2006

Ecological studies of the European corn borer (Lepidoptera: Crambidae): nosema dose-response, pheromone trapping, and adult dispersal

Brendon James Reardon

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Entomology Commons

Recommended Citation


https://lib.dr.iastate.edu/rtd/1293

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Ecological studies of the European corn borer (Lepidoptera: Crambidae):

*Nosema* dose-response, pheromone trapping, and adult dispersal

by

**Brendon James Reardon**

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Entomology

Program of Study Committee:
Thomas W. Sappington, Co-major Professor
Leslie C. Lewis, Co-major Professor
Richard L. Hellmich
Jon J. Tollefson
Alicia L. Carriquiry

Iowa State University

Ames, Iowa

2006
This is to certify that the doctoral dissertation of

Brendon James Reardon

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Committee Member

Signature was redacted for privacy.

Co-major Professor

Signature was redacted for privacy.

Co-major Professor

Signature was redacted for privacy.

For the Major Program
Even with insects—

some can sing,

some can't.

Kobayashi Issa
# TABLE OF CONTENTS

## LIST OF FIGURES

| vi |

## LIST OF TABLES

| viii |

## CHAPTER 1. GENERAL INTRODUCTION

| 1 |

- Dissertation Organization 1
- Introduction 1
- Objectives 1
- Literature Review 2
- References Cited 12

## CHAPTER 2. GROWTH, DEVELOPMENT, AND SURVIVAL OF *NOSEMA PYRAUSTA*-INFECTED EUROPEAN CORN BORERS (LEPIDOPTERA: CRAMBIDAE) REARED ON MERIDIC DIET AND CRY1AB

| 23 |

- Abstract 23
- Introduction 24
- Materials and Methods 25
- Results and Discussion 28
- Acknowledgments 30
- References Cited 30

## CHAPTER 3. IMPACT OF TRAP DESIGN, WINDBREAKS, AND WEATHER ON CAPTURES OF THE EUROPEAN CORN BORER (LEPIDOPTERA: CRAMBIDAE) IN PHEROMONE-BAITED TRAPS

| 37 |

- Abstract 37
- Introduction 38
- Materials and Methods 39
- Results 45
- Discussion 46
- Acknowledgments 53
- References Cited 53
LIST OF FIGURES

CHAPTER 2

Figure 1 Percent of both first and second, third, and both fourth and fifth instars of Nosema pyrausta-infected and uninfected Ostrinia nubilalis at different concentrations of purified Cry1Ab in meridic diet after 10 d. 34

Figure 2 Mean (±SE) larval weights of Nosema pyrausta-infected and uninfected Ostrinia nubilalis fed different concentrations of purified Cry1Ab in meridic diet for 10 d. 35

Figure 3 Percent survival (±SE) of Nosema pyrausta-infected and uninfected Ostrinia nubilalis fed different concentrations of purified Cry1Ab in meridic diet for 10 d. 36

CHAPTER 3

Figure 1 Three trap designs used to examine the relative efficiency of traps in capturing Ostrinia nubilalis moths in 2003 and 2004: a) the Intercept™ wing trap (wing trap), b) the Intercept™ bucket/funnel UNI trap (bucket trap), and c) the Hartstack wire-mesh, 75-cm diameter cone trap (large metal cone trap) (Hartstack et al. 1979). In 2005, the large metal cone trap was compared to: d) a modified Hartstack wire-mesh, 35-cm diameter cone trap (small metal cone trap), and e) the Heliothis, 35-cm diameter cone trap (small nylon cone trap). All traps were baited with an Iowa-strain pheromone lure, changed biweekly. 62

Figure 2 Mean number of Ostrinia nubilalis moths collected daily in Hartstack wire-mesh, 75-cm diameter cone traps per week. 63

Figure 3 Mean (±SE) number of Ostrinia nubilalis moths collected daily with different pheromone-baited trap designs in a) 2003 and 2004 (pooled), and b) 2005. 64

Figure 4 Mean (±SE) number of Ostrinia nubilalis moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps in different locations relative to a windbreak at different wind speeds in 2003, 2004, and 2005. Captures at different trap locations within wind speed category with different letters were significantly different based on least-squares differences of means (P < 0.05). 65
CHAPTER 4

Figure 1  Schematic of the study arena at each farm for release of marked *Ostrinia nubilalis* moths via pupal rings. The numbers within the squares correspond to randomly-assigned treatments in small-grain aggregation plots. Areas between aggregation plots were fallow alleyways.

