Additionally, his attempts to rear more than two laboratory generations of western bean cutworms failed at a point where the second generation did not produce any viable eggs.

Doyle (1994) worked on rearing western bean cutworms in 1992 and had similar success

with 3.6% of the eggs developing to adults and 0% of the second generation adults laying

viable eggs.

Antonelli (1974) reported two meridic diets on which western bean cutworms

could complete their development, but only individuals that fed on the modified Shorey-

Hale diet were able to produce viable eggs. Doyle (1994) recommended using a

modified black cutworm diet (Results, #F9875, Bio-Serve., Frenchtown, NJ) with no

formalin containing 0% bean pods (Kentucky blue green snap beans, *Phaseolus vulgris*

L.), but the second generation adults did not produce any viable eggs.

The common problems mentioned in all these studies that make rearing western

bean cutworms difficult are low egg viability, high mortality, susceptibility to disease and

mold, and a long development time.

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CHAPTER 2: Flight Characteristics of the Western Bean Cutworm

(Lepidoptera: Noctuidae)

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ABSTRACT The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) is a pest of corn and dry beans in the central United States. It has recently expanded its distribution from western Iowa in 2000 to western Ohio in 2006. There has been no research conducted on the basic flight characteristics of this insect. Understanding their basic flight characteristics may help improve management techniques and understand their dispersal potential. We hypothesis that the sex, age, mating status, and Microsporidia infection level affects the flight characteristics of the western bean cutworm. Adults were raised in laboratory colonies and then tethered and flown in the laboratory on a flight mill for one night. The effects that sex, age (1-2, 3-4, 5-6, 7-8, and 9-10 days post emergence), and mating had on their flight characteristics were then determined. However, there were not enough infected adults to evaluate the effect that a Microsporidia infection had on their flight characteristics. Females flew 18.9±3.2 km, which was significantly further than males (13.2±2.1 km). The average total distance flown by virgin males was relatively low when they were 1-2 days old, then rapidly increased once they were 3-4 days old followed by a gradually decrease as age increased. Virgin females initially followed the same pattern, but their mean flight

distance did not decrease by age 9-10 days post emergence. Virgin 3-4 day old females

flew on average significantly further (24.3±3.9 km) than mated females (10.7±2.6 km).

These data suggest that the western bean cutworm is a strong flyer and adds to the basic

understanding of this insect that may be important for developing resistance management

strategies.

KEY WORDS *Striacosta albicosta*, flight mill, flight characteristics, tethering

techniques, Microsporidia

Introduction

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera:

Noctuidae) is a pest of corn (*Zea mays* L.) and dry beans (*Phaseolus* spp.), and is native

to North and Central America (Smith 1887, Douglass et al. 1957, Lafontaine 2004).

Since its description in 1887, it has steadily expanded its range northward and eastward

from Arizona (Crumb 1956, Antonelli 1974, Blickenstaff 1979). In 1935 it was reported

for the first time in Nebraska (Walkden and Whelan 1942) although it was not listed as an

economic pest. By 1969 it was causing economic damage throughout Nebraska and was

reported for the first time on its eastern border (Keith et al. 1970). Then for

approximately the next 30 years it was occasionally found in western Iowa, but

significant damage was not reported. In 2000, the first report of significant damage (95%

of ears heavily damaged with up to six larvae per ear) was observed in a northwest Iowa

cornfield (Rice 2000) and was reported for the first time in Minnesota (O'Rourke and

Hutchison 2000). Two years later, larvae or their damage could be found in most western

Iowa counties and adults were being collected in blacklight traps in central Iowa (Pope and Rice 2003). In 2004, adults were collected in eastern Iowa and were assumed to occur in every Iowa County (Rice et al. 2004). That year they were also reported for the first time in Missouri and Illinois (Dorhout and Rice 2004). In 2005 they were found in eastern Illinois and by 2006 they were found for the first time in Indiana, Ohio, and Michigan (Rice and Dorhout 2006, Pope 2007).

On corn, the female primarily lays her eggs in the whorl where the larvae hatch, feed on the tassel and then quickly move into the ear (Douglass et al. 1957, Hagen 1962). The larvae then feed in the ear for the remainder of the larval stages until they are ready to overwinter underground. Adults emerge from the soil the following summer, with the flight period ranging from late June to mid August in Iowa (Dorhout and Rice 2007). Females typically emerge about two weeks before the males which can cause a discrepancy between blacklight and sex pheromone trap catches (Blickenstaff 1979).

Managing the western bean cutworm has become more important since it started to expand its distribution again because it now has the ability to damage more corn and dry beans. However there is very little information published on their natural flight behavior (Blickenstaff 1979) and none published using a flight mill. Flight mills are useful tools to examine intrinsic flight behavior such as the flight differences between sexes and age groups, but the results must be interpreted with caution because the flight mill is an artificial system that restricts flight behavior (Dingle 1985, Gatehouse and Woodrow 1987, Sappington and Showers 1992).

The objective of this study was to examine the effect that sex, age, mating atatus, and Microsporidia infection level has on the flight characteristics of the western bean cutworm using a flight mill.

Methods and Materials

Experiment Design. The effects that sex, age, Microsporidia infection level, and mating status had on the flight characteristics of *S. albicosta* were examined. The differences between the sexes were examined by pooling all ages and by comparing between sexes by age group. All individuals used when comparing between sexes were virgins. Age was divided into five groups, 1-2, 3-4, 5-6, 7-8, and 9-10 days post emergence. Age is here-on referred to the age of the adult since it emerged. The effect that Microsporidia had on flight characteristics was going to be examined by correlating flight attributes with spore counts after each moth had flown, but there were only two adults that emerged and was abandoned. The effect of mating was examined by comparing the flight characteristics of females that were mated with virgin females. The number of replications depended on the available adults and varied by year.

Laboratory Colonies. Laboratory colonies were established using eggs oviposited by adult western bean cutworms collected from blacklight traps near Ames, IA during 2005 and 2006. The adults were placed in 30x30x60 cm cages with 2-3 corn whorls serving as oviposition sites. Egg masses were then cut from the corn leaves and approximately 75 to 100 eggs were placed in empty 19 ml (0.63 oz.) plastic cups. The cups were set in the center of 180 ml (6 oz.) wax cups (Sweetheart Cup Company,

Owings Mills, MD) filled with approximately 130 ml (4.5 oz.) a modified black cutworm

diet with aureomycin and benlate omitted (Hendrix et. al 1991, Lewis and Lynch 1969).

When larvae reached the third or fourth instar, they were placed in individual 19 ml (0.63

oz.) plastic cups with 12ml (0.4 oz.) (2005) or 9ml (0.3 oz.) condos (Oliver Products,

Grand Rapids, MI) filled with 6ml (0.2 oz.) (2006). Cups were sealed with laminated

paper lids to reduce larval desiccation. Condos were sealed with a semi-permeable

plastic sheet. All larvae were reared in continuous darkness (2005) or 16:8 hr (L:D)

(2006) at 27°C and 80% RH (relative humidity).

After pupation, individuals were separated by sex to prevent mating and

transferred to 8x8 cm cylindrical cages made from copper hardware cloth (4 mm^2 mesh).

Pupae were held at 27°C, 16:8 (L:D) hr and approximately 80% RH until adult

emergence. Emerged adults were placed in 15x15 cm cylindrical holding cages made

from steel hardware cloth (25 mm^2 mesh). They were provided a stale 1:1 beer/honey diet

and held at 27°C, 16:8 (L:D) hr and approximately 80% RH until they were of the

appropriate age to be flown on the flight mill.

Microsporidia Infection. In 2006, western bean cutworm adults infected with an

unidentified Microsporidia were collected in Scott's Bluff County, NE. and shipped

overnight on dry ice. Their wings were removed (to minimize extraneous debris) and the

bodies were homogenized with a tissue grinder in approximately 100 ml of deionized

water. This solution was filtered through cheesecloth and the concentration of

Microsporidia spores was determined using a haemocytometer (Levy, Horsham, PA)

under a 400x phase contrast microscope. The solution was then further diluted with

deionized water and pipetted onto the meridic diet at a concentration of 2,000 spores per

mm^2, covered, and allowed to dry overnight. The next day, approximately 2,000 second

instars were placed on the infested diet and allowed to develop to the fourth or fifth instar

at 27°C, 16:8 hr (L:D), and 70% RH. Once the larvae reached the fourth or fifth instar

they were singly place inside individual condos with fresh non-infested diet and held at

27°C, 16:8 hr (L:D), and 70% RH. After adult emergence, they were flown, and then

frozen (-20°C) and later homogenized using a tissue grinder (Raun et al. 1960). Spores

were then counted using a haemocytometer under a 400x phase contrast microscope.

Mating Status. One day old males and females were placed in a holding cage for

two or three days (2006 and 2005, respectively). In 2005, eight females were placed with

five males. In 2006, four females were placed with five males. Mated females were

flown at four days (2005) or three days (2006) of age. Female mating was confirmed

after they had flown by dissection and checking for a spermaphore that would have been

inserted into the female by the male during mating.

Tethering. A moth harness was held in place for 20 seconds, allowing the glue to

set. Each harness was constructed from two 6.4 mm (0.25 inch) wide garden twist ties

laminated together with the same glue to form an inverted "Y," then bent and trimmed to

allow for more natural wing movement (Fig. 1). This procedure was modified from

Luo's et. al (2002) procedure where they used double insulated wire instead of garden

twist ties to construct the harnesses. Adults were sedated by placing them in a 100 ml

glass vial for approximately 5-8 seconds containing approximately one ml of ether

absorbed into a tissue. This exposure sedated the moths for the entire time that it took for

the harness to be attached (45-60 seconds). A harness was then glued (Insta-Cure+ glue,

Bob Smith Industries, Atascadero, CA) to the sedated moth about two mm behind the

base of the posterior wings on the dorsal and lateral surface near the junction of the metathorax and abdomen.

Flight Mill. Individually harnessed moths were attached to a mechanical flight arm on a flight mill. The flight arm allowed the moth to fly a radius of 15.92 cm (1 meter circumference) in 2005 or a radius of 23.87 cm (1.5 meter circumference) in 2006. We used a 16 flight mill system conceptually similar to those used by Clarke et al. (1984) and Beerwinkle et al. (1995). Each flight mill was enclosed within a clear vinyl tent that deflected external air movement. In 2005, the moths flew in total darkness for 12 h at $\approx 24°C$, $\approx 70\%$ RH. In 2006, flights ran for 12 h at $24°C$, $\approx 80\%$ RH but the first 210 min were light, followed by a 30 min dimming period that simulated dusk. The rest of the flight occurred in darkness.

Data Analysis. The flight numbers and distance of each adult during a 12 h period was electronically recorded in 60 s intervals. A flight was considered separate from another flight if the data recorder did not record any distance traveled for 60 s or more. Flight information was recorded by a Hewlett Packard Vectra VL Series 4 microcomputer (Hewlett Packard Company, Palo Alto, CA). Software and hardware designs were obtained from Beerwinkle et al. (1995). Only the first eight h of the 2005 flights and only the first eight hours of the dark flight in 2006 were analyzed. Individuals that flew less than 100 m during the eight hr flight period were considered as trivial and were removed from the data set. The data were analyzed using the general linear models procedure (GLM) (SAS Institute 2007, 9.1.3) ($P \leq 0.05$) and means separated using Tukey's adjustment for multiple comparisons.

Results

2005. A pathogenic mold occurred in the environmental growth chamber that contained the laboratory colony of larvae and from the initial 2,500 eggs, only 48 larvae survived to the adult stage. These adults were flown as soon as they reached the desired age.

There were no significant differences in any of the categories between virgin males and females with age pooled except the number of flights (Table 1.) There were no significant differences by age in any categories divided by sex except the total number of female flights (Table 2). There was a trend for virgin males' total flight distance to increase dramatically and then gradually decrease as they got older (Fig. 2). Virgin females initially followed the same pattern, but their mean flight distance did not decrease by age 9-10 days post emergence (Fig 2). Males tended to take their longest duration flight earlier in the night, peaking after two hours, and gradually decrease while mated and virgin females tended to take their longest duration flight after about five hours (Fig. 3).

One to two day old virgin females took an average of 22.33± 4.10 flights compared to the range of 7.2±1.91 to 9.0±2.02 flights for the other age groups. There was a significant difference between mated and virgin females in all categories except between the number of flights (Table 3).

2006. Again, as in 2005, mold significantly destroyed a majority of the laboratory colony so that only 30 adults from the uninfected colony properly emerged and flew more than 100 meters. From the Microsporidia infected colony, only 3 adults properly emerged and only 2 flew more than 100 meters. At this point the Microsporidia

component of this experiment was abandoned. The adults flew as soon as they reached

the desired age but in 2006, only flew with individuals of the same sex.

There were no significant differences in the categories by sex, age, or mating

status (Table 1).

Discussion

Of the approximately 20,000 larvae in the laboratory colony designed to produce

adults for experimentation, only 2.18% and 0.14% properly emerged during 2005 and

2006, respectively. Similarly, only 0.15% of the estimated 2,000 larvae in the infected

Microsporidia colony properly emerged as adults. Antonelli (1974) worked on rearing

techniques for western bean cutworms and had 0.8-19.6% F_1 adult emergence.

Additionally, his attempts to continually rear western bean cutworms failed when the

eggs produced by the F_1 adults failed to develop in sufficient numbers. Doyle (1994) had

similar success with 3.6% of the eggs emerging as adults and 0% of the F_2 adults laying

viable eggs. The common problems in all these studies that make rearing western bean

cutworms difficult are: high mortality, susceptibility to pathogens, and a long

development time.

Harnessing insects and tethering them to a flight mill is inherently intrusive and

proper caution must be applied to interpreting any data, however, the flight mill is very

good at discovering intrinsic flight behavior (Dingle 1985, Gatehouse and Woodrow

1987, Sappington and Showers 1992). The low numbers of adult western bean cutworms

that properly emerged add further caution to the application of the results from this study,

but even with such a low number there are some very interesting results.

There were insufficient replications in 2006 and the methods differed significantly enough that those data will be excluded from this discussion. In 2005 there was a general trend for sex and age to greatly affect flight characteristics (Table 1, 2) and there was a significant difference between mated and virgin females in all categories except the number of total flights (Table 3).

By sex, there was a trend for virgin females to fly further than virgin males within the same age group (Fig. 2). Similar results have been reported with other noctuids (Sharp et al. 1975, Parker and Gatehouse 1985, Sappington and Showers 1991) and may be related to the development of the ovaries (Luo et al. 2002), or some evidence suggest that in general males may have a lower flight ability because they require more energy for flight (Rankin and Burchsted 1992, Li and Lou 1999).

Males were the most likely to take their longest flight (≥30 min) two hours after the flight test started (Fig. 3). In contrast, mated and virgin females tended to take their longest flight (≥30 min) five hours after the flight test started (Fig 3), but the trend was less evident in mated females. This behavior is probably related to mating and suggests that males are more likely to be mate searching earlier in the night while females are calling.

Both males' and females' average flight distances followed the same initial trend in the total distance that they flew by age (Fig. 2). The average distance that they flew started relatively low for the first two days and then dramatically increased once they were three or four days old. However, the females' average flight distance did not decrease, even at age 9-10 days old. The males' average flight distance exhibited a distinct peak at three to four days old and then steadily declined as they got older (Fig.

2b). This follows the same general trends seen in many migratory insects which had a general low average flight distance immediately after emergence that was followed by sudden peak in flight potential and then a slow decline as age continued to increases (Johnson 1976, Dingle 1985) including other Noctuids (Sharp et al. 1975, Zhang and Li 1985, Armes and Cooter 1991, Sappington and Showers 1991, 1992, Luo et al. 1995, 2002). However, there is no information suggesting that western bean cutworms migrate, and this flight behavior may just be intrinsic of insects that are strong flyers and are able to readily expand their distribution, such as this species (Keith et al. 1970, Antonelli 1974, Blickenstaff 1979, Rice 2000, Pope and Rice 2003, Dorhout and Rice 2004, Rice and Dorhout 2006, Pope 2007).

There was a significant difference between the number of flights that one to two day old virgin females made (22.3 flights) compared to all other ages (7.0-9.0 flights) (Table 2). Blickenstaff (1979) states that the pre-oviposition period is four days and these frequent, short flights may represent part of female mating behavior.

There was a significant difference between mated and virgin 3-4 day old females in all categories except the total number of flights (Table 3). The mated females took shorter; more frequent flights compared to their virgin counterparts and may represent a switch in behavior from mate calling to oviposition sit searching. These general results are similar to those found by Arms and Cooter (1991) using *Helicoverpa armigera* (Lepidoptera: Noctuidae) who found mating affects flight behavior. However, several other studies with other Lepidoptera do not show any significant effect between mated and virgin females (Shirai 1998, Hughes and Dorn 2002, Shirai and Kosugi 2000, Shirai et al. 1998).

The western bean cutworms' pre-oviposition period and the average flight distance during their first two days may be important for resistance management. The western bean cutworms' preoviposition period (Douglass 1957, Blickenstaff 1979) indicates that most females are mated relatively soon after they emerged, but not necessarily with individuals near where they emerged because the flight mil data indicated that even a 1-2 day old adult can fly on average 3.5 km in 8 hrs. This behavior may increase or reduce the accumulation of resistance to an insecticide within a population (Korman et al. 1993).