Figure 2  Mean (±SE) number of marked *Ostrinia nubilalis* adults collected by sweep net per aggregation plot per sample date at four distances from the point of moth emergence from pupal rings.

Figure 3  Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the first flight of 2003.

Figure 4  Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the first flight of 2004.

Figure 5  Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the second flight of 2004.
LIST OF TABLES

CHAPTER 2

Table 1 Toxicity of Cry1Ab to *Nosema pyrausta*-infected and uninfected *Ostrinia nubilalis*. 33

CHAPTER 3

Table 1 Model statistics for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather. 59

Table 2 Parameter coefficients for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather. 60

Table 3 Partial $r^2$ values for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather. 61

CHAPTER 4

Table 1 Expected and observed mean number of marked *Ostrinia nubilalis* adults recovered from aggregation plots with a sweep net within five days of moth release. 94
CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This thesis is organized into five chapters. Chapter 1 is a general introduction and literature review of the European corn borer, *Ostrinia nubilalis* (Hübner). Chapter 2 is a bioassay study of growth, development, and survival of *Nosema pyrausta*-infected larvae reared on meridic diet impregnated with Cry1Ab, the toxin expressed in transgenic corn to protect it against *O. nubilalis*. Chapter 3 is a field study of the impact of trap design, windbreaks, and weather on moth captures in pheromone-baited traps. Chapter 4 is a field study that examines the dispersal of newly-eclosed moths from corn into small-grain aggregation plots. The general conclusions of this dissertation are presented in Chapter 5.

Introduction

*Ostrinia nubilalis* is estimated to have entered the US from Hungary and Italy between 1909 and 1914 in broom corn (Fracker and Fluke 1926). Since its arrival, it has established itself as a major pest of corn, *Zea mays* L (Cyperales: Poaceae). At present, *O. nubilalis* is found in most states east of the Rocky Mountains, several Canadian provinces, Africa, Asia, and Europe (Guthrie et al. 1985a). Yield losses and control measures associated with this insect may exceed one billion dollars annually in the US alone (Mason et al. 1996). It is important to understand the ecology and behavior of this pest to better manage it, reducing damage and injury to crops.

Objectives

The objectives of the work presented in this dissertation were the following:
1. To examine the effect of purified Cry1Ab on growth, development, and survival of Nosema-infected and uninfected O. nubilalis larvae.

2. To examine differences in O. nubilalis-moth captures among the Intercept™ wing trap, the Intercept™ bucket/funnel UNI trap, and the large Hartstack wire-mesh cone trap. Furthermore, to compare capture efficiency among three cone-trap designs, including the large metal, small metal, and small nylon cone traps.

3. To examine the influence of the location of the large metal cone trap relative to a windbreak on the number of O. nubilalis moths captured.

4. To examine the relationship between nightly mean air temperature, relative humidity, wind speed, precipitation, and the numbers of O. nubilalis moths captured in the large metal cone traps.

5. To examine the influences of pheromone lure, aggregation-site plant density, and aggregation-site plant species on the distributions of marked-released and feral O. nubilalis adults among aggregation plots of small grains.

Literature Review

The Ostrinia nubilalis adult was initially described in Europe in 1796 (Parks 1926). The first record of it being an economic pest was in 1835 (Parks 1926). In that year, Joseph Schmidt described O. nubilalis damage in Carniola—then a part of Hungary—where it attacked broom corn millet. The first plant reported as a host was the hop plant (Parks 1926). In April of 1948, Dr. H. M. Harris of Iowa State College circulated letters suggesting the possibility of organizing station entomologists of the “North Central Region” (i.e., Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Dakota,
Ohio, South Dakota, and Wisconsin) into a committee to develop a regional approach to
tomological issues including the *O. nubilalis* problem (Brindley and Showers 1978).

*Ostrinia nubilalis'* cosmopolitan distribution is probably a result of a combination of
factors. These include a moderate dispersal capability; some moths can travel at least 50 km
(Showers et al. 2001), and they may rest on land or water before taking off again
(Anonymous 1955). Exploitation of a broad range of host plants (>200) also contributes to
its wide range. The ability of larvae to diapause and to endure internal freezing further
increases *O. nubilalis'* geographical distribution (Hanec and Beck 1960).

Young larvae feed in the whorl, leaf sheath, and midrib of the corn plant. Most
damage is done by fourth and fifth instars that bore into stalks and ears. Tunneling disrupts
nutrient flow and leads to incomplete ear development. It also weakens stalks and ear
shanks, and exposes internal tissues to stalk rots and other diseases. Laboratory and
greenhouse studies indicate that *O. nubilalis* is capable of transmitting the bacterium *Erwinia
carotovora* (van Hall), the causal agent of potato blackleg (Anderson et al. 1981).

Infestations of *O. nubilalis* increase the chance of contamination by aflatoxin (a carcinogen)
produced by *Aspergillus* spp. (Jarvis et al. 1984a). *Fusarium*, a mycotoxin and carcinogen,
may be introduced through wounds induced by *O. nubilalis* (Munkvold et al. 1997), and other
stalk rots have been associated with *O. nubilalis* damage as well (Jarvis et al. 1984b).

**Biology.** *Ostrinia nubilalis* is a holometabolous insect with life stages of egg, larva,
pupa, and adult. The egg stadium is about 3 d at 27°C, 75–80% RH, and constant light
(Guthrie et al. 1985b). The larval stadium is about 11–16 d, and the pupal stadium is about
1–3 d under similar conditions (Guthrie et al. 1985b). Thus, it takes about 15–22 d from the
egg stage to reach adulthood. The five larval instars can be distinguished based on a combination of head capsule width, the length of the prothoracic shield, and body size (Dewitt and Stockdale 1983). Got (1988) concluded that larval head capsule width cannot be used alone to determine instar because the ranges of measurements of consecutive developmental stages may overlap. After eclosion, larvae feed on leaves before boring into stems or stalks as third instars to continue feeding. The remaining larval and pupal development occurs within these plant structures. Adults live about 10–14 d in the field. Female moths are generally larger and lighter in color than males.

Females attract males for mating by releasing a species-specific pheromone, a behavior referred to as “calling.” Responding males fly upwind towards the pheromone source. This phenomenon is known as optomotor anemotaxis, and an internal program of self-steered, counterclocks or zig zags is characteristic of such (Foster and Frérot 1994). There are two pheromone strains of *O. nubilalis* in North America. Females that release, and males that are attracted to, a mixture of 97% cis-11-tetradecenyl acetate and 3% trans-11-tetradecenyl acetate are designated as Iowa- or Z-borers. Those releasing, and responding to, a mixture of 1% cis-11-tetradecenyl acetate and 99% trans-11-tetradecenyl acetate are designated as New York- or E-type borers (Mason et al. 1996). Hybridization between the two strains can occur.

Males are promiscuous, and there is a refractory period of 1 d between successive matings (Loughner 1971, Royer and McNeil 1993). Females have been documented to be occasionally polyandrous (Pesho 1961, Loughner 1971, Elliott 1977), and the number of times a female has mated can be determined by the number of spermatophores in her bursa
copulatrix (Showers et al. 1974). Elliott (1977) indicated that the majority of *O. nubilalis* females mate once. However, it has been documented that 15–37% of females mated twice, 0–13% mated thrice, and less than 1% mated four times (Elliott 1977). Another report indicated that after 11 d, 12% of females had mated twice (Loughner 1971). Pesho (1961) found that 8–43% of females mated multiple times.