There are many opportunities for basic research regarding the flight and mating behavior of western bean cutworms and laboratory rearing. It is possible that there are differences between the flight characteristics of western bean cutworms from different geographic areas, reflecting differences between the expanding population and the original population. Flight mill experiments would be ideal for testing for such differences.

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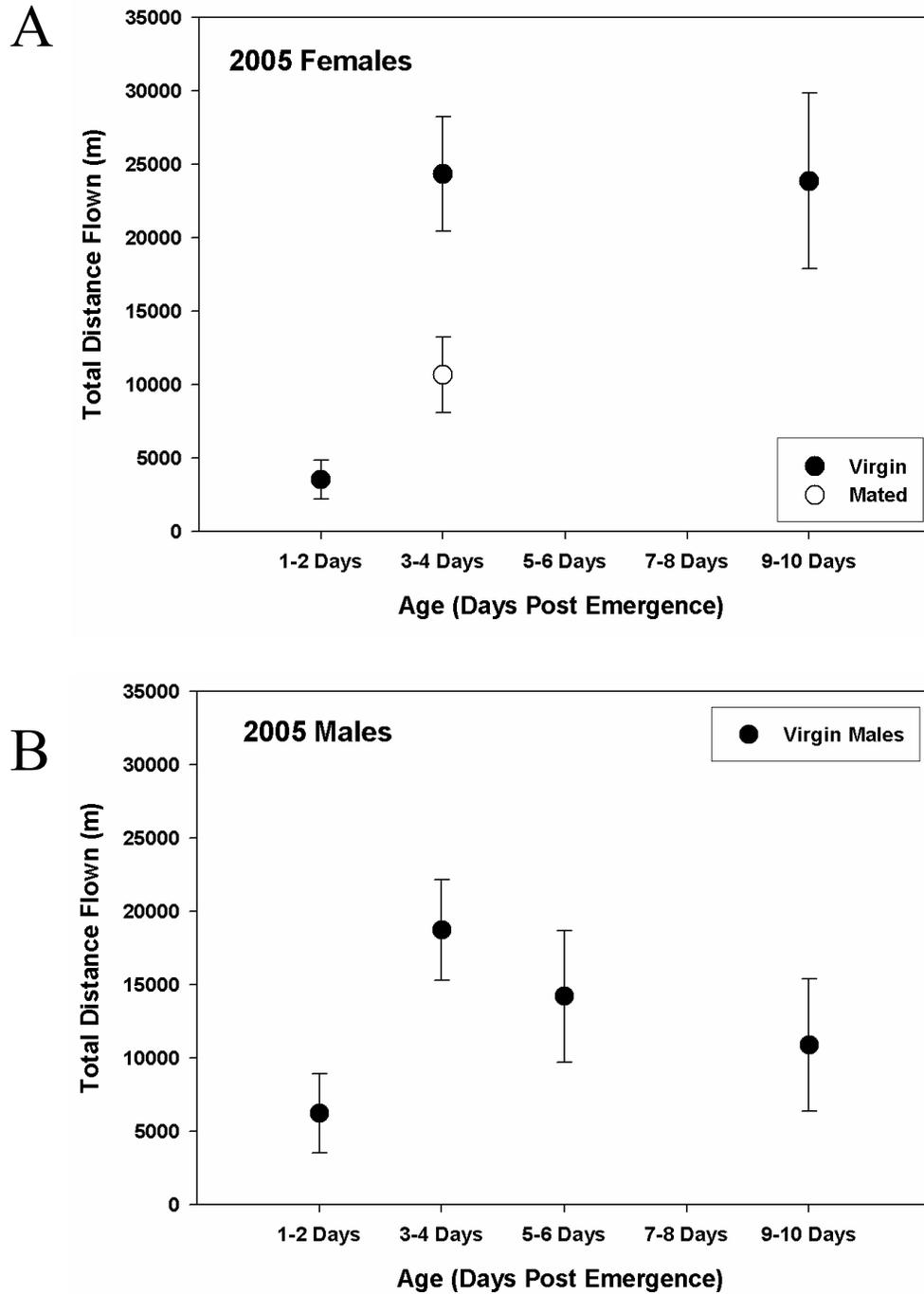**Fig. 1.** Western bean cutworm with flight mill harness.

Fig. 2. A. Females (virgin and mated), and B. Males. Mean±SEM total distance flown in eight hours. If *N*=1, then data not shown.
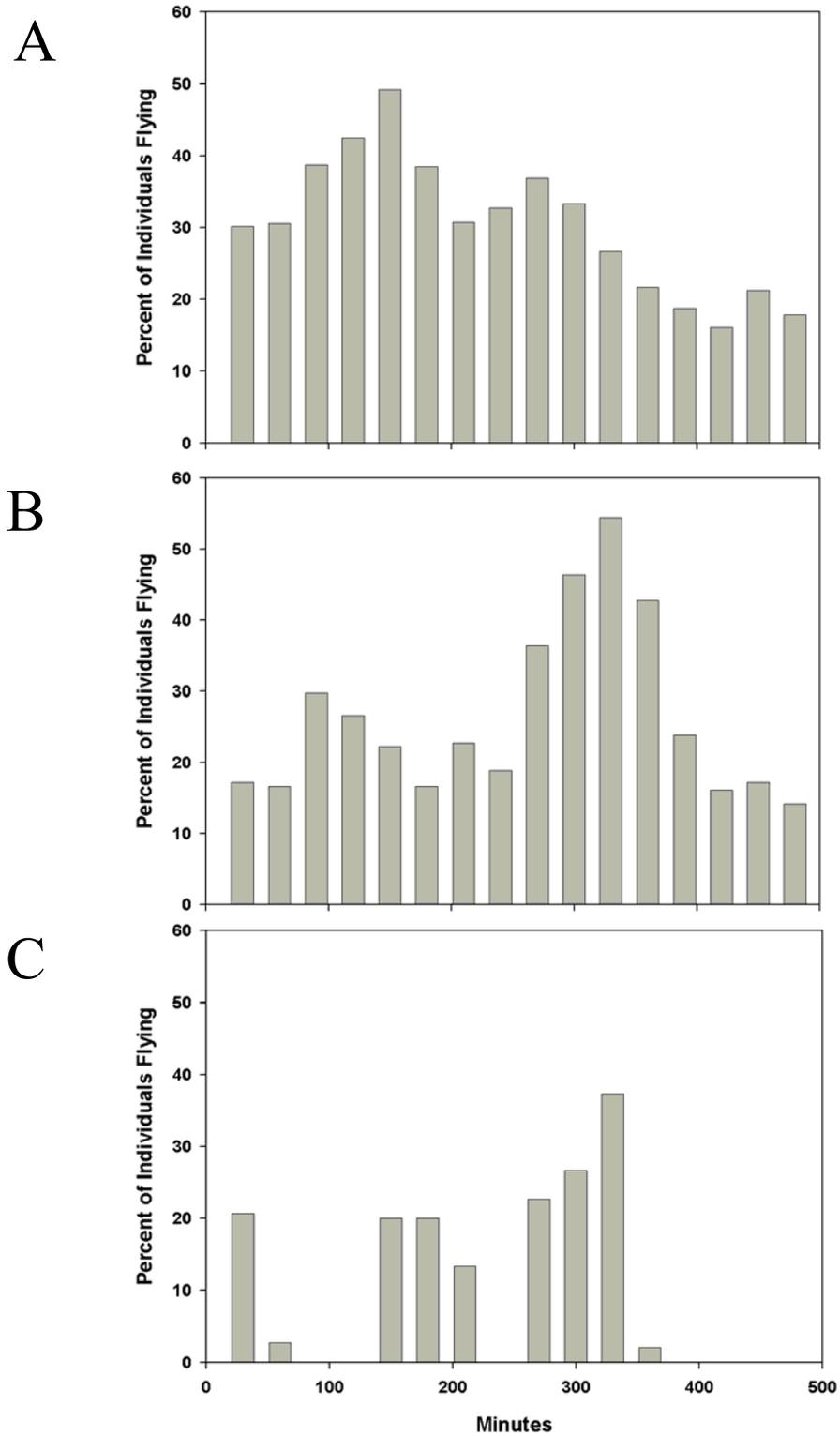
Fig. 3. Percent of individuals participating in their longest duration flight divided into 30 min blocks starting at 17:00 hr. A. virgin males; B. virgin females; C. mated females

Table 1. Statistics for western bean cutworm flight characteristics, 2005-2006.

	Test	df	n	Total distance (m)		Total no. of flights		Farthest single flight		Mean distance flown		Longest single flight (min)		Total time flying		Mean time flying	
				F	P	F	P	F	P	F	P	F	P	F	P	F	P
2005	Virgin Females vs. Virgin Males	1	40	2.48	0.1236	0.04	0.8476	2	0.1659	1.77	0.1916	1.91	0.1751	4.12	0.0495	1.4	0.2441
	Virgin Females by Age	4	15	2.6	0.1004	4.87	**0.0194**	1.98	0.1741	1.09	0.4126	1.76	0.2138	1.46	0.2865	1.19	0.3745
	Virgin Males by Age	4	25	1.7	0.1889	1.64	0.2026	0.87	0.5003	1.66	0.1984	0.45	0.7721	0.88	0.4962	0.94	0.4615
	Mated females vs. Virgin females	1	13	9.33	**0.0110**	0.72	0.4157	9.36	**0.0108**	5.29	**0.0421**	10.67	**0.0075**	9.8	**0.0096**	5.82	**0.0344**
2006	Virgin Females vs. Virgin Males	1	22	2.00	0.1727	0.34	0.567	1.25	0.2768	0.57	0.4602	0.67	0.4215	0.85	0.3682	0.17	0.6802
	Virgin Females by Age	4	13	0.55	0.6635	0.63	0.6161	0.32	0.8131	0.56	0.6534	0.43	0.7336	1.28	0.3397	0.7	0.5766
	Virgin Males by Age	4	9	0.27	0.7721	0.23	0.8015	0.11	0.8951	0.14	0.8718	1.18	0.3709	1.82	0.2407	0.7	0.5338
	Mated females vs. Virgin females	1	8	0.36	0.5700	0	0.9754	0.63	0.4564	0.41	0.5444	0.08	0.7865	0.06	0.8079	0.28	0.6167

ANOVA using type III sum of squares in Proc GLM (SAS 2007). P≤ 0.05 are considered significant and are highlighted.

Table 2. Virgin western bean cutworm flight characteristics presented by sex and age flown for 8 hrs in darkness, 2005.*

	Sex	n	Total distance (m)	Mean no. of flights	Farthest single flight (m)	Mean distance flown (m/flight)	Longest flight (min)	Total time flying (min)	Mean time flying (min/flight)
	Female	15	18,935±3,180	10.9±1.9	10,156±2,195	3,277±926	140±22.9	286.6±31.3	44.7±10.4
	Male	25	13,205±2,085	11.4±1.4	6,729±1,344	2,038±464	98.4±18.8	207.4±23.7	30.6±6.8
Female	1-2	3	3,525±1,339	22.3±4.1a	1,580±585	152.4±37.4	55.3±29.0	164.0±83.0	6.7±2.2
	3-4	5	24,331±3,898	7.2±1.9b	13,673±3,515	5,311±2,044	168.6±42.0	305.6±41.7	66.4±23.9
	5-6	1	22,628	9.0b	4,506	2,514	38	209	23.2
	7-8	1	9,885	7.0b	3,285	1,412	135	389	55.6
	9-10	5	23,856±5,993	9.0±2.0b	14,288±3,975	3,642±1,448	183.6±35.0	336.2±51.9	47.9±14.1
Male	1-2	4	6,236±2,691	11.8±2.8	2,808±2,184	520±235	62.5±43.8	150.3±58.8	14.1±5.93
	3-4	9	18,701±3,431	9.0±1.9	9,192±2,413	3,309±893	114.6±30.8	238.6±28.8	39.2±9.2
	5-6	5	14,198±4,494	9.4±2.5	6,934±2,542	2,339±1,144	114.2±43.2	240.8±68.2	46.0±27.1
	7-8	1	596	26	110	22.9	5	59	2.3
	9-10	6	10,880±4,499	14.0±4.2	6,582±3,254	1,226±652	100.5±47.1	195.5±55.7	20.5±8.4

*Means with the same letter are not significantly different, $P \leq 0.05$ Mean±SEM.

Table 3. Mated 3-4 day old female western bean cutworm flight characteristics presented by sex and age

flown for 8 hrs in darkness, 2005.*

	n	Total distance (m)	Mean no. of flights	Farthest single flight (m)	Mean distance flown (m/flight)	Longest flight (min)	Total time flying (min)	Mean time flying (min/flight)
Virgin	5	24,331±3,898a	7.2±1.9a	13,673±3,515a	5,311±2,044a	168.6±42.0a	305.6±41.7a	66.4±23.9a
Mated	8	10,669±2,582b	10.75±3.1 a	4,106±1,223b	1,472±438b	49.3±13.3b	155.5±27.7b	19.7±4.8b

*Means with the same letter are not significantly different, P≤ 0.05 Mean±SEM.

CHAPTER 3: An Evaluation of Pheromone Trapping Techniques for Western Bean

Cutworms (Lepidoptera: Noctuidae)

A paper to be submitted to *The Journal of Economic Entomology*

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ABSTRACT Pheromone traps can be used to monitor adult western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) and to time of field scouting. Understanding the effects of different trapping techniques on adult catches could help corn producers make better pest management decisions. Several approaches to trapping adults were evaluated in 2005 and 2006 using two different pheromone traps (sticky wing and jug traps) in two different environments (corn or corn/soybeans) at three heights (0.6, 1.2 and 1.8 m). There was no significant difference in the trap catches by trap type in either 2005 or 2006. There were significantly more adults captured in traps placed between two corn fields than traps placed between corn/soybean fields during both years. Trap height also was significant with the traps at 1.2 and 1.8 m catching more moths than traps at 0.6 m during both years. These results show that trapping techniques affect trap catches and should be considered when comparing trap totals from different traps. These results also suggest that either trap type placed between two corn fields at either 1.2 or 1.8 meters above the ground will maximize trap catches of the methods examined.

KEY WORDS *Striacosta albicosta*, monitoring, pest management

Introduction

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera:

Noctuidae) is a pest of corn (*Zea mays* L.) and dry beans (*Phaseolus* spp.). It has steadily

expanded its range northward and eastward from Arizona since its description in 1887

(Smith 1887, Crumb 1956, Keith et al. 1970, Antonelli 1974, Blickenstaff 1979). It is a

common pest of field corn in Colorado (Hanstbarger 1969) and Nebraska (Hagen 1976),

but prior to 2000, the western bean cutworm was considered to be extremely rare in Iowa

and economic damage had never been reported. The first significant damage (95% of ears

heavily damaged with some containing six larvae) in Iowa was observed in 2000 in a

cornfield in Holstein (Rice 2000). Two years later, larvae or their damage could be found

in most western Iowa counties and adult moths were being collected in blacklight traps in

central Iowa. In 2004, sex pheromone traps for males were placed throughout eastern

Iowa including counties bordering the Mississippi River. Adult moths were collected in

every trap placed in eastern Iowa and the insect was assumed to occur in every Iowa

County. That year, traps were also placed for a single night in several northeast Missouri

and northwest Illinois counties where adults were trapped and reported there for the first

time in those two states (Dorhout and Rice 2004). This species continued to move

eastward and in 2006 adults were trapped for the first time in Indiana, Michigan, and

Ohio (Rice and Dorhout 2006, Pope 2007).

On corn, the female primarily lays her eggs in the whorl where the larvae hatch

and quickly move into the ear (Douglass et al. 1957, Hagen 1962). The larvae then feed

in the ear for the remainder of their larval development after which they exit the ear and

overwinter underground. The adults emerge the next summer and rest on corn leaves during the day before calling for a mate during the night (Blickenstaff 1979).

Management of this insect has traditionally required the use of insecticides. Insecticide timing is critical because larvae are most vulnerable immediately after they've hatched and are nearly impossible to control once they have entered the ear (Blickenstaff and Peckenpaugh 1981, Seymour et al. 1998).

Pheromone traps can be used to monitor for western bean cutworms and determine when surrounding fields should be scouted for eggs (Seymour et al. 1998). Two common pheromone trap types used for monitoring are sticky wing traps and "milk jug" traps. Mahrt et al. (1987) found that trap height and the surrounding habitat significantly affected traps catches in dry bean fields, but that the type of trap used did not differ significantly. Because trapping techniques affected trap totals within a dry bean cropping environment, it seemed appropriate to examine these same kinds of effects within a corn production system.

The objective of this study was to evaluate two pheromone trap types at three heights within two field environments and examine the effects on adult western bean cutworm captures.

Materials and Methods

Experimental Design. Pheromone traps were operated in Ames, IA, from July 13 through August 10, 2005 and from June 24 through August 7, 2006. Two types of pheromone traps were placed in two environments at three heights following a complete 2x2x3 factorial design. The first factor was trap type and compared the trapping efficacy

of sticky wing (Scentry Biologicals, Inc., Billings, MT) and milk jug (Seymour et al. 1998) pheromone traps. The second factor examined the effect that the trapping environment had on trap catches when the traps were placed between either two corn fields or between a corn and soybean field (Fig. 1). All traps were placed on the southern edge of a 600 m wide corn field, starting 25 meters from the corner of the field and continuing every 25 meters. A grass access road ran the length of the northern corn field and separated it and the traps from adjacent corn and soybean fields by approximately 10 meters. The third factor was trap height where traps were placed 0.6, 1.2, or 1.8 meters above the ground. Each treatment was replicated twice.