Peak oviposition occurs about a week after adult eclosion, but may begin within three days (Guthrie et al. 1985b). Fecundity estimates average about 300 eggs per female, and a clutch contains 20–30 eggs (Guthrie et al. 1985b). Virgin females live longer than mated ones (Fadamiro and Baker 1999). As virgin females age, they call earlier in the night and spend more time calling to increase the probability of attracting mates (Royer and McNeil 1991).

Adult males emerge from the pupal case with a near full complement of sperm that are delivered to a female in a spermatophore (Chaudhury and Raun 1966). The mating sequence usually consists of a male approaching a calling female with its wings held vertically above its abdomen, fluttering slightly with its claspers extruded (Schlaepfer and McNeil 2000). The male thorax is then aligned perpendicular to the female thorax in an attempt to copulate. Short-range, male-produced pheromones may play a role in courtship, and scent-releasing hair pencils are found on the eighth sternite of males (Royer and McNeil 1992).

**Host Plants.** Many feeding and reproductive hosts of *O. nubilalis* have been identified. The most comprehensive work done in the US was conducted in New England by Hodgson (1928), who identified eggs, larvae, or pupae on 131 genera of plants from 40
families. Overall, *O. nubilalis* larvae were found on more than 200 plant species, including over 30 economic crops.

**Seasonal History.** In the US, there are three geographic ecotypes—northern, central, and southern (Showers et al. 1975), which are differentiated by incidence of diapause when reared under similar conditions (Reed et al. 1978). Characteristics of *O. nubilalis* diapause include arrested gonadal development, failure to pupate shortly after cessation of feeding, and reduction of oxygen consumption. The fifth instar of multivoltine *O. nubilalis* enters a facultative diapause to overwinter, and univoltine larvae undergo an obligate diapause. Voltinism is correlated with geography in the US, and there are usually 1–3 generations annually in Iowa (Hanec and Beck 1960, Mason et al. 1996). Showers et al. (1972) suggested that incidence of diapause is a quantitative trait controlled by more than one gene. Reed et al. (1981) indicated that at least some of the genes are sex-linked. Diapause is mediated by photoperiod and temperature (Reed et al. 1981). Cold-hardening is part of the diapause phenomenon and enables larvae to survive internal freezing (Hanec and Beck 1960). Polyols in the hemolymph likely confer, in part, cold resistance. Cold-hardening occurs during the fall and early winter (Lynch et al. 1972). Hanec and Beck (1960) reported that cold-hardening in *O. nubilalis* in the northern portions of the US begins in August and reaches a maximum by late November.

In the spring, the overwintered larvae break diapause, pupate, and the adults emerge from the corn stubble. This comprises the first flight of moths. Oviposition during the first flight extends over a three to four week period (Mason et al. 1996). The tallest corn plants are most attractive to ovipositing females, and eggs are laid on abaxial leaf surfaces (Everett
et al. 1958). The resulting larvae feed on leaves in the whorl until about third instar, and then they bore into the stalk to continue feeding. During the second flight, oviposition lasts four to six weeks (Mason et al. 1996). Volatile compounds such as farnesol and farnesene moderately deter and attract females, respectively (Binder et al. 1995). The aromatic compound phenylacetaldehyde is attractive to adult *O. nubilalis* (Cantelo and Jacobson 1979), and nonvolatile compounds such as carbohydrates on the surface of the corn leaf serve as oviposition stimulants (Derridj et al. 1986). Second-flight moths preferentially oviposit on corn plants with emerging silks and that are shedding pollen. Young larvae feed on accumulated pollen in leaf axils, but most of the feeding is on sheath and collar tissues (Brindley et al. 1975). Older larvae tunnel into the stalk and feed on the pith. Larvae cause injury and damage to corn by herbivory and tunneling, which causes disruption to vascular translocation vessels and predisposes plants to lodging.

**Management Tactics.** Early management practices for *O. nubilalis* relied on mechanical and cultural practices. Clean plowing, stalk shredding, and burning lead to significant reductions in numbers of overwintering *O. nubilalis*, but were only effective when all farmers in an area practiced them or when they were used in conjunction with a planting-date adjustment (Caffrey 1926).

Host-plant resistance to *O. nubilalis* was probably the most frequently employed control measure over the last 80 years in North America. With the advent of efficient insect-rearing facilities and methodologies, research on host-plant resistance increased (Guthrie et al. 1965, Lewis and Lynch 1969, Brindley et al. 1975). The physiological age of a corn plant affects the degree of resistance it exhibits (Reed et al. 1972). Klun and Robinson (1968)
demonstrated that the concentrations of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxzin-3-one), the resistance factor in resistant corn, was highest in seedling plants and decreased as the plants developed. Thus, DIMBOA-related resistance is effective against first-generation infestations.


Chemicals are often used to manage insects, and arsenicals, fluosilicates, and nicotine dusts were used initially to control *O. nubilalis*. As insecticide chemistry improved, novel insecticides were tested on *O. nubilalis* (Anonymous 1955, Brindley et al. 1975). Gould and Wilson (1957) conducted work with chlorinated hydrocarbons and the early phosphate-based insecticides. Hudon (1962) reported on efficacy of some of the early carbamates used as management tools. Many other insecticides have been evaluated for *O. nubilalis* control as well (Harding et al. 1968, Berry et al. 1972, Brindley et al. 1975, McWhorter et al. 1976, and Koziol and Witkowski 1981, Rice 2000).
Timing of insecticide applications to corn is critical for effective control of larvae. Larvae that have bored into plants are not susceptible to conventional insecticides. If insecticides are applied before a majority of the larvae hatch, then control will not be efficacious. A single application for first-generation *O. nubilalis* is made ideally at 10–14 days after egg masses are first spotted on corn, and applications for second-generation should be made during egg hatch, but before larvae bore into stalks (Mason et al. 1996). Insecticides with relatively long-lasting residuals are desirable for late-season control because oviposition during the second flight occurs over more days than during the first flight. Many techniques are used to scout fields so that insecticides are applied at the appropriate times. Scouting for first generation infestations may include the use of light traps and pheromone-baited traps to detect first-flight adults, degree-day models to estimate the onset of oviposition, assessment of corn phenology, and randomly sampling corn plants for larval infestations (Mason et al. 1996, Witkowski and Wright 1997, Gesell and Calvin 2000, Rice 2000, Bessin 2004, Sorensen 2005). Second-generation scouting is similar except that the focus is on detecting egg masses instead of larvae, and more insecticide applications are generally required.

Mating disruption to control *O. nubilalis* has been evaluated. Under mating disruption conditions, moths either do not mate or mating is delayed, which reduces the quality and quantity of eggs oviposited (Fadamiro et al. 1999). Both the metered-semiochemical, timed-release system (MSTRSTM) and Shin-Etsu rope formulations have been evaluated as mating-disruption tools (Fadamiro et al. 1999). Although mating disruption was not correlated with an increase in crop yield, fewer matings occurred in areas treated with MSTRSTM or Shin-Etsu ropes, and mating was apparently delayed in the
treatment areas based on the reduced number of females with spermatophores (Fadamiro et al. 1999). Fadamiro et al. (1999) further suggested that this tactic of moth control is promising because mating disruption tools only need to be placed where moths mate—mainly in or near patches of certain grasses (Showers et al. 1976, Showers et al. 1980).

Showers et al. (1990) evaluated genetic tactics to reduce *O. nubilalis* populations with some success, and found that when the progeny of reciprocal crosses of univoltine and multivoltine parents are released into the northern US, some may not undergo a timely larval diapause. Conversely, the resultant progeny of the reciprocal crosses may detrimentally enter diapause early when released in the southern US. Because incidence of diapause is a heritable trait (Reed et al. 1981) and correlated with geography (Showers et al. 1975), artificial releases of bivoltine moths in univoltine areas may result in progeny that are not able to enter diapause at the appropriate time to ensure winter survival. Releasing univoltine moths into naturally multivoltine areas may disturb *O. nubilalis* populations too, although more work is needed on *O. nubilalis* genetics.

Genetically-modified corn hybrids that express crystal protein endotoxin genes from *Bacillus thuringiensis* Berliner (*Bt*) (Bacillales: Bacillaceae) for the control of *O. nubilalis* have been available commercially since 1996 (Koziel et al. 1993) and are an increasingly popular management tactic (Pilcher et al. 2002). However, development of resistance by pestiferous Lepidoptera may reduce the effective life of this transgenic technology.