Trap Design. The sticky wing traps consisted of a plastic top with a replaceable paperboard bottom that was pre-coated with an adhesive. The top and bottom were separated with spacers creating a 10 cm gap between the two trap pieces. The milk jug traps were constructed following the procedure of Seymour et al. (1998) by removing approximately 60 cm^2 from each of the four side panels of a new, 3.8 liter (one gallon), plastic milk jug (Containers & Packaging Supply, Inc., Eagle, ID) while leaving a 6 cm deep reservoir in the bottom. The reservoir was filled with a 4:1 mixture of water and ethylene glycol (Antifreeze, Prestone Inc., Danbury, CT) and a couple of drops of liquid soap (Palmolive Original, Colgate-Palmolive Company, New York, NY) to reduce the surface tension of the water. Both traps were baited with *S. albicosta* sex pheromone lures that attract only males (Scentry Biologicals, Inc., Billings, MT). Pheromone lures were held in the center of the sticky wing traps by the pheromone holder and were hung inside the milk jugs approximately 5cm from the lid with a 5 cm piece of wire.

Data Collection. Adults in traps were counted every two days after which the sticky paperboard bottoms were replaced and the reservoirs in the jug traps were refilled. The pheromone lures in all the traps were replaced every eight days to minimize the temporal variance in the concentration of the pheromone plume. In 2006 the wind direction was recorded each hour from 9 pm to 5 am to determine the prevailing wind direction by a local weather station (Iowa State University 2007).

Data Analysis. A factorial analysis of variance (ANOVA) using PROC GLM (SAS 2007) looking at type III sum of squares was used to analyze the data expressed as the number of moths collected per trap for the year. Data were log transformed to normalize the variance. Means were compared using Tukey's adjustment for multiple comparisons with $P \leq 0.05$ considered significant. Trap catch data are expressed as mean±the standard error.

<div align="center">

Results

</div>

Traps in 2005 were placed in the field after the beginning of the moth flight (Fig 1a); therefore the entire flight period was not sampled. Traps in 2006 were placed two weeks earlier than the previous year and the entire flight period was sampled (Fig 1b).

In 2005, the grand total of all moths captured in all of the traps during the entire season was 2,488 moths. The greatest number of moths captured by all traps during one period was 496 moths on July 13. The last day that any moths were captured in 2005 was on August 8.

In 2006, the grand total of all moths captured in all of the traps during the entire season was 8,856 moths. A total of three moths were captured by all of the traps during

the first period, June 24. The greatest number of moths captured by all traps during one

period was 975 moths on July 6. A total of twelve moths were captured during the last

period on August 7. After August 7 the traps were removed and the western bean

cutworm flight presumably ended shortly thereafter.

In 2006 the wind direction was recorded every hour from 9 PM to 5 AM. The

wind blew from a northerly direction (315-44°) 33.4%, easterly direction (45-134°)

13.1%, southerly direction (135-224°) 35.0%, and from a westerly direction (225-314°)

18.5% of the time (Iowa State University 2007).

There was a significant difference between the number of moths captured in 2005

and 2006 pooled across all factors (df = 1, F = 168.9, P< 0.001). There was no

significant difference between the total adults captured by the sticky wing or milk jug

traps pooled across trapping environment and trap height in 2005 (df = 1, F = 0.37, P =

0.555) and 2006 (df = 1, F = 0.94, P = 0.351) (Table 1). There were significantly more

adults captured in traps located between two corn fields than traps located between a corn

and soybean field in 2005 (df = 1, F = 9.16, P = 0.0.011; 126.1±12.2 and 81.3±13.1

moths, respectively) and 2006 (df = 1, F= 6.35, P= 0.027; 404.9±30.7 and 346.0±48.8

moths, respectively). Trap height was significant with traps 0.6 meters high catching

significantly fewer total adults in 2005 (df = 2, F = 8.08, P = 0.006) and 2006 (df = 2, F =

23.07, P< 0.001) than traps at 1.2 and 1.8 meters in both trapping environments. No

other interactions were significant.

Discussion

The significant difference between the number of western bean cutworm adults captured in 2005 and 2006 was a consequence of missing the beginning of the 2005 flight, but fig. 1 shows that fewer adults flew during the same time period in 2005 than in 2006. The difference in adults captured between 2005 and 2006 is probably just the normal population fluctuations that have been seen elsewhere in Idaho (Blickenstaff 1979), Nebraska (Hagen 1976) and Iowa (Pope and Rice 2003 and Rice et al. 2004).

Adult trap captures between the sticky wing and jug traps were not significantly different, but there seemed to be a trend that the jug traps captured more moths for a short while after the peak than the sticky wing traps (Fig. 1). This may indicate that the jug traps are able to capture more moths quicker than the sticky wing traps.

In both years, more western bean cutworms were captured in the traps that were bordered by corn on both sides than traps adjacent to both corn and soybeans. This may be because there was more acceptable or suitable habitat in a corn/corn environment that is preferred by the adults during the day when they are at rest, or at night when they are mating. Similarly, Derrick et al. (1992) found that pheromone traps placed in the middle of corn fields captured more European corn borers, *Ostrinia nubilalis* (Hübner), than traps outside the fields. However, it is interesting that the traps in the corn/corn environment did not capture twice as many adults as the traps in the corn/soybean environment (Table 2). This may be explained by examining the direction of the prevailing winds at night. The weather station data showed that the prevailing wind blew in a southerly direction approximately 35% of the time. This would have caused the pheromone from these traps to be blown away from the corn and into the soybeans,

thereby reducing trap effectiveness because western bean cutworm adults settle in corn fields (Blickenstaff 1979). Mahrt et al. (1987) reported a similar situation where the prevailing wind may have caused fewer adults to be captured in traps whose pheromone was blown over alfalfa fields and pastures and away from the preferred crop.

Results from both years show that the higher traps (1.2 and 1.8 m) captured significantly more moths than the shorter traps (0.6 m) (Table 2). The affect of trap height may be related to the female's preference to oviposit at the top of the corn plant in the whorl (Douglass et al. 1957, Hagen 1962). It was interesting that there were no environment by trap height interactions, because it would be expected that the lower soybean canopy would cause more moth to fly lower and consequently be captured in the 0.6 m traps or to fly higher and be captured in the 1.8 m traps.

The results from this study indicate that the trapping environment and the height of the trap significantly affect the number of western bean cutworms captured and should be considered when comparing trap totals from different traps. These results also indicate that there is no significant difference in captures between the sticky wing and jug traps although the jug traps were easier and cheaper to maintain. These results suggest that the most effective trapping technique would be to place either trap type between two corn fields between 1.2 and 1.8 meters above the ground for maximized trap catches of the methods examined. Additional research should examine if the period between counting and replacing the sticky bottoms in the wing traps affects the overall significant between the trap types, if the distance that the trap is placed from a corn field affects the trap catches, and if crop rotation affects the optimized placement of trap catches.

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Table 1. Statistical analysis from western bean cutworm trap catch data (2005 and 2006)*

2005	Source	df	F	P
	Trap Type	1	0.37	0.555
	Height	2	8.08	**0.006**
	Trap Type x Height	2	0.02	0.982
	Environment	1	9.16	**0.011**
	Trap Type x Environment	1	0.11	0.746
	Height x Environment	2	0.97	0.407
	Trap Type x Height x Environment	2	0.25	0.781
2006	Source	df	F	P
	Trap Type	1	0.94	0.351
	Height	2	23.07	**<0.001**
	Trap Type x Height	2	0.93	0.422
	Environment	1	6.35	**0.027**
	Trap Type x Environment	1	0.01	0.913
	Height x Environment	2	3.80	0.053
	Trap Type x Height x Environment	2	3.53	0.062

*Analyzed using a factorial analysis separated by year using type III sum of squares. P≤ 0.05 are considered significant.

Table 2. Mean trap catches of western bean cutworms collected

in pheromone traps, 2005 and 2006.

	Environment	Trap height (m)	Mean ± SE
2005	pooled	0.6	56.1±13.4a
	pooled	1.2	126.9±16.1b
	pooled	1.8	121.1±13.6b
	corn/corn	0.6	87.8±15.7a
	corn/corn	1.2	153.8±14.6b
	corn/corn	1.8	136.8±19.8b
	corn/soybeans	0.6	38.3±11.4a
	corn/soybeans	1.2	100.0±22.6b
	corn/soybeans	1.8	105.5±17.4b
2006	pooled	0.6	235.3±32.5a
	pooled	1.2	413.4±35.0b
	pooled	1.8	477.8±37.5b
	corn/corn	0.6	297.3±42.3a
	corn/corn	1.2	468.0±33.0b
	corn/corn	1.8	449.5±40.2b
	corn/soybeans	0.6	173.3±23.9a
	corn/soybeans	1.2	356.8±51.4b
	corn/soybeans	1.8	506.0±66.4b

*By environment, means with the same letter are not

significantly different, P≤ 0.05. Mean±SEM

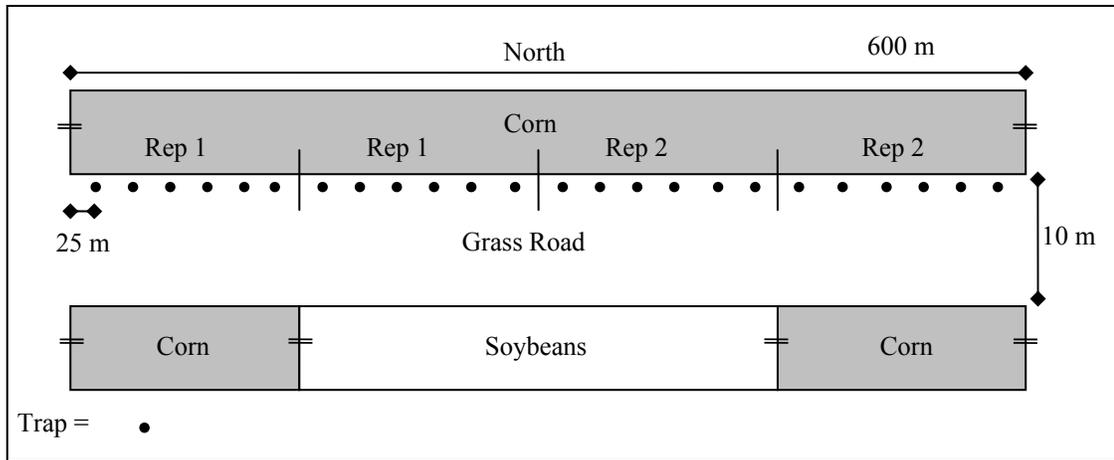**Fig. 1.** Schematic of field map showing the relative placement of western bean cutworm pheromone traps. Note: Fields not drawn to scale.
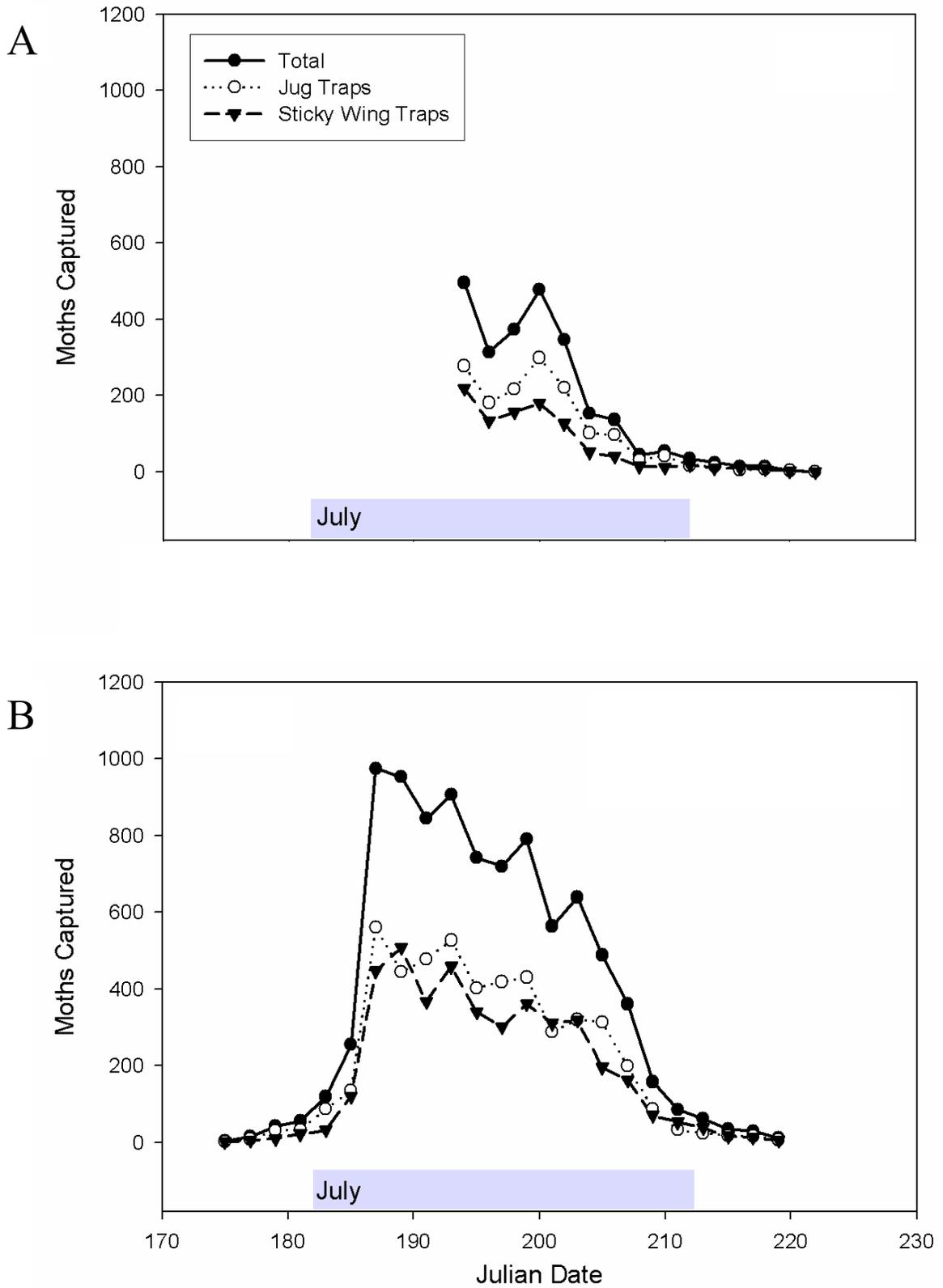
Fig. 2. Two day trap totals of adult western bean cutworms; A 2005, B 2006.

CHAPTER 4: Efficacy of Transgenic Maize for the Control of the Western Bean

Cutworms (Lepidoptera: Noctuidae)

A paper to be submitted to *The Journal of Economic Entomology*

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ABSTRACT Transgenic corn (Herculex[®]) expressing an endotoxin (Cry1F) from *Bacillus thuringiensis* (*Bt*) (Berliner) is labeled as a method for controlling western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae). In 2005 and 2006 Herculex, YieldGard[®] CornBorer (CB), and non *Bt* hybrids were evaluated for their performance against the western bean cutworm at four locations. The hybrids were evaluated for western bean cutworm infestation levels and ear damage. A total of 3,100 ears were examined for western bean cutworms in 2005 and 1,100 in 2006 and a total of 340 ears were scored for damage in 2005 and 220 in 2006. In all, a total of 17 hybrids were compared in 2005 and 11 in 2006. Infestation levels and ear damage in 2005 and 2006 were significantly lower in the Herculex hybrids than in the check or YieldGard CB hybrids across all locations. Interestingly, in 2005, YieldGard CB hybrids had significantly higher levels of infestation and more ear damage than the non *Bt* hybrids. However, in 2006, there was no significant difference between the YieldGard CB and the non *Bt* hybrids scores, but this may be because there was no YieldGard CB data from the location with the highest western bean cutworm infestations and injury levels. There was no YieldGard CB data from that location because that location only had Herculex and

non *Bt-* hybrids. These data indicate that Herculex offers the best protection of the

current technology traits for management of western bean cutworms

KEY WORDS *Striacosta albicosta*, YieldGard, Herculex, biotechnology, *Bt* corn

Introduction

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera:

Noctuidae), is native to North and Central America (Douglass et al. 1957, Lafontaine

2004) and is a pest of dry beans (*Phaseolus* spp.) and corn (*Zea mays* L.). It has steadily

expanded its range northward and eastward from Arizona since its description in 1887

(Smith 1887, Crumb 1956, Keith et al. 1970, Antonelli 1974, Blickenstaff 1979). It is a

common pest of field corn in Colorado (Hanstbarger 1969) and Nebraska (Hagen 1976),

but prior to 2000, the western bean cutworm was considered to be extremely rare in Iowa

and economic damage had never been reported. The first significant damage (95% of

ears heavily damaged with some ears containing six larvae) in Iowa was observed in

2000 in a cornfield in Holstein (Rice 2000). In the years that followed, the western bean

cutworm expanded its distribution across Iowa and by late summer 2004, it could be

found in most eastern Iowa counties and was captured for the first time in Illinois and

Missouri (Dorhout and Rice 2004). It has since continued to expand its distribution

eastward and in 2006, adults were trapped for the first time in Indiana, Michigan, (Rice

and Dorhout 2006) and Ohio (Pope 2007).

Corn and dry beans are the two primary host for western bean cutworms

(Blickenstaff 1979). It is possible for them to complete their development on tomatoes,

but survival is very low (Blickenstaff 1979). On corn, the female primarily lays her eggs in the whorl where the larvae hatch and quickly move into the ear (Douglass et al. 1957, Hagen 1962). The larvae then feed in the ear for the remainder of their larval development after which they exit the ear and overwinter underground. The adults emerge the next summer and rest on corn leaves during the day before searching for a mate during the night (Blickenstaff 1979).

The movement of the western bean cutworm across the Corn Belt is a concern because it directly damages corn by feeding on the kernels in the ear. Each larva feeds on corn for a considerable length of time, taking an average of 55.9 days to complete their development at 27°C, 16:8 (L:D) (Antonelli 1974). One larva per corn plant at dent stage reduces yields by 2.32 q/ha (3.7 bu/acre) (Appel et al. 1993), but because the larvae are not cannibalistic, heavily infested corn may have as many as 20 larvae per ear (Seymour 1998).

Management of this insect has traditionally required the use of insecticides. However, insecticide timing is critical because larvae are most vulnerable immediately after hatching and are nearly impossible to control once they enter the ear (Blickenstaff and Peckenpaugh 1981, Seymour et al. 1998). The introduction of corn hybrids producing crystalline (Cry) protoxins from the *Bacillus thuringiensis* (*Bt*) (Berliner) bacterium has given producers a new management tool. There are currently two commercial *Bt* traits for controlling Lepidopteran pests in corn, YieldGard® CornBorer (hereafter, YieldGard CB) (event MON810 from *B. thuringiensis* sp. *kurstaki*) (Monsanto Company, St. Louis, MO) expressing the endotoxin, Cry1Ab and Herculex® I or Herculex® Xtra (hereafter, Herculex) (event TC 1507 from *B. thuringiensis* sp. *aizawa*i

(Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc.,
Indianapolis, IN and Des Moines, IA, respectively) expressing the endotoxin, Cry1F. Of
these two events, only Herculex is labeled for suppressing the western bean cutworm.
Our hypothesis is that Herculex hybrids will have significantly lower infestation levels of
western bean cutworms and less ear damage than the YieldGard and non-*Bt* hybrids. Our
second hypothesis is that the infestation levels of western bean cutworms and ear damage
caused by them will not be different between the YieldGard and non-*Bt* hybrids.

The objective of this study was to evaluate the effectiveness of Herculex and
YieldGard CB hybrids for controlling western bean cutworms in Iowa using natural field
populations.

Methods and Materials

Experimental Design. Natural western bean cutworm infestations in non-*Bt*,
Herculex and YieldGard CB hybrids were quantified in four locations in central Iowa
during 2005 (New Providence, Independence, Jesup, Hubbard) and 2006 (New
Providence, Independence, Jesup, Conrad). Each location was selected from a list of
Pioneer Hi-Bred Intl. strip trials that had high infestation levels in recent years and were
planted with at least one Herculex and one non-*Bt* corn hybrid. Preference was given to
locations with a Herculex hybrid, its near-iso-line counterpart (a non-*Bt* corn hybrid), and
a YieldGard CB hybrid. The hybrids were planted in strips four or six rows wide and at
least 91 m (300 ft) long and managed independently by Pioneer sales agents or their
personnel at each location using current farming practices. Replication at each location

was by trait (non-*Bt*, Herculex, and YieldGard CB) and the number of replications at each location depended on the location.

Sampling Infestation Levels and Ear Injury. Corn ears were examined for the presence of western bean cutworm larvae during August 8-12, 2005 and August 16-23, 2006. In 2005, a large sample size of 200 ears in each replication was chosen because the infestation level was expected to be between 0 and 8% and because the ears were scored only for the presence or absence of western bean cutworm larvae. A sample size of 100 ears was used in a few replications at Jesup. In 2006, a sample size of 100 ears in each replication was chosen. In 2006, the number and species of Lepidoptera larvae present in each ear were also recorded Conrad, Jesup, and Independence. In both years, the ears were sampled from the middle two rows in each strip plot beginning 20 feet inside the plot in groups of ten sequential ears. Each group of ten sequential ears were sampled approximately 3 m (10 ft) apart and alternated between the two rows. Each ear was examined for the presence of a western bean cutworm by pulling back the husk.

In both 2005 and 2006, 20 ears from each replicate were scored for injury using a nonlinear 9-1 injury scale (Table 1). These ears were sampled from the middle two rows in each strip plot beginning 7 m (20 ft) inside the plot in four groups of five sequential ears. Each group of five sequential ears were sampled approximately 3m (10 ft) apart and alternated between the two rows. Ears were not sampled if they had their husks previously pulled back while checking for the presence of larvae earlier in the season.

Data Analysis. An analysis of variance (ANOVA) using PROC GLM (SAS 2007) looking at type III sum of squares was used to analyze the data and compare the infestation, ear injury levels and yield differences between the three traits: non-Bt,

Herculex and YieldGard CB. Data were analyzed both by location and with locations

pooled. Means were compared using Tukey's adjustment for multiple comparisons with

$P \leq 0.05$ considered significant. Data are expressed as mean±SEM.

Results

2005. Ear scores were signficant by hybrids at all four locations: New Providence

(df= 2, F= 20.96, P= <0.0001), Independence (df= 4, F= 34.33, P= <0.0001), Jesup (df=

6, F= 19.89, P= <0.0001), and Hubbard (df= 1, F= 10.71, P= 0.0023) (Table 2). Ear

damage was significantly lower ($P \leq 0.05$) in the Herculex hybrids than the YieldGard CB

and check hybrids in all locations except between one of the YieldGard Hybrids at Jesup

(Table 2). YieldGard CB hybrids at New Providence and Independence had significantly

more ($P \leq 0.05$) ear damage than the non-*Bt* corn hybrids (Table 2).

Ear scores pooled by trait by location were significant at all locations: New

Providence (df= 2, F= 20.96, P= <0.0001), Independence (df= 2, F= 69.27, P= <0.0001),

Jesup (df= 6, F= 24.27, P= <0.0001), and Hubbard (df= 1, F= 10.71, P= 0.0023) (Table

3). Ear injury was significantly lower ($P \leq 0.05$) in the Herculex hybrids than the

YieldGard CB and check hybrids in all locations except New Providence (Table 3). In

New Providence, the YieldGard CB hybrids had significantly more damage than the

Herculex or check hybrids (Table 3). Infestation levels were significant pooled by trait at

Independence (df= 2, F= 38.51, P= 0.0253) and Jesup (df= 6, F= 25.70, P= 0.0052)

(Table 3). In Independence, the YieldGard CB hybrids had a significantly higher

infestation level than the Herculex or check hybrids (Table 3). In Jesup, the Herculex

hybrids had a significantly lower infestation level than the YieldGard CB or check hybrids (Table 3).

Traits pooled over all locations were significantly different by ear scores (df= 2, F= 84.48, P= <0.0001) and infestation levels (df= 2, F= 29.20, P= <0.0001) (Table 4). Herculex hybrids had significantly lower infestation levels and ear damage than the check or YieldGard CB hybrids and the check hybrids had significantly lower infestation levels and ear damage than the YieldGard CB hybrids (Table 4).

2006. Ear scores were significantly different by trait at two of the four locations: New Providence (df= 1, F= 21.48, P= <0.0001), Independence (df= 2, F= 9.45, P= <0.0003), while Jesup (df= 2, F= 1.95, P= 0.1517), and Conrad (df= 2, F= 0.24, P= 0.7901) were not (Table 5). Ear damage was significantly lower ($P \leq 0.05$) in the Herculex hybrids than the YieldGard CB and check hybrids at New Providence and Independence (Table 5). Traits pooled over all location were significantly different by ear scores (df= 2, F= 14.18, P= <0.0001) and by European corn borer infestation levels (df= 2, F= 105.25, P= <0.0001) (Table 6). Herculex hybrids had significantly lower ear damage than the check or YieldGard CB hybrids and the check hybrids had significantly lower ear damage than the YieldGard CB hybrids (Table 6). European corn borer infestation levels were significantly lower in the Herculex and YieldGard CB hybrids than the check hybrids (Table 6). Traits pooled over all location were not significant for western bean cutworm (df= 2, F= 1.55, P= 0.2696) and corn earworm (df= 2, F= 1.5, P= 0.2963) infestation levels (Table 6).

Discussion

In general, Herculex hybrids had significantly lower infestation levels of western bean cutworms and less ear damage than YieldGard CB or the check hybrids in both 2005 and 2006. The Herculex hybrids did not completely control western bean cutworms however, the infestation levels and ear damage was approximately one fifth of that sustained by the YieldGard CB hybrids and one-fourth of that sustained by the check hybrids in 2005. Interestingly, the YieldGard CB hybrids pooled had approximately 40% higher infestation levels of western bean cutworms and approximately 24% more ear damage than the check hybrids in 2005. However, in 2006 the YieldGard CB hybrids had significantly lower infestation levels and less damage than the non-*Bt* hybrids, but this may be because there was no YieldGard CB data from the location with the highest western bean cutworm infestations and injury levels. There was no YieldGard CB data from that location because that location only had Herculex and non *Bt*- hybrids. The data from the ear damage is consistent with other research where Herculex ears had less injury than YieldGard or non-*Bt* hybrids and where YieldGard ears had more injury than the non-Bt hybrids at most locations or years (Catangui and Berg 2006 Rice 2007). The results of these studies further support laboratory research by Dorhout and Rice (2007) suggesting that YieldGard CB hybrids are changing the proportion of Lepidopteron larvae that damage YieldGard CB hybrids and are indirectly favoring the western bean cutworm's survival which cause more damage than the previous pest corn earworms, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). This phenomenon, known as pest replacement (Pedigo and Rice 2006), may be a partial explanation for the recent eastern explanation of the western bean cutworm.

Future research should examine any change in the ratio of western bean cutworms, corn earworms, and European corn borers in an area as YieldGard CB hybrids are introduced and differences between the composition of Lepidoptera larvae in YieldGard CB, Herculex, and non-*Bt* hybrids. Those results may help explain the recent eastern expansion of western bean cutworms. Research should also be focused as developing an economic threshold for using Herculex hybrids.

Acknowledgments

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Table 1. Rating scale (9-1) used to rate western bean cutworm injury to corn ears.

9-No damage to ear tips or kernels, slight damage to silks or husks (i.e. a nibble or two).

8-Slight damage to silks, husks, eartips but no kernel damage.

7-Small damage to silks, husks, eartips and slight damage to kernels (1-2 kernels damaged/lost).

6-Small damage to silks, husks, eartips, and 0.1-1.0 cm (> 2) kernels damaged/lost.

5-Moderate damage to silks, husks, cob tips and/or 1.1-2.0 cm of kernels lost.

4-Moderate damage to silks, husks, cob tips and/or 2.1-3.0 cm of kernels lost.

3-Heavy damage to silks, husks, cob tips and/or 3.1-4.0 cm of kernels lost.

2-Heavy damage to silks, husks, cob tips and/or 4.1-5.0 cm of kernels lost.

1-Heavy damage to silks, husks, cob tips and/or 5.1 (+) cm of kernels lost.

note: centimeters, measured from the tip toward the butt of the ear.

Table 2. Western bean cutworm damage to Herculex I or Herculex Xtra (HX), YieldGard CB (YG), and a check (X) at four Iowa locations, 2005*.

Location	Trait	Hybrid	No. ears examined	Percent infested	No. ear scored	Ear score (9-1)
New Providence	HX	34A16	200	9.0	20	8.9±0.4a
New Providence	YG	33R78	200	55.0	20	5.6±0.4c
New Providence	X	34A15	200	80.5	20	7.4±0.4b
Independence	HX	34A16	200	10.5	20	8.4±0.4a
Independence	HX	33B55	200	16.5	20	8.5±0.4a
Independence	YG	33B51	200	79.5	20	4.1±0.4c
Independence	YG	34M95	200	95.5	20	4.5±0.4c
Independence	X	34A15	200	37.0	20	6.2±0.4b
Jesup	HX	34A16	200	29.5	20	7.3±0.4a
Jesup	HX	33A87	200	16.0	20	7.6±0.4a
Jesup	YG	33P67	100	88.0	20	4.4±0.4bc
Jesup	YG	33B51	100	87.0	20	4.7±0.4bc
Jesup	YG	36B03	100	71.0	20	7.5±0.4a
Jesup	X	34A15	200	60.0	20	3.7±0.4c
Jesup	X	33A84	200	71.0	20	5.3±0.4b
Hubbard	HX	34A16	200	5.5	20	8.5±0.4a
Hubbard	X	34A15	200	34.0	20	6.9±0.4b

*Mean±SEM at the same location and followed by the same letter are not significantly different using Tukey's adjustment for multiple comparisons with P≤ 0.05 considered significant.

Table 3. Western bean cutworm damage (summarized by location) to Herculex I or Herculex Xtra (HX),

YieldGard CB (YG), and a check (X) at four Iowa locations, 2005*.

Location	Traits	reps	No. ears examined	Percent ears infested	No. ears scored	Ear score (9-1)
New Providence	HX	1	200	9.0	20	8.9±0.4a
New Providence	YG	1	200	55.0	20	5.6±0.4b
New Providence	X	1	200	80.5	20	7.4±0.4a
Independence	HX	2	400	13.5±6.0a	40	8.5±0.4a
Independence	YG	2	400	87.5±6.0b	40	4.3±0.3c
Independence	X	1	200	37.0a	20	6.2±0.4 b
Jesup	HX	2	400	22.8±6.5a	40	7.4±0.3a
Jesup	YG	3	300	82.0±5.3b	60	5.5±0.3b
Jesup	X	2	400	65.5±6.5b	40	4.5±0.3b
Hubbard	HX	1	200	5.5	20	8.5±0.4a
Hubbard	X	1	200	34.0	20	6.9±0.4b

*Mean±SEM at the same location and followed by the same letter are not significantly different using

Tukey's adjustment for multiple comparisons with P≤ 0.05 considered significant.

Table 4. Western bean cutworm damage by trait to Herculex I or Herculex Xtra

(HX), YieldGard CB (YG), and a check (X) at four Iowa locations, 2005*.

Traits	reps	Ears examined	Percent ears infested	Ears scored	Ear score (9-1)
HX	6	1,200	14.5±6.1a	120	8.2±0.2a
YG**	6	900	79.3±6.1c	120	5.1±0.2c
X	5	1,000	56.5±6.7b	100	5.9±0.2b

*Mean±SEM followed by the same letter are not significantly different using

Tukey's adjustment for multiple comparisons with P≤ 0.05 considered significant.

**No data from Hubbard.

Table 5. Western bean cutworm damage to Herculex I or Herculex Xtra (HX), YieldGard CB (YG), and a check (X) at four Iowa locations, 2006*.

				Percent Infested				
Location	Trait	Hybrid	No. ears examined	WBC	CEW	ECB	No. ears scored	Ear score (9-1)
New Providence	HX	34A16	100	12	-	-	20	7.9±0.5a
New Providence	X	35T06	100	70	-	-	20	4.8±0.5b
Conrad	HX	34A16	100	0	0	1	20	8.6±0.3a
Conrad	YG	33B51	100	10	1	0	20	8.4±0.3a
Conrad	X	34A15	100	3	1	8	20	8.7±0.3a
Jesup	HX	34A18	100	3	0	0	20	8.0±0.5a
Jesup	YG	34H39	100	7	0	0	20	7.2±0.5a
Jesup	X	34A19	100	15	0	6	20	6.8±0.5a
Independence	HX	34A16	100	4	0	0	20	8.7±0.5a
Independence	YG	34H38	100	40	0	0	20	6.6±0.5b
Independence	X	34A15	100	32	1	7	20	6.0±0.5b

WBC= western bean cutworm, CEW= corn earworm, ECB= European corn borer

*Mean±SEM at the same location and followed by the same letter are not significantly different using Tukey's adjustment for multiple comparisons with P≤ 0.05 considered significant.

Table 6. Western bean cutworm damage by trait to Herculex I or Herculex Xtra (HX), YieldGard CB

(YG), and a check (X) at four Iowa locations, 2006*.

			Percent Ears Infested				
Traits	reps	Ears examined	WBC	CEW**	ECB**	Ears scored	Ear score (9-1)
HX	4	400	4.8±10.2	0.0±0.3a	0.3±0.3a	80	8.3±0.2a
YG[§]	3	300	19.0±11.7	0.3±0.3a	0.0±0.3a	60	7.4±0.3c
X	4	400	30.0±10.2	0.7±0.3a	7.0±0.3b	80	6.5±0.2b

WBC= western bean cutworm, CEW= corn earworm, ECB= European corn borer

*Mean±SEM followed by the same letter are not significantly different using Tukey's adjustment for multiple

comparisons with $P \leq 0.05$ considered significant.

**CEW and ECB were only counted in three of the four locations.

[§]No data from New Providence.