**Resistance Management.** Insects have evolved resistance to all classes of synthetic insecticides, and may evolve resistance to transgenic insecticidal crops at some time in the future. Widespread use of *Bt* corn is imposing high selection pressure for resistance to the *Bt*
toxin. Thus, the chance that a resistance allele, or alleles, will increase in frequency and spread through a population has been increased, potentially rendering conventional *Bt* insecticide formulations and genetically-engineered insecticidal plants ineffective in the future. The resistance management strategy for *O. nubilalis* on *Bt* corn is predicated on the expression of a high dose of *Bt* toxin in the plants, and provision of untreated, non-transgenic refugia (Andow and Ives 2002). A proposed definition of high dose is 25 times the toxin concentration needed to kill susceptible larvae (EPA 1998). Further, the management strategy presumes that resistance is recessive and that heterozygotes are susceptible to the toxin. Resistance in *O. nubilalis* to a commercial formulation of *Bt* (Dipel® ES) appears to be inherited as an incompletely dominant autosomal gene (Huang et al. 1999). However, Dipel® ES contains several Cry toxins including Cry1Aa, Cry1Ab, and Cry1Ac as well as toxic spores (Huang et al. 2002). *Bt* corn usually contains the *cry1Ab* gene, although other genes have been used including the *cry1Ac* gene, the *cry9C* gene, and the *cry1F* gene (Hellmich et al. 2001). A theoretical model suggests that recessive resistance alleles must be detected at frequencies of less than 0.005 to provide enough time to adapt management practices to prevent the build up of resistance in a population to the point of control failure (Andow and Ives 2002). Methods to monitor resistance include screening field-collected egg masses, screening field-collected larvae, an in-field *Bt*-corn screen, and an F2 screen (Venette et al. 2000, Andow and Ives 2002).

A near century of *O. nubilalis* research notwithstanding, additional information is required to improve methods of managing this important pest of corn. Particularly, examination of the interactions of *O. nubilalis*, disease, and the toxin found in genetically-
modified corn will improve resistance monitoring. Elucidation of abiotic factors that affect nightly moth captures in pheromone-baited traps will aid workers in interpreting changes in numbers collected. Further, a better understanding of adult aggregation and dispersal behavior will improve resistance management strategies for this insect relative to Bt corn.

References Cited


http://www.uky.edu/Ag/Entomology/entfacts/fldcrops/ef106.htm


Caffery, D. J. 1926. How to fight the European corn borer this fall. USDA Misc. Cir. No. 84.


[EPA] Environmental Protection Agency. 1998. *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn; exemption from the requirement of a tolerance in corn. 10 April. Fed Reg. 3(69).


http://www.ento.psu.edu/extension/factsheets/european_corn_borer.htm


Hudon, M. 1962. Field experiments with Bacillus thuringiensis and chemical insecticides for the control of the European corn borer, Ostrinia nubilalis, on sweet corn in southwestern Quebec. J. Econ. Entomol. 55: 115–117.


http://www.ces.ncsu.edu/depts/ent/notes/Vegetables/veg2.html


Steinhaus, E. A. 1952. Microbial infections in European corn borer larvae held in the laboratory. J. Econ. Entomol. 45: 48–51.


http://entomology.unl.edu/ecb/ecb1.htm#Item4
CHAPTER 2. GROWTH, DEVELOPMENT, AND SURVIVAL OF
NOSEMA PYRAUSTA-INFECTED EUROPEAN CORN BORERS
(LEPIDOPTERA: CRAMBI DaE) REARED ON MERIDIC DIET AND CRY1AB


Brendon J. Reardon, Richard L. Hellmich, Douglas V. Sumerford, and Leslie C. Lewis