CHAPTER 5: Intraguild Competition and Enhanced Survival of Western Bean

Cutworm (Lepidoptera: Noctuidae) on Transgenic Corn

A paper to be submitted to *The Journal of Economic Entomology*

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ABSTRACT The effect of genetically engineered corn (YieldGard[®] CB) expressing the

Bacillus thuringiensis sp. *kurstaki* (Berliner) (Bt) endotoxin, Cry1Ab, on the survivability

of western bean cutworm, *Striacosta albicosta* (Smith), larvae during intraguild

competition studies with either European corn borer, *Ostrinia nubilalis* (Hübner), or corn

earworm, *Helicoverpa zea* (Boddie), larvae were examined. Competition scenarios were

constructed by using either a laboratory or field competition arena containing one of five

different diets and one of thirteen different larval size by species scenarios. The

survivability of *S. albicosta* competing with *H. zea* in the laboratory arenas on a meridic

diet and iso-line corn silks was significantly lower than the controls (P≤0.01) in 13 out of

14 competition scenarios and was frequently zero. However, the survivability of *S.

albicosta* competing with *H. zea* in the laboratory arenas with YieldGard[®] CB corn silk

diet were significant lower (P≤0.01) than the controls in only 2 out of 6 competition

scenarios. Meaning that the survivability of western bean cutworms competing with corn

earworms on YieldGard CB silks were not significantly different from the controls in 4

our of 6 scenarios compared with 1 out of 13 scenarios on iso-line silks. The

survivability of *S. albicosta* in the three way competition scenarios in the laboratory

arenas using the iso-line corn silks were significantly lower (P≤0.01) from the controls in 7 out of 7 competition scenarios, but the survivability of *S. albicosta* on the YieldGard® CB corn silk diet was significantly lower (P≤0.01) than the controls in only 5 out of 7 competition scenarios. Again, western bean cutworms competing with corn earworms and European corn borers on iso-line silks had near zero survivability while western bean cutworms competing with corn earworms and European corn borers on YieldGard CB silks had the same survivability as the controls in 2 out of 7 competition scenarios. These data indicate that YieldGard® CB confers a competitive advantage to *S. albicosta* larvae within the presence of intraguild competition from *O. nubilalis* and *H. zea* larvae and that western bean cutworms become equal competitors only when they are of equal of larger size and the diet is YieldGard CB silks.

KEY WORDS maize, competition, *Bacillus thuringiensis*, *Striacosta albicosta*, YieldGard

Introduction

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), is native to North and Central America (Douglass et al. 1957, Lafontaine 2004) and is a pest of dry beans (*Phaseolus* spp.) and corn (*Zea mays* L.). The species was described in 1887 from specimens collected in Arizona (Smith 1887), and since that time it has expanded its distribution northward into Rocky Mountain states and eastward into the central Great Plains. By 1970, it was causing significant damage throughout Nebraska and a few small populations were found as far east as counties bordering the

Missouri River in western Iowa (Crumb 1956, Keith et al. 1970, USDA 1970, Antonelli 1974, USDA 1977, Blickenstaff 1979). However, *S. albicosta* did not become a serious pest in Iowa as it had in Nebraska and its occurrence was only sporadically documented in Iowa. Approximately 30 years later in 2000, a commercial corn field in west central Iowa was heavily infested with *S. albicosta* larvae, with kernel damage noted on 95 percent of the ears and some ears containing 6 larvae per ear (Rice 2000). In the years that followed, the western bean cutworm expanded its distribution across Iowa and by late summer, 2004, it could be found in most eastern Iowa counties and was captured for the first time in Illinois and Missouri (Dorhout and Rice 2004). Two years later, it was documented for the first time in Indiana, Ohio, and Michigan (Rice and Dorhout 2006, Pope 2007).

Western bean cutworm expansion across the Corn Belt is a concern because it damages corn by directly feeding on the kernels in the ear. One larva per corn plant at dent stage reduces yields by 2.32 q/ha (3.7 bu/acre) (Appel et al. 1993). Each Larva feed on corn for a considerable length of time, taking an average of 55.9 days to complete their larval development at 27°C, 16:8 (L:D) (Antonelli 1974) with only one generation per year. Larvae are not cannibalistic and heavily infested corn may have as many as 20 per ear (Seymour 1998). Western bean cutworms, along with European corn borers, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and corn earworms, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) are the principal larva causing corn ear damage in Iowa. These three lepidopteran species represent a guild, which is a group of species that exploits the same class of environmental resources in the same way (Root 1967).

The European corn borer is found in all the corn growing regions of North America from the Atlantic coast to the Rocky Mountains (Steffey et. al 1999). Larvae damage corn principally by tunneling through the stalk and they may directly feed on corn kernels. Larvae take approximately 14 days to complete their development at 27°C, 16:8 hour light dark period (L:D) (USDA ARS, Ames, Iowa). There are two, and occasionally three, generations a year in Iowa (Mason et al. 1996). They are not cannibalistic and multiple larvae may be found throughout the corn stalk, in leaf midribs, and ear.

The corn earworm is found throughout the United States wherever corn is grown (Steffey et. al 1999), but it can not overwinter north of $40°$ latitude and must migrate into these areas each summer. Larvae damage corn by feeding directly on the corn kernels in the ear and they require 18.9 days to complete their larval development at 27°C, 16:8 (L:D) (Butler 1976). There may be two or three generations a year in Iowa but they are considered a minor pest of field corn (Steffey et. al 1999). Larvae of *H. zea* are aggressive and cannibalistic (Barber 1936), which typically results in only one late stage larva per ear.

Since the western bean cutworm distribution has rapidly expanded from 2000 to 2006, it seemed appropriate to examine any farming practices that changed just prior to their resurgence and that may be affecting their population dynamics. One major change in farming that occurred in 1996 was the introduction of genetically engineered corn to control the European corn borer. YieldGard® CornBorer (hereafter YieldGard CB) (event MON810) (Monsanto Company, St. Louis, MO), is now the major technology in the corn market and expresses the *Bacillus thuringiensis* sp. *kurstaki* (Berliner) (Bt) endotoxin,

Cry1Ab (Koziel et. al. 1993, Armstrong et al. 1995). YieldGard CB controls 100% of

European corn borer larvae and 75-88% of corn earworm larvae (Gould 1994, Burkness

et. al 2001, Horner et. al 2003.) while not negatively affecting western bean cutworms

(Helms and Wedberg 1976, Catangui and Berg 2006). YieldGard CB has also been

shown to reduce the cannibalistic behavior of corn earworms (Horner and Dively 2003.).

Since YieldGard CB controls European corn borer and corn earworm while not

negatively affecting western bean cutworm, it therefore seems plausible that YieldGard

CB might be influencing their survivability and facilitating their expanding distribution

by providing a relatively exclusive habitat that, of these guild members, only western

bean cutworms can utilize.

The objective of this study was to examine the possible effect that genetically

engineered corn (YieldGard CB) has on the survivability of western bean cutworms

during intraguild competition from European corn borer and corn earworm larvae.

Methods and Materials

Experimental Design. A completely randomized design was used with

treatments replicated based on availability of *S. albicosta* and *H. zea* larvae. The

experiment had three factors and followed a 2 x 5 x 13 treatment design that was

unbalanced and incomplete (Fig. 1). The three factors were competition arena, larval

diet, and intraguild competition.

The first factor, competition arena, was divided into field trials using cages on

corn ears or laboratory trials using plastic cups containing diet. The field trials used

cages constructed from aluminum hardware cloth (4 mm^2 mesh) cut into 30 cm squares.

One edge of the hardware cloth was placed half way along the major axis of the corn ear, which allowed approximately 15 cm of the hardware cloth to extend beyond the ear tip. The hardware cloth was then rolled two or three times around the corn ear, thereby forming a cylinder. The bottom of the cage was held tight around the corn ear by cinching a cable tie over both the hardware cloth and the corn ear. Larvae were placed on the corn silks, then the top of the cage was closed by folding the end back on itself and stapling it while leaving an internal space of approximately 10 cm between the ear tip and the end of the cage. Corn ear cages were not used in 2005 because of limited western bean cutworm availability. The laboratory trials used clear plastic cups (18ml or 30ml in 2005 and 2006, respectively) (Fill Rite, Newark, NJ) filled halfway with a particular diet.

The second factor was divided into five levels based on the type of diet used within the competition arenas. These were a modified meridic black cutworm diet, YieldGard CB corn silks (Corn States 304 YG, Des Moines, IA), non-transgenic iso-line corn silks (Corn States 304, a near iso-line for Corn States 304 YG) YieldGard CB corn ears (Corn States 304 YG, Des Moines, IA), and non-transgenic iso-line corn ears (Corn States 304, a near iso-line for Corn States 304 YG). Diet preparation is described below.

The third factor was divided into thirteen levels based on intraguild competition scenarios comprised of different instar and species combinations (Fig. 1). There were a total of eight treatments (2005) or 30 treatments (2006) across all diets and competition arenas.

Insect Cultures. In 2005, adult western bean cutworm, European corn borer, and corn earworm were collected from blacklight traps near Ames, IA for the purpose of establishing laboratory colonies. In 2006, corn earworm eggs were commercially

purchased (French Agricultural Research, Inc. Lamberton, MN) while western bean cutworm and European corn borer adults were again collected from blacklight traps near Ames, IA to establish laboratory colonies.

Diet Preparation. Prior to use in an experiment, European corn borers were maintained on a standard wheat germ meridic diet (Lewis and Lynch 1969) while western bean cutworms and corn earworms were maintained on a modified meridic black cutworm diet with aureomycin and benlate deleted (Hendrix et. al 1991). All larvae were held in an environmentally controlled room at 27°C, 16:8 (L:D), and approximately 80% RH until they were used for experimentation.

Corn silks from YieldGard CB and the iso-line hybrid were removed from the respective ears as needed, washed in a 2% bleach solution for 4 minutes, and then rinsed in water for 4 minutes. Only green silks were used.

Treatments. Two-way intraguild competitions were constructed by placing a western bean cutworm larva with a corn earworm larva, each of a particular size, and recording western bean cutworm survival after nine days. Three-way intraguild competitions included the previous two species plus an European corn borer of a particular size. The larvae for a particular competition were selected from three relative size categories (small, medium and large). Larvae were placed in a particular size category based on their overall body size and their similarity to a larva of a different species within the same size category (Table 1). Thus, in a treatment version of a small vs. small competition, a second instar western bean cutworm competed with a third instar *H. zea*, however, the two species were of approximate equal sizes. Competitions among larvae are presented as sizes (small, medium or large) of western bean cutworm versus

corn earworm, or in the case of three-way competitions, the last species is always the European corn borer, i.e. MvMvL represents a medium western bean cutworm staged against a medium corn earworm and a large European corn borer.

Controls were constructed by using two (or three for the three-way competitions) western bean cutworms of the same sizes as the two or three larvae in the competition treatments. These controls mimicked the effect of resource and spatial competition, but not the behavior so that the unique effects of corn earworm and European corn borer behavior on western bean cutworm survivability could be determined. Each competition in the laboratory was conducted at 27°C, 16:8 (L:D) and approximately 80% RH for nine days in a walk-in environmental chamber. The field competitions on corn ears were conducted for nine days from July 27 to August 5, 2006.

Statistical Analyses. Data from each competition were analyzed for independence using a Chi-square test (CHISQ option of the SAS procedure PROC FREQ; SAS Institute 2007) between the survivability of the treated western bean cutworm and its corresponding control. Averages are expressed as Mean±SEM with treatment differences determined to be significant at $P \leq 0.01$.

Results

A limited supply of western bean cutworm larvae in 2005 prevented some competition treatments from being conducted. Additionally, a pathogenic mold problem in the environmental chamber killed larvae in some of the replicates; these replicates were then removed from the study.

Competitions on Meridic Diet. In 2005, the survivability of *S. albicosta* competing with *H. zea* was significantly lower than the controls across all scenarios on the meridic diet (Table 2, Fig. 3, P≤0.01). *Striacosta albicosta* competing with *H. zea* had zero percent survivability in the small vs. large and large vs. large competition scenarios. There was a small increase in the survivability of *S. albicosta* in the small vs. small and large vs. small scenarios, 16.7±15.2 and 28.6±17.1 percent survivability, respectively. The survivability of all of the control *S. albicosta* scenarios were 100 percent except for the large vs. small scenario that had a survivability of 91.7±8.0 percent.

In 2006, Survivability of *S. albicosta* in 2006 (Fig. 3) followed the same trends observed in 2005 (Fig. 2). The survivability of *S. albicosta* competing with *H. zea* were significantly lower than the controls across all scenarios on the meridic diet (Table 3, P≤0.01). Treated *S. albicosta* again had zero percent survivability in the small vs. large and large vs. large competition scenarios. There was an increase in the survivability of *S. albicosta* in the small vs. small and large vs. small scenarios, 16.0±15.0 and 48.0±20.4 percent survivability, respectively. Survivability of the controls ranged from 74.0±17.9 to 92.0±11.1 percent survivability, respectively.

Competitons on Corn Silk Diets. In 2005, similar results were observed in both scenarios (small vs. small and large vs. large) on the iso-line silk diets where the *S. albicosta* competing with *H. zea* had zero percent survivability while the control *S. albicosta* had 100 percent survivability (Table 2, Fig. 3). During this time the progress of the competitions were examined to determine the fate of the *S. albicosta* and it was observed that the *H. zea* were consuming the *S. albicosta*. In the small vs. small

competition scenario all of the *S. albicosta* had been consumed by the *H. zea* within the first 72 hours of the experiment.

Very different results were observed in both scenarios (small vs. small and large vs. large) on the YieldGard® CB silk diet where there was no significant difference between the survivability of the treated and control treatments. The treated *S. albicosta* competing with *H. zea* had 100 percent survivability in both scenarios while the survivability of the controls in the small vs. small and large vs. large scenarios was 83.3±10.8 and 91.7±8.0 percent, respectively.

In 2006, two more 2-way interactions on YieldGard® CB and non-transgenic corn silks were added (Fig. 1). On the iso-line silk diets (Fig. 4), there were again results similar to those observed in 2005 (Table 3, Fig. 3) where the survivability of *S. albicosta* competing with *H. zea* was significantly lower than the controls across all scenarios except in the large vs. small competition where the treatment was not significantly different from the control, 60.0±20.0 and 76.0±17.4 percent survivability, respectively.

The survivability of treated *S. albicosta* on the YieldGard® CB silk diet (Table 3, Fig. 4) was again different than the survivability of treated *S. albicosta* on the meridic or iso-line silk diets. The survivability of treated *S. albicosta* in the small vs. small or large vs. small competition scenarios (68.0±19.0 and 100 percent, respectively) on the YieldGard® CB silk diet were not significantly different from the controls (90.0±12.3 and 83.3±15.2 percent, respectively). However, the survivability of treated *S. albicosta* in the small vs. large or large vs. large competition scenarios (0 and 18.2±15.8 percent, respectively) on the YieldGard® CB silk diet were significantly different from the controls (93.8±9.9 and 64.3±19.6 percent, respectively).

In 2006, seven 3-way interactions with *O. nubilalis* were added (fig. 1). The survivability of the treated *S. albicosta* in the three way competition (Table 3, Fig. 4) on the iso-line were significantly lower than the survivability of the control *S. albicosta* in all scenarios ($P \leq 0.01$, Fig. 4). In contrast, there were two scenarios (small vs. small vs. small and large vs. small vs. small) on the YieldGard[®] CB silk diet where the treated *S. albicosta* survivability (62.5 ± 19.8 and 75.0 ± 17.7 percent, respectively) was not significantly different from the controls (95.8 ± 8.2 and 79.2 ± 16.6 percent, respectively). Adding *O. nubilalis* for the three way competition scenarios (Table. 4) did not significantly affect the survivability of the treated *S. albicosta* when compared to the similar two way competition (Fig. 3) (i.e. comparing small vs. small to small vs. small vs. small and small vs. small vs. large).

Field Trial. During 2005, there were insufficient western bean cutworm larvae available for conducting experiments in the field. In 2006, sufficient numbers of larvae were available and the field trial was initiated. The survivability of western bean cutworms in all the treatment competitions on the YieldGard CB and iso-line corn hybrids were not significantly different from the controls (Fig. 3 and Table 3). There was a natural population of western bean cutworms in the plot and they confounded these data. At the end of the experiment, more western bean cutworms were found in the ear cages than were originally placed in them. It is suspected that early instar western bean cutworms were already in the ears before the cages were attached and went undetected at the beginning of the experiment.

Discussion

The objective of this study was to measure the potential effects of genetically engineered YieldGard CB corn expressing the *Bt* endotoxin, Cry1Ab (Koziel et. al. 1993, Armstrong et al. 1995) on the survivability of western bean cutworm larvae in the presence of potential intraguild competitors: corn earworms and European corn borers. The survivability of western bean cutworms in the presence of corn earworm larvae on either a meridic diet or iso-line corn silks was near zero and significantly lower than the control survivability of almost 100 percent (Figs. 2-4). Corn earworm larvae out compete western bean cutworms by attacking and sometimes killing them, even if the western bean cutworm was initially larger than the corn earworm. This is probably because corn earworm larvae develop approximately three times faster than western bean cutworms in the same conditions (Antonelli 1974, Butler 1976) and are aggressive, even against conspecifics (Barber 1936). In contrast, western bean cutworm survivability on YieldGard CB corn silks was near 100 percent when the larvae were of equal or larger size than the corn earworms and was not significantly different than the controls. These results also show that European corn borers, in the three way interactions, generally do not significantly alter the competition outcomes seen in the two way interactions and are probably not the primarily guild member responsible for reducing the survivability of western bean cutworms (Fig. 3-5).

These data indicate that YieldGard® CB confers a competitive advantage to *S. albicosta* larvae within the presence of intraguild competition from *O. nubilalis* and *H. zea* larvae and that western bean cutworms become equal competitors only when they are of equal of larger size and the diet is YieldGard CB silks. Thus, if competition from corn

earworm populations had been suppressing either the movement or population increase of western bean cutworms east of Nebraska, then the recent introduction and widespread planting of YieldGard CB could have acted as an environmental release for western bean cutworms by suppressing corn earworm populations and enabling western ben cutworms to become competent competitors.

Future research should repeat the field component of this experiment, but exclusion cages should be placed over the corn after it is planted so that natural infestations of western bean cutworms do not confound the results.

Acknowledgments

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Vol. 20.

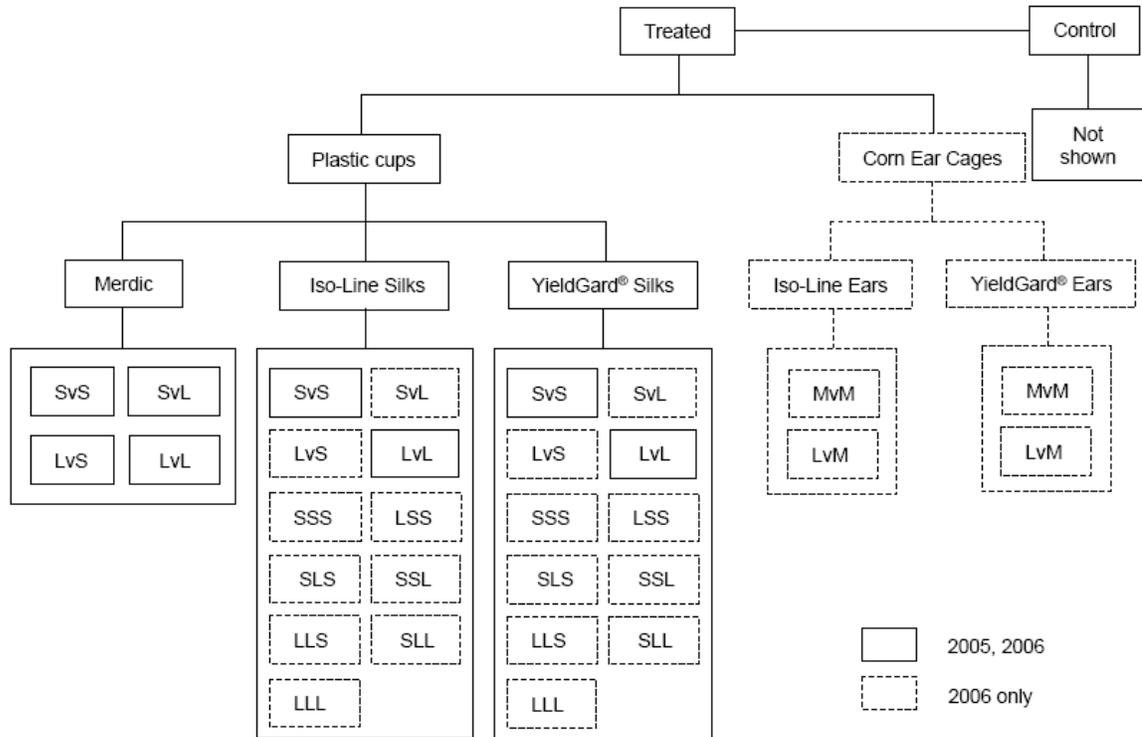**USDA, Animal and Plant Health Inspection Service. 1977.** Coop Plant Pest Report.

Vol. 2.

Fig. 1. Flow chart showing the order of the factors examined in this study. The order of the factors for the control is the same as the treated, but is not shown. First letter corresponds to the size of the *Striacosta albicosta* larva and the second and third letters corresponds to the size of the *Helicoverpa zea* and *Ostrinia nubilalis* larvae, respectively. S=small, M=medium, L=large; v=versus and denotes a two way competition.
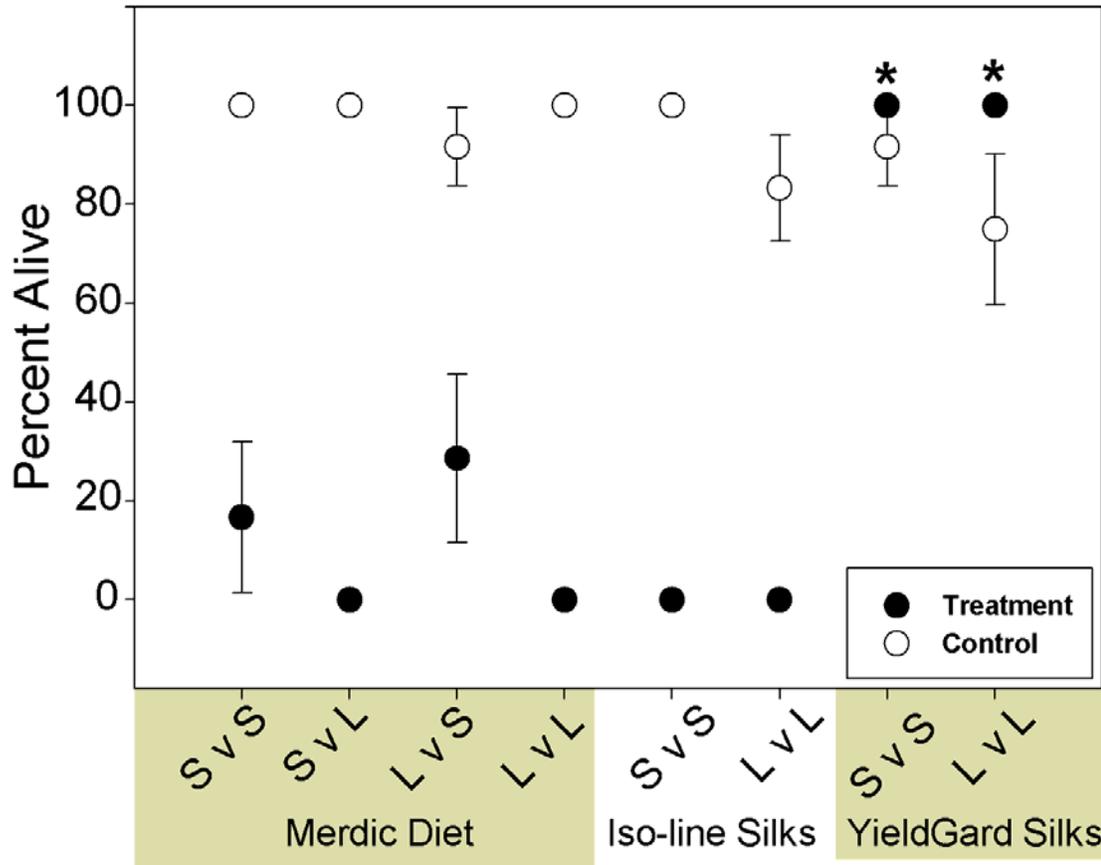
Fig. 2. Average survivability of *Striacosta albicosta* competing with *Helicoverpa zea* in 2005 (mean±SEM). First letter corresponds to the size of the *Striacosta albicosta* larvae and the second letter corresponds to the size of the *Helicoverpa zea* larvae.

* no significant difference between the survivability of the treated and control *Striacosta albicosta* using a Chi-square test, P ≤0.01.
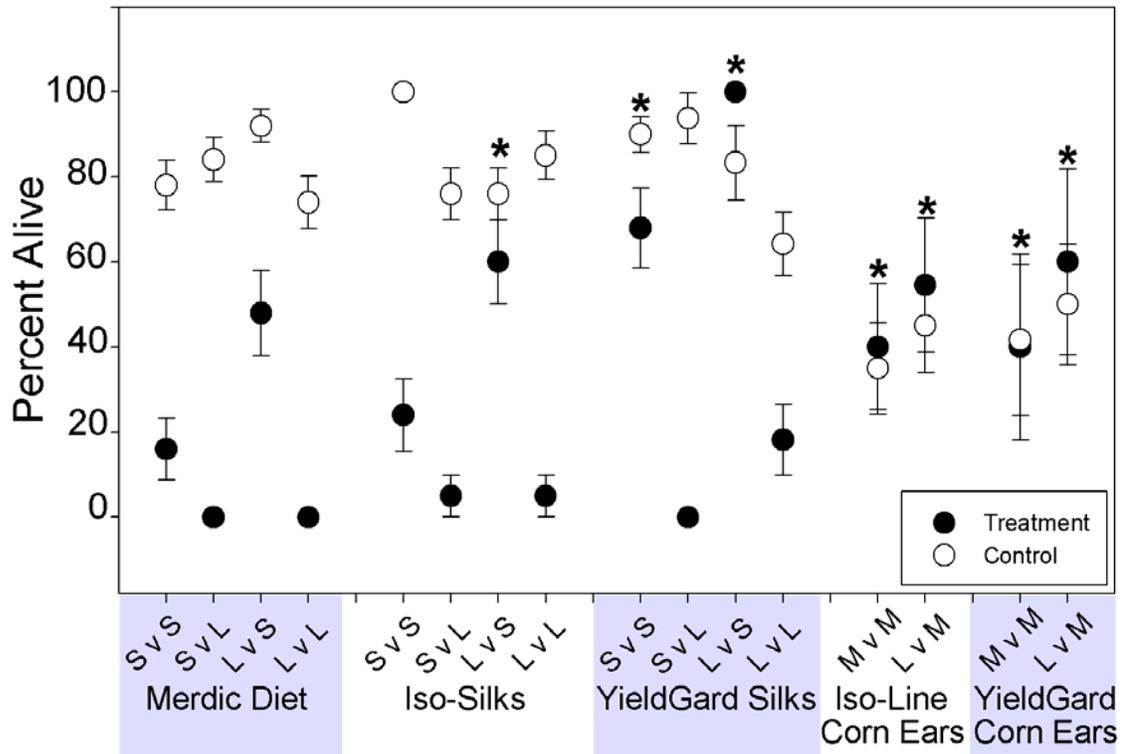
S= Small, L= Large, v=versus.

Fig. 3. Average survivability of *Striacosta albicosta* competing with *Helicoverpa zea* in 2006 (mean±SEM). First letter corresponds to the size of the *Striacosta albicosta* larvae and the second letter corresponds to the size of the *Helicoverpa zea* larvae.

* no significant difference between the survivability of the treated and control *Striacosta albicosta* using a Chi-square test, P ≤0.01.
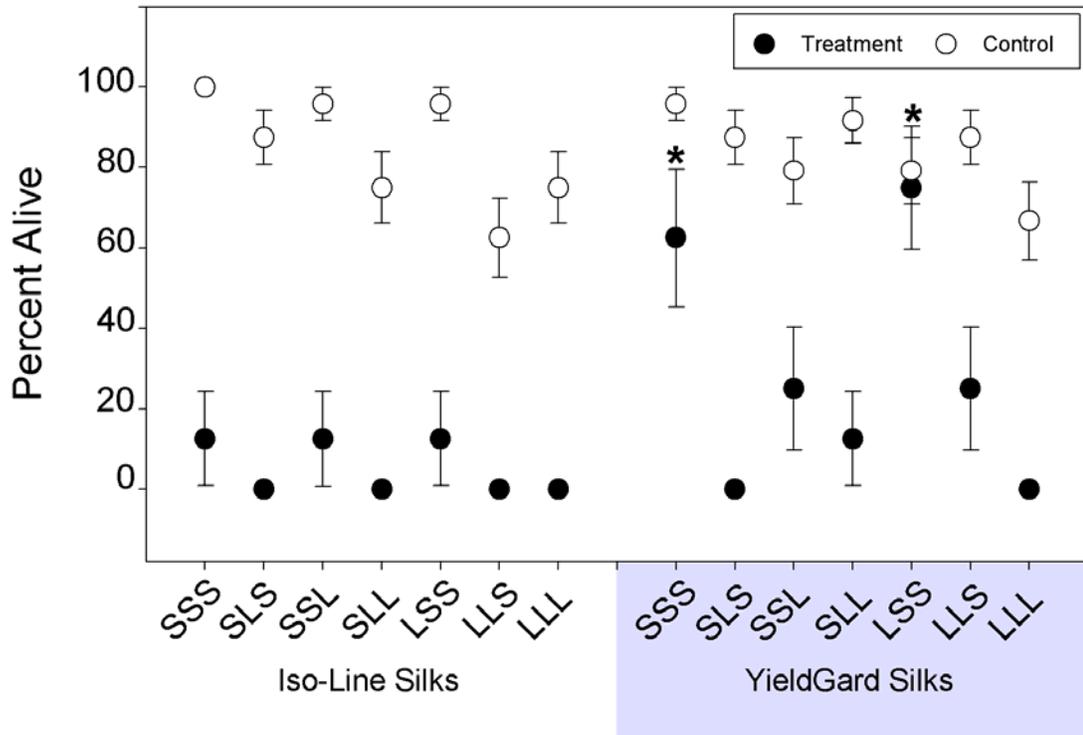
S=Small, M=Medium, L=Large, v=versus

Fig. 4. Average survivability of *Striacosta albicosta* competing with *Helicoverpa zea* and *Ostrinia nubilalis* in 2006 (mean±SEM). First letter corresponds to the size of the *Striacosta albicosta* larvae and the second and third letters corresponds to the size of the *Helicoverpa zea* and *Ostrinia nubilalis* larvae, respectively.

* no significant difference between the survivability of the treated and control *Striacosta albicosta* using a Chi-square test, P ≤0.01.

S=Small, L=Large, v=versus

Table 1. Species, instar sizes, and relative size

categories used in competition studies.

Insect	Instar	Size category
Striacosta albicosta	2,3	Small
	3	Medium
	4,5	Large
Helicoverpa zea	3,4	Small
	4	Medium
	5	Large
Ostrinia nubilalis	3,4	Small
	6	Large

Table 2. Competition results from 2005. First letter corresponds to the size of the *Striacosta albicosta* larvae and the second letter corresponds to the size of the *Helicoverpa zea* larvae.

Diet	Scenario	df	Chi-squared value	Chi-squared probability	Treated Alive *S. albicosta*	Treated Number of reps	Control Alive *S. albicosta*	Control Number of reps
Artificial	sm vs. sm	1	13.8462	0.0002	1	6	12	12
Artificial	sm vs. lg	1	19.0000	< 0.0001	0	7	12	12
Artificial	lg vs. sm	1	8.1458	0.0043	2	7	11	12
Artificial	lg vs. lg	1	19.0000	< 0.0001	0	7	12	12
Iso line silks	sm vs. sm	1	19.0000	< 0.0001	0	7	12	12
Iso line silks	lg vs. lg	1	12.3148	0.0004	0	7	10	12
YG silks	sm vs. sm	1	0.5294	0.4669	6	6	11	12
YG silks	lg vs. lg	1	0.9167	0.3384	3	3	6	8

sm=small, lg=large, vs=versus P ≤0.01.

Table 3. Competition results from 2006. Scenarios ordered *S. albicosta*, *H zea*, and then *O. nubilalis* sm=small, lg=large, vs.=versus P ≤0.01.

Diet	Scenario	df	Chi-squared value	probability	Treated Alive *S. albicosta*	Number of reps	Control Alive *S. albicosta*	Number of reps
Artificial	sm vs. sm	1	26.1900	<0.0001	4	25	39	50
Artificial	sm vs. lg	1	47.7273	<0.0001	0	25	42	50
Artificial	lg vs. sm	1	18.4077	<0.0001	12	25	46	50
Artificial	lg vs. lg	1	36.5132	<0.0001	0	25	37	50
Iso line silks	sm vs. sm	1	50.8929	<0.0001	6	25	50	50
Iso line silks	sm vs. lg	1	29.1869	<0.0001	1	20	38	50
Iso line silks	lg vs. sm	1	2.0583	0.1514	15	25	38	50
Iso line silks	lg vs. lg	1	35.1086	<0.0001	1	20	34	50
YG silks	sm vs. sm	1	5.6297	0.0177	17	25	45	50
YG silks	sm vs. lg	1	20.0000	<0.0001	0	8	15	16
YG silks	lg vs. sm	1	1.5072	0.2196	8	8	15	18
YG silks	lg vs. lg	1	12.2872	0.0005	4	22	27	42
YGCornEar	lg vs. md	1	0.1238	0.7249	3	5	4	8
YGCornEar	md vs. md	1	0.0040	0.9493	2	5	5	12
IsoCornEar	lg vs. md	1	0.2589	0.6109	6	11	9	20
IsoCornEar	md vs. md	1	0.0718	0.7888	4	10	7	20
Iso3way	lg vs. lg vs. lg	1	13.7143	0.0002	0	8	18	24
Iso3way	lg vs. lg vs. sm	1	9.4118	0.0022	0	8	15	24
Iso3way	lg vs. sm vs. sm	1	22.2222	<0.0001	1	8	23	24
Iso3way	sm vs. lg vs. lg	1	13.7143	0.0002	0	8	18	24
Iso3way	sm vs. lg vs. sm	1	20.3636	<0.0001	0	8	21	24
Iso3way	sm vs. sm vs. lg	1	22.2222	<0.0001	1	8	23	24
Iso3way	sm vs. sm vs. sm	1	26.88	<0.0001	1	8	24	24
YG3way	lg vs. lg vs. lg	1	9.9388	0.0016	0	8	16	25
YG3way	lg vs. lg vs. sm	1	11.5942	0.0007	2	8	21	24
YG3way	lg vs. sm vs. sm	1	0.6100	0.8050	6	8	19	24
YG3way	sm vs. lg vs. lg	1	18.6023	<0.0001	1	8	22	24
YG3way	sm vs. lg vs. sm	1	20.3636	<0.0001	0	8	21	24
YG3way	sm vs. sm vs. lg	1	7.8038	0.0052	2	8	19	24
YG3way	sm vs. sm vs. sm	1	6.0952	0.0136	5	8	23	24

Table 4. Comparison between the survivability of *Striacosta albicosta* in two way and three way competition scenarios where the size of the *Striacosta albicosta* and *Helicoverpa zea* larvae was held constant and *Ostrinia nubilalis* larvae of two different sizes were added. Chi- Square probabilities less than 0.01 would indicate that adding the *Ostrinia nubilalis* larvae significantly altered the survivability of *Striacosta albicosta*. First letter corresponds to the size of the *Striacosta albicosta* larvae and the second and third letters corresponds to the size of the *Helicoverpa zea* and *Ostrinia nubilalis* larvae, respectively.

Diet	Two way scenario	Three way scenario	df	Chi- square value	Chi- square probability
Iso-line corn silks	sm vs. sm	sm vs. sm vs. sm	1	0.48	0.49
		sm vs. sm vs. lg	1	0.48	0.49
	sm vs. lg	sm vs. lg vs. sm	1	0.41	0.52
		sm vs. lg vs. lg	1	0.41	0.52
	lg vs. sm	lg vs. sm vs. sm	1	5.47	0.02
		lg vs. sm vs. lg		-	-
	lg vs. lg	lg vs. lg vs. sm	1	0.41	0.52
		lg vs. lg vs. lg	1	0.41	0.52
YieldGard® CB corn silks	sm vs. sm	sm vs. sm vs. sm	1	0.08	0.77
		sm vs. sm vs. lg	1	4.59	0.03
	sm vs. lg	sm vs. lg vs. sm	1	0.00	1.00
		sm vs. lg vs. lg	1	1.07	0.30
	lg vs. sm	lg vs. sm vs. sm	1	2.29	0.13
		lg vs. sm vs. lg		-	-
	lg vs. lg	lg vs. lg vs. sm	1	0.17	0.68
		lg vs. lg vs. lg	1	1.68	0.20

sm=small, lg=large, vs.=versus , "-" indicates competition scenario not performed because of limited numbers of *Striacosta albicosta*

CHAPTER 6: Spatial Distribution in the Great Plains of a Microsporidia in Adult Western Bean Cutworms (Lepidoptera: Noctuidae)

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ABSTRACT The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) in an economically important pest that has expanded its distribution from Colorado in 1896 eastward into Ohio and Michigan by 2007. However, for approximately 30 years from 1970 to 1999, western bean cutworm expansion appeared to stall on the border of Nebraska and Iowa. Beginning in 1999, the western bean cutworm started to expand its eastward distribution again, expanding into seven new states and more than 1,000 km (650 miles) in six years. Understanding why the western bean cutworm is expanding its distribution again and what apparently stalled its eastward expansion for 30 years may help management efforts. Research examining *Nosema pyrausta* in European corn borers found that flight fitness decreased as the spore count increased. Western bean cutworms are known to be infected with an unidentified microsporidium that may affect the western bean cutworm's flight characteristics in the same detrimental way that *N. pyrausta* does European corn borers. This study examined the frequency and the level of infection from an unidentified Microsporidium (Zygomycota: Microsporidia) in western bean cutworms collected in 17 locations from Wyoming to Indiana and between individuals collected at a central Iowa location in 2004

and 2006. The results indicate that the frequency of infected individuals in central Iowa was higher in 2006 (27 infected out of 48) than in 2004 (6 infected out of 48). Results from earlier research suggested that 100% of the individuals would be infected; however infection levels differed significantly by location and no location had an infection frequency of 100%. The 2006 distribution data also indicated that the number of infected individuals were significantly different east and west of the Missouri River. The mean number of spores was also significantly different between locations. These results indicated that there is a spatial relationship with the Microsporidium.

KEY WORDS *Striacosta albicosta*, *Nosema*, distribution expansion, *Bacillus thuringiensis* sp. *kurstaki*

Introduction

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), is native to North and Central America (Douglass et al. 1957, Lafontaine 2004) and is a pest of dry beans, *Phaseolus* spp. and corn, *Zea mays* L. The species was described in 1887 from specimens collected in Arizona (Smith 1887), and since that time it has expanded its distribution northward into Rocky Mountain states and eastward into the central Great Plains. By 1970, it was causing significant damage throughout Nebraska and a few small populations were found as far east as counties bordering the Missouri River in western Iowa (Crumb 1956, Keith et al. 1970, USDA 1970, Antonelli 1974, USDA 1977, Blickenstaff 1979). However, the western bean cutworm did not continue to expand its distribution and become a serious pest in Iowa as it had in

Nebraska and its occurrence was only sporadically documented in Iowa during those years. Then, approximately 30 years later in 2000, a commercial corn field in west central Iowa was heavily infested with western bean cutworm larvae, with kernel damage reported on 95 percent of the ears and some ears containing 6 larvae per ear (Rice 2000). In the years that followed, the western bean cutworm expanded its distribution across Iowa and by late summer, 2004, it could be found in most eastern Iowa counties and was reported for the first time in Illinois and Missouri (Dorhout and Rice 2004). Two years later, it was reported for the first time in Indiana, Ohio, and Michigan (Rice and Dorhout 2006, Pope 2007).

Western bean cutworms expansion across the Corn Belt is a concern because it damages corn by directly feeding on the kernels in the ear and can cause serious economic lost. Larvae feed on corn for a considerable length of time, taking an average of 55.9 days to complete their development at 27°C, 16:8 (L:D) (Antonelli 1974) and there is only one generation per year. One larva per corn plant at dent stage reduces yields by 2.32 q/ha (3.7 bu/a) (Appel et al. 1993). Larvae are not cannibalistic and heavily infested corn may have more than 20 larvae per ear (Seymour et al. 1998).

Su (1976) described western bean cutworms in Nebraska as infected with a *Nosema loxagrotidis* sp. N, but the name was never published. She did report that, during the period 1970-75, 100% of western bean cutworm larvae examined contained either spores or vegetative stages of the Microsporidium. *Nosema* is a Microsporidia that are obligate parasites that can reduce the vigor of its host and may even kill it. Microsporidia typically infect their host after being consumed and injecting themselves into the midgut cells. Dorhout et al. (2007) reported that the flight potential of European

corn borers, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) heavily infected with

Nosema pyrausta (Paillot) was severely reduced and could not fly as far as the

individuals with a low level infection. Helms and Wedberg (1976) used commercial

formulations of *Bacillus thuringiensis* (*Bt*) var. *alesti* and *kurstaki* (Nutrilite Products,

Inc., Lakeview, CA) to "test the effectiveness of several commercial formulations of the

bacterium" for control western bean cutworms. *Bacillus thuringiensis* infects the host

after being consumes where crystalline protoxins produced by the bacterium create pores

in the midgut cell that disrupt the osmotic pressure in the cell causing the cell to burst and

die. The different varieties of *B. thuringiensis* are highly selective with each variety only

affecting a narrow spectrum of insects. Helms and Wedberg's results for controlling

western bean cutworms were inconclusive but they reported, "that the bacterium and/or

its toxin may have affected the insect in a positive [reduced the infection] rather than a

debilitative manner in relation to its affect on *Nosema*-infected midgut epithelium."

Results from a different study found that European corn borers fed a commercial

formulation of *B. thuringiensis* var. *kurstaki* (Dipel ES, Abbott, North Chicago, IL) in

their diet had a 70 fold decrease in the number of spores in them compared to the controls

(85-103 thousands and 7.6-8.7 million, respectively). YieldGard[®] CornBorer (hereafter

YieldGard CB) (event MON810) (Monsanto Company, St. Louis, MO) corn expresses

the protoxin Cry1Ab from *B. thuringiensis* var. *kurstaki* (Koziel et. al. 1993, Armstrong

et al. 1995) was introduced in 1996 and is widely used today to control European corn

borers, but is not toxic to western bean cutworms. Since commercial formulations of *B.

thuringiensis* var. *kurstaki* have reduced the number of spores in both European corn

borers and western bean cutworms, it seamed plausible that YieldGard CB corn might be

affecting the number of spores and the number of infected western bean cutworms individuals because it expresses the Cry1Ab protoxin that was derived from *B. thuringiensis* var. *kurstaki*. If this was true, then one possible consequence would be for the mean spore count and frequency of infected individuals to vary between geographic populations of western bean cutworms. Geographic variability between the mean spore count and frequency of infected western bean cutworms would not necessarily mean that YieldGard CB corn caused it, but if there was no difference and 100% of the western bean cutworms were infected with the Microsporidium, then it would be very unlikely that YieldGard CB corn affected the Microsporidium infections within western bean cutworms in any meaningful way.

Therefore, since Microsporidia have been reported to severely reduce the flight potential of other Lepidopterans and the western bean cutworm has rapidly expanded its distribution shortly after the introduction of YieldGard CB corn that might possibly reduce the Microsporidia count in western bean cutworm; we decided to examine Microsporidia infection levels in western bean cutworm across the Corn Belt. It also seemed appropriate to determine if the frequency and level of infection in a newly colonized area changed over time.

The objectives of this study were to examine western bean cutworms infected with this Microsporidia and determine if there were differences in the frequency and intensity of the infection by geographic location. This study also examined the frequency and intensity of infected individuals in a newly colonized area over a four week period during 2004 with data from the same location in 2006.

Methods and Materials

Adult Collections. In 2004, adult western bean cutworms were collected near Ames, IA on July 10, 19, 26, and August 4 using a blacklight trap. These collection dates covered the western bean cutworm peak flight period in Iowa for that year (Rice 2007). Collected specimens were stored at -20°C and 12 individuals from each date were randomly selected and examined for Microsporidia in 2007.

In 2006, adult males were collected using pheromone traps were constructed following the Seymour et al. (1998) procedure.

Pheromone traps were placed approximately 113 km (70 miles) apart in public right-of-ways next to corn fields along a west-to-east transect from eastern Wyoming (Goshen County) following Highway 26 into Nebraska to Interstate 80, then Interstate 74, and finally Interstate 70 to western Ohio (Preble County) (Table 3, Fig. 1). Two traps were placed at each location approximately 25 m apart and each trap was hung approximately 1.22 m off the ground on a 1.52 m (0.635 cm dia) steel reinforcement rod within 1 m of a corn field. The traps were placed in locations west of Ames, IA on July 1 and east of Ames, IA on July 3. Adults were removed from the westward traps on July 8 and the eastward traps on July 10. Collected western bean cutworms from each location were placed in 0.95 liter Ziploc self sealing plastic bags (S. C. Johnson & Son, Inc., Racine, WI), labeled, placed on ice, returned to the laboratory and stored at -20°C.

Microsporidia Data Collection. In the laboratory, collected western bean cutworm adults were rinsed with deionized water and allowed to air dry for approximately 15-20 min. A random subsample of 48 individuals from each location was selected unless there was less than 48 adults, then all the individuals at that location were

used. The abdomens were excised approximately 3 mm behind the junction between the metathorax and abdomen, and then the last 2 mm of the last segment containing the claspers were removed. The truncated abdomen was then cut in half longitudinally and weighed separately. The sections were then placed into individual wells within a 96 DeepWell Microplate (2 ml) (VWR Scientific, Batavia, IL). One section of the abdomen later would be homogenized while the other would remain intact and archived. After samples were loaded onto the plate they were stored at -20°C for six months. Processed samples were first thawed and then a 0.64 cm (0.25 inch), 1.05 g (0.037 oz.) steel shot (Crosman Corp., East Bloomfield, NY) was loaded into each well. The plates were then sealed by placing two layers of parafilm wax (American National Can, Menasha, WI) between the plate and the lid (UniSeal Capmat) (VWR Scientific, Batavia, IL). Sealed plates were loaded onto a random axis paint shaker and shaken for approximately 15 minutes to homogenize the abdomens. The Microsporidia in the homogenized samples were then counted using a haemocytometer (Levy, Horsham, PA) under a 400x phase contrast microscope following the procedure of Raun et al. (1960).

Data Analysis. The data were first analyzed comparing infected and non-infected individuals using a Chi-square test (CHISQ option of the SAS procedure PROC FREQ; SAS Institute 2007) across all locations and between locations east and west of the Missouri River. The Microsporidia counts were then analyzed by location (ANOVA, PROC GLM; SAS Institute 2007). Data were log transformed to normalize the variance. Means were compared using Tukey's adjustment for multiple comparisons with $P \leq 0.05$ considered significant. Microsporidia count data are expressed as the mean±SEM.

Results

Between 2004 and 2006 at Story County, IA, there were significantly fewer
infected individuals in 2004 (12.5%, n= 48) than in 2006 (56.3%, n= 48) (df= 1, x^2=
20.36, $P<0.0001$). In 2004, there was no significant difference (df= 1, x^2= 0.76, P=
0.8586) between the frequency of infected individuals collected on the four sampling
dates July 10 (n= 12, infected= 1, spore count= 440), 19 (n= 12, infected= 2, mean spore
count= 164±44), 26 (n= 12, infected= 2, mean spore count= 64±52), and August 4 (n=
12, infected= 1, spore count= 8). The six infected individuals in 2004 had significantly
higher Microsporidium counts (150.7±26.5) than the 27 infected individuals in 2006
(7.8±12.5) (df=1, F= 23.75, $P<0.0001$) (Table 1).

In 2006, traps were placed at 24 locations (Fig. 1) ranging in latitude from 42ºN to
39ºN, longitude from 104ºW and 84ºW, and elevations 1,290-230 m (Table 2). There
were no data from location 16 because the traps were destroyed by vandals. Only six
adults were collected in the nine most-easterly traps, therefore these data were not
included in the analysis. There was a significant difference between the frequency of
infested individuals across locations (df= 14, x^2= 182.90, $P<0.0001$). There was also a
significant difference between the frequency of infected individuals located west of the
Missouri River in Nebraska and Wyoming and east of the river in Iowa, Illinois, and
Indiana (df= 1, x^2= 40.69, $P <0.0001$) (Fig 3). The frequency of infected individuals was
significantly different by between locations (F=27.10, $P<0.0001$) (Fig. 3). The mean
spore counts were also different by location (F=17.19, $P<0.0001$) with the highest mean
level of Microsporidia located west of the Missouri River and lowest mean level of
Microsporidia was east of the river (Fig. 2).

Discussion

The intensity and frequency of the Microsporidia infection levels within western bean cutworms did not vary significantly between the four dates in 2004 (Table 1). However, this experiment should be repeated because the data are from only one location in one year and the intensity and frequency of infected individuals was very low and these results may differ at locations that have a relatively high number of infected individuals with a high infection level.

There were significantly more infected adult western bean cutworms at the central Iowa location in 2006 than in 2004 (Table 1). The individuals were most likely infected with Microsporidia that had been transported to that location by western bean cutworms from earlier generations because Microsporidia are obligate parasites and typically have a very narrow host range. Thus, western bean cutworms most likely took the Microsporidium with them. Also, western bean cutworms were reported for the first time in central Iowa in 2003 (Pope and Rice 2003, Rice 2003) and the lower infection frequency observed in 2004 than in 2006 may be related to the number of years that the western bean cutworm had been at that location. The increase of infected individuals in a population over time after the introduction of a microsporidium to an area was similarly reported with *Nosema pyrausta* in European corn borers (Kramer 1959). *Nosema pyrausta* is a microsporidium that infects European corn borers and was first reported in United States in 1951 (Steinhaus 1951). In Illinois from1956 to1957 live, first generation European corn borer larvae were collected in 23 counties and the percent of infected larvae increased from 20 to 24%, respectively and the frequency of infected live, second generation larvae increased every year from 41 to 48 to 57 to 69% from 1954-1957,

respectively (Kramer 1959). This suggests that the number of infected individuals accumulates over time.

The distribution data indicated that there were significant differences between the frequencies of infected individuals located east and west of the Missouri River and between locations in most trapping sites (Fig. 3). The locations were divided by the Missouri River because prior to 2000 (Rice 2000) the western bean cutworm was not known to cause economic damage east of the Missouri River even though it had been sporadically reported in Iowa (Crumb 1956, Keith et al. 1970, USDA 1970, Antonelli 1974, USDA 1977, Blickenstaff 1979). The locations were also divided by the Missouri River because the Missouri River Valley largely consists of high clay content soil. Soil composition is instrumental to the emergence of western bean cutworm adults with fewer adults emerging in clay soils compared to sandy soils (Hoerner 1948, Hanstbarger 1969, Douglass et al. 1955) suggesting that the Missouri River Valley would be poor habitat for western bean cutworms because they wouldn't be able to emerge in very high numbers.

Infection levels were significant by locations (Fig. 2). The highest infection level was west of the Missouri River at Location 6 and the lowest level was east of the Missouri River at Location 14 (Fig 2). The frequency and the number of Microsporidia within western bean cutworms significantly vary by location, suggesting a spatial effect on the Microsporidia within the western bean cutworm populations.

Future research should examine the effect that recent changes in farming practices have on the Microsporidia infection within western bean cutworms. The effect thata commercial formulations of *B. thuringiensis* var. *kurstaki* has on the Microsporidium infection within western bean cutworm, because Helms and Wedberg (1976) reported

that the infection within the larvae was less sever after they had fed on the bacterium. Pierce et al. (2001) similarly reported a 70 fold decrease (7.6-8.7 million to 85-103 thousand spores per larvae) in the number of *N. pyrausta* spores produced by European corn borer larvae treated with a commercial formulation of *B. thuringiensis* var. *kurstaki* (Dipel ES) after 15 days compared to untreated larvae. Research should also examine the effect that YieldGard® CornBorer (event MON810) (Monsanto Company, St. Louis, MO) has on the Microsporidia infection levels in western bean cutworms because its endotoxin (Cry1Ab) is derived from *B. thuringiensis* var. *kurstaki* (Koziel et. al. 1993, Armstrong et al. 1995) and may similarly reduce the Microsporidia infection creating healthier, more fit western bean cutworms that are able to fly further and expand their distribution faster.

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Table 1. Microsporidia counts (Means± SEM) in adult western bean cutworms in

Ames, IA, 2004 and 2006.*

Year	Date collected	Sample number	Number infected	Microsporidia counts*
2004	710-8/4	48	6	150.7±26.5a
2006	7/1-8	48	27	7.8±12.5b

* Means with the same letter are not significantly different, P≤ 0.05.

Table 2. Microsporidia densities (means± SE) in adult western bean cutworms at 24

locations across the Great Plains, 2006.

Trap No.	n	Microsporidia means± SEM	County, state	Latitude and longitude	Elevation Meters	Feet
1	21	14.9±7.6	Goshen, WY	42° 5'587N, 104° 20'548W	1,290	4,231
2	48	46.3±8.1	Morrill, NE	41° 46'376N, 103° 18'887W	1,176	3,857
3	48	52.6±10.1	Garden, NE	41° 21'122N, 102° 9'752W	1,009	3,311
4	48	47.7±8.5	Keith, NE	41° 6' 707N, 101° 21'170W	905	2,970
5	48	63.4±10.1	Dawson, NE	40° 52'379N, 100° 9'758W	787	2,583
6	48	124.9±18.5	Kearney, NE	40° 44'807N, 98° 37'386W	573	1,879
7	48	24.2±6.2	York, NE	40° 50'589N, 97° 27'463W	471	1,544
8	48	9.6±3.6	Cass, NE	40° 59'184N, 96° 23'347W	353	1,159
9	48	11.5±3.5	Pottawattamie, IA	41° 16'967N, 95° 43'299W	395	1,297
10	48	39.9±6.9	Audubon, IA	41° 30' 710N, 94° 59'829W	417	1,368
11	48	12.8±4.0	Dallas, IA	41° 32' 245N, 93° 56'412W	265	868
12	48	4.4±1.1	Story, IA	41° 59' 985N, 93° 45' 112W	248	815
13	48	5.5±2.2	Jasper, IA	41° 41'830N, 93° 15'364W	230	754
14	48	0.1±0.0	Iowa, IA	41° 41'656N, 92° 9'679W	276	905
15	48	3.0±1.4	Cedar, IA	41° 30'437N, 90° 54'985W	234	769
16*	0	-	Knox, IL	40° 54' 914N, 90° 14'751W	240	786
17	3	0.3±0.3	McLean, IL	40° 35'5N, 89° 8'846W	228	748
18	2	0.5±0.5	Champaign, IL	40° 7' 427N, 88° 2'767W	200	655
19	1	2	Montgomery, IN	40° 6'695N, 87° 4'268W	243	797
20	0	-	Boone, IN	39° 57'917N, 86° 37'251W	289	947
21	0	-	Hancock, IN	39° 49'682N, 85° 47'553W	312	1,024
22	0	-	Preble, OH	39° 48'577N, 84° 46'773W	358	1,175
23	0	-	Preble, OH	39° 45'55N, 84° 46'774W	346	1,135
24	0	-	Preble, OH	39° 48'56N, 84° 43'975W	367	1,205

*traps missing or vandalized

Fig. 1. Approximate location of 24 western bean cutworm pheromone traps, 2006.
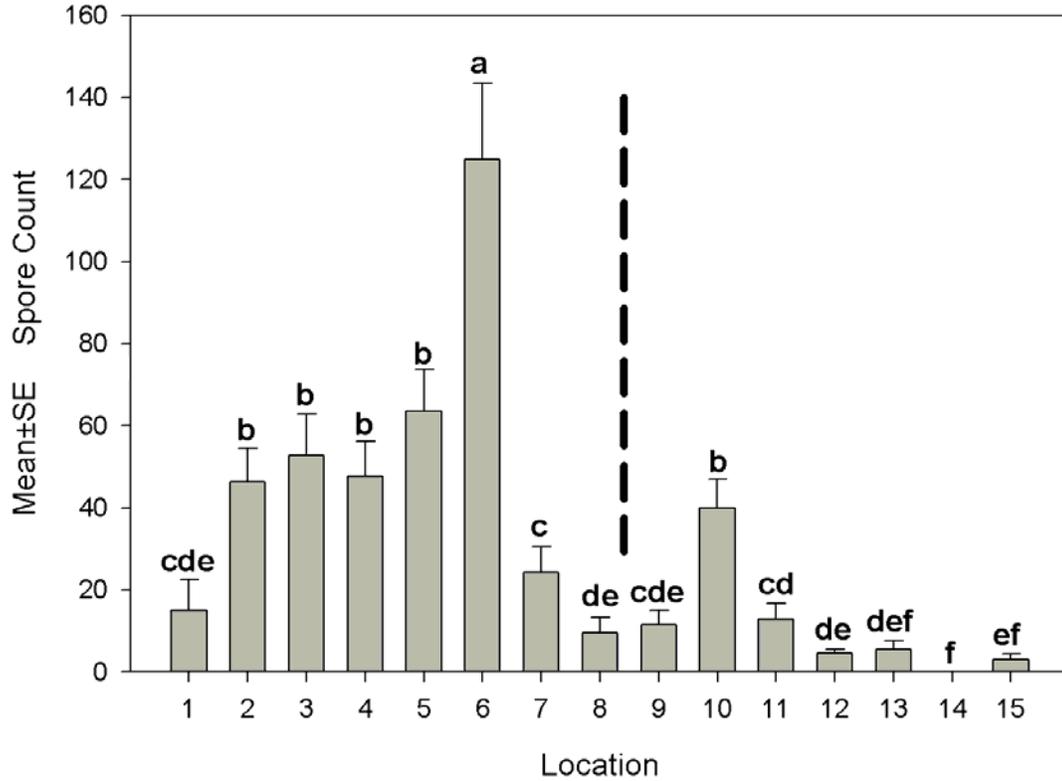
Fig. 2. Adult western bean cutworm Microsporidia counts (mean± SE) at 15 trap locations across the Great Plains, 2006*   Means followed by the same letter are not significantly different by ANOVA. P≤ 0.05 are considered significant. (df= 14, *F*= 17.19, *P*= <0.0001) Dash line separates traps located west (1-8) and east (9-15) of the Missouri River.

*Excludes locations 16-24 because of small sample size or no adults collected.
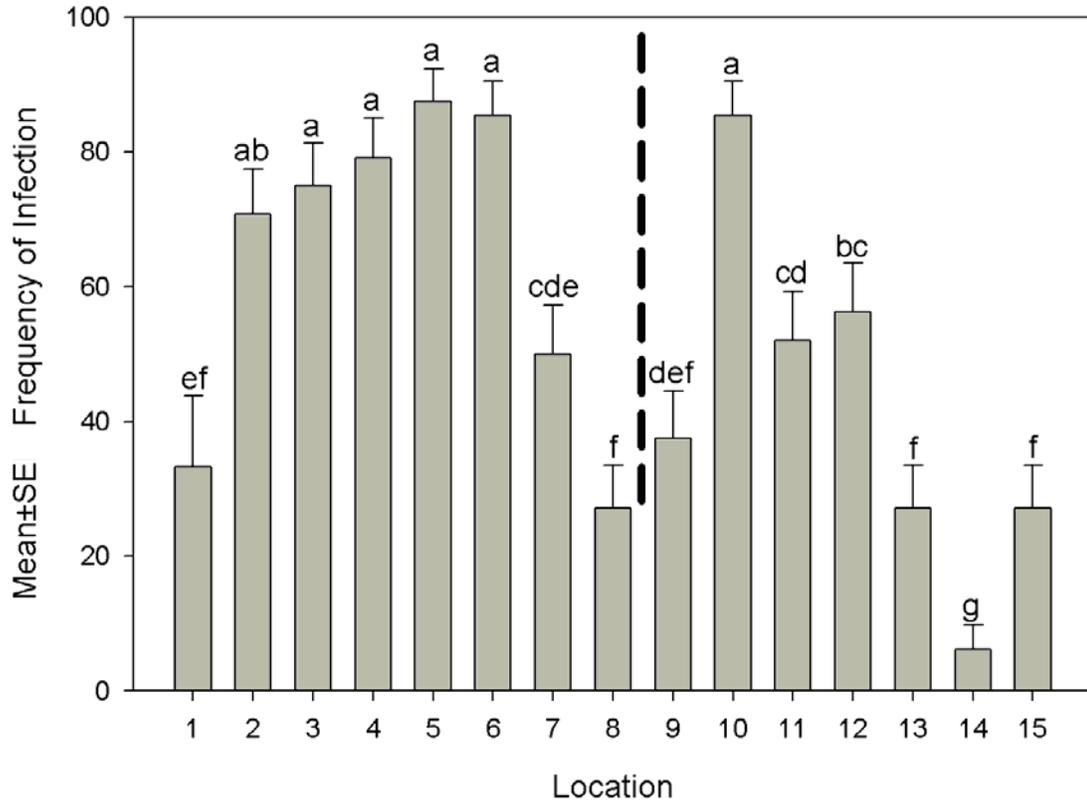
Fig. 3. Frequency (mean± SE) of adult western bean cutworms infected with Microsporidia at 15 trap locations from Wyoming to Iowa.* Means followed by the same letter are not significantly different, P≤ 0.05. (df= 14, *F*= 27.10, *P*= <0.0001) Dash line separates traps located east and west of the Missouri River.

*excludes locations 16-24 because of small sample size or no adults collected.

CHAPTER 7: General Conclusions

The importance of managing the western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), has risen dramatically since it resumed its eastern expansion (O'Rourke and Hutchison 2000, Dorhout and Rice 2004, Rice and Dorhout 2006, Pope 2007). At the beginning of this research program the western bean cutworm had just been reported east of Interstate 35 in northeast Iowa (Rice 2003) and appeared to be steadily expanding eastward and would continue to do so. It therefore seemed urgent to construct research projects that would help better understand its movement and explore reasons for why the western bean cutworms' eastward expansion stalled for approximately 30 years on the Nebraska-Iowa border.

This research program initially focused on three questions related to the overall expansion question of the western bean cutworm: its flight potential, surveying methods, and the influences of new farming practices on the movement of the western bean cutworm. The influence of new farming techniques was limited to the introduction of transgenic corn because YieldGard® CornBorer (CB) (Monsanto Company, St. Louis, MO) was introduced in 1996, does not control it, and the first report of western bean cutworms beginning to expand their distribution came in 1999. So, two projects were developed to examine the efficacy of transgenic corn controlling western bean cutworms and the effect that YieldGard CB has on the survivability of the western bean cutworms in intraguild completion with other Lepidoptera larvae.

In 2005, field observations of the frequency of infected western bean cutworm larvae with a Microsporidia collected in central Iowa were not as expected after reading the literature. So, an additional study was constructed to examine the spatial distribution

of the Microsporidia and examine the possibility that it was related to the western bean cutworms' expansion.

The flight potential of the western bean cutworm was examined by tethering the moths to a flight mill and examining the effect of sex, age and mating on the western bean cutworms' flight characteristics. Virgin females flew significantly farther than virgin males and mated females. The mean distance flown by virgin males started low at 1-2 days old, rapidly increased at age 3-4 days, and then slowly decreased as they got older, 9-10 days old. Virgin females' mean flight distance started relatively low at 1-2 days old and then rapidly increased at age 3-4 days old, but then their mean flight distance did not decrease significantly by age 9-10 days old. These results indicate that western bean cutworms have the potential to be strong flyers and good dispersers.

Improving surveying techniques was examined by evaluating several different trapping techniques by placing two different sex pheromone traps at three different heights in two different environments. The results showed that the type of pheromone trap was not significant, but the traps placed 1.2 or 1.8 meters above the ground caught significantly more moths that those placed 0.6 meters above the ground. Also, traps placed between two corn fields caught significantly more moths than traps placed between a corn and soybean field. These data suggest that if an individual wanted to capture the most western bean cutworm adults with their pheromone traps, they should place their traps between two corn fields and 1.2- 1.8 meters above the ground and that the placement of the trap should be considered when comparing trap catches from different traps.

The impact of transgenic corn on western bean cutworms were examined by evaluating the efficacy of Herculex® I or Herculex® Xtra (Herculex) (event TC 1507 from *B. thuringiensis* sp. *aizawa*i (Mycogen Seeds c/o Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc., Indianapolis, IN and Des Moines, IA, respectively), expressing the *Bacillus thuringiensis* (Berliner) (*Bt*) endotoxin Cry1F and is labeled to suppress western bean cutworms, YieldGard CB (event MON810 from *B. thuringiensis* sp. *kurstaki*) expressing the endotoxin, Cry1Ab and is not labeled for western bean cutworms, to non-*Bt* hybrids in central Iowa in 2005 and 2006. The three traits were evaluated for the percent of ears infestated with western bean cutworm larvae, ear injury, and yield. The Herculex hybrids had significantly lower infestation and injury levels than the YieldGard CB and non-*Bt* hybrids. Interestingly, the YieldGard CB hybrids had significantly higher infestation levels and ear injury than the non-*Bt* hybrids in 2005. In 2006 there was no difference between the YieldGard CB and non-*Bt* hybrids, but this may be because there was no YieldGard CB data from the location with the highest western bean cutworm infestations and injury levels. There was no YieldGard CB data from that location because that location only had Herculex and non *Bt*- hybrids.

The effect that YieldGard CB hybrids has on the survivability of western bean cutworm larvae during intraguild competition with European corn borer larvae, *Ostrinia nubilalis* (Hübner), and corn earworm larvae, *Helicoverpa zea* (Boddie) was examined using competition scenarios. Competition scenarios were constructed by placing different sized larvae in arenas on one of three diets: meridic, non-*Bt* silks, and YieldGard CB silks. On a meridic or non-*Bt* silk diet, corn earworm larvae out competed western bean cutworms by attacking and sometimes killing them, even if the western bean

cutworm was initially larger than the corn earworm. However, on YieldGard CB corn silks western bean cutworm survivability was near 100 percent when they were of equal or larger size than the corn earworms. Adding European corn borers and creating three way interactions did not significantly affect the survivability of the western bean cutworm larvae. These results indicate that YieldGard CB confers a competitive advantage to western bean cutworm larvae within the presence of intraguild competition from corn earworm larvae and may be facilitating their recent eastward expansion.

The frequency and the infection level of a Microsporidia infecting western bean cutworms was investigated by examining individuals collected in 15 locations from eastern Wyoming (Goshen County) to western Iowa (Cedar County) in 2006. Western bean cutworms were also examined that had been collected in 2004. The frequency of infected individuals was lower east of the Nebraska-Iowa border and it was lower in 2004 than it was in the same location in 2006. The frequency and the number of Microsporidia within western bean cutworms significantly vary by location, suggesting a spatial effect on the Microsporidia within the western bean cutworm populations.

The western bean cutworm will most likely continue to increase in important as its distribution expansion continues eastward and as it becomes more established east of Nebraska. Future research should examine the effect that recent changes in farming practices have on the Microsporidia infection within western bean cutworms. The effect that commercial formulations of *B. thuringiensis* var. *kurstaki* has on the Microsporidium infection within western bean cutworms should be examined, because Helms and Wedberg (1976) reported that the infection within the larvae was less sever after they had fed on the bacterium. Pierce et al. (2001) similarly reported a 70 fold decrease (7.6-8.7

million to 85-103 thousand spores per larvae) in the number of *N. pyrausta* spores produced by European corn borer larvae treated with a commercial formulation of *B. thuringiensis* var. *kurstaki* (Dipel ES) after 15 days compared to untreated larvae. Research should also examine the effect that YieldGard® CB has on the Microsporidia infection levels in western bean cutworms because its endotoxin (Cry1Ab) is derived from *B. thuringiensis* var. *kurstaki* (Koziel et. al. 1993, Armstrong et al. 1995) and may similarly reduce the Microsporidia infection creating healthier, more fit western bean cutworms that are able to fly further and expand their distribution faster.

Research should also examine the effect that the soils (Hoerner 1948, Hanstbarger 1969, Douglass et al. 1955) of the Missouri River Valley have on the emergence of western bean cutworms and determine if western bean cutworms infected with the Microsporidium were more likely to fly shorter distances and thus laid their eggs on corn plants growing in soil that would have prevented emergence compared to healthier, less infected individuals that would might have then been more likely to fly further and laid their eggs on corn plants growing in soil that would have allowed for adult emergence. This research would then determine if YieldGard CB hybrids reduced the infection levels of Microsporidia enough to allow western bean cutworms to fly far enough east from Nebraska so that when they laid their eggs, their offspring could emerge from the soil in high enough numbers to create a founding population.

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