Abstract

Transgenic corn hybrids expressing crystal protein endotoxin genes from Bacillus thuringiensis Berliner are an increasingly popular tactic for managing the European corn borer, Ostrinia nubilalis (Hübner), in North America. Ostrinia nubilalis populations also are often vulnerable to the ubiquitous entomopathogenic microsporidium Nosema pyrausta (Paillot). We examined the effect of feeding meridic diet incorporated with purified Cry1Ab on growth, development, and survival of Nosema-infected and uninfected neonate O. nubilalis. Infected larvae developed more slowly than uninfected larvae. Increasing concentrations of Cry1Ab in diet reduced larval development, and this phenomenon was amplified by microsporidiosis. Infected larvae weighed significantly less than uninfected larvae. The relationship among Nosema infection, Cry1Ab concentration, and larval weight was fitted to an exponential function. The LC$_{50}$ of infected larvae was one-third that of uninfected larvae, indicating that infected larvae are more vulnerable to toxin. This work has implications for resistance management of O. nubilalis and demonstrates that it is important to determine whether N. pyrausta is present when testing susceptibility of larvae to transgenic corn hybrids.
Introduction

The European corn borer, *Ostrinia nubilalis* (Hübner), is estimated to have entered the US from Europe between 1909 and 1914 (Smith 1920, Fracker and Fluke 1926). Since its arrival, *O. nubilalis* has established itself as a major pest of corn, *Zea mays* L. Rice (1994) suggested that yield loss due to *O. nubilalis* may reach 21 quintals per hectare. Genetically-modified corn hybrids that express crystal protein endotoxin genes from *Bacillus thuringiensis* Berliner (*Bt*) for the control of *O. nubilalis* have been available commercially since 1996 (Koziel et al. 1993) and are an increasingly popular management tactic (Pilcher et al. 2002). However, development of resistance by pestiferous Lepidoptera may reduce the duration of efficacious use of transgenic technology.

*Nosema pyrausta* (Paillot) (Microspora: Nosematidae) is likely the most chronically detrimental, naturally occurring pathogen of *O. nubilalis* in the US (Lewis and Lynch 1978). A ubiquitous microsporidium, *N. pyrausta* reduces egg hatch, development rate, fecundity, and life span of *O. nubilalis* (Zimmack and Brindley 1957, Windels et al. 1976). Pierce et al. (2001) examined the interactions of Dipel® ES (a spray formulation of *Bt*), *N. pyrausta*, and *O. nubilalis*, and they concluded that the susceptibility of *O. nubilalis* to Dipel® ES increased when infected with *N. pyrausta*. Dipel® ES, however, contains bacterial spores and several different endotoxins active against Lepidoptera including Cry1Aa, Cry1Ab, Cry1Ac, and others (Rukmini et al. 2000, Huang et al. 2002) that may maximize the toxicity of this *Bt* preparation to *O. nubilalis* (Mohd-Salleh and Lewis 1982). In contrast to insecticidal sprays for *O. nubilalis*, gene transfer technology currently uses one or a few genes, including the *cry1Ab* crystal protein endotoxin gene (Pilcher et al. 2002). It is, therefore, important to
assess the interactions of *O. nubilalis*, *N. pyrausta*, and Cry1Ab. The objective of this research was to examine the effect of purified Cry1Ab on growth, development, and survival of *Nosema*-infected and uninfected *O. nubilalis*.

**Materials and Methods**

**Insect and Pathogen Cultures.** Adult *O. nubilalis* collected during the summer of 2002 from light traps were used to establish a colony at the Corn Insects and Crop Genetics Research Unit, Ames, IA. To ensure that the colony was *Nosema*-free, eggs were heat-treated (Raun 1961). Moths were reared following procedures similar to Guthrie et al. (1965).

*Nosema pyrausta* spores were isolated from field-collected *O. nubilalis* in 2002. Infected larvae were homogenized in a glass tissue grinder with 10X phosphate buffered saline (PBS), and the homogenate was filtered through cheesecloth. Aureomycin (50 mg/ml solution) was added to solution to inhibit microbial growth. The concentration of spores was determined by using a haemocytometer (Levy, Horsham, PA) under 400X phase contrast microscopy. The suspension was frozen at −20°C when not in use.

Purified trypsin activated Cry1Ab (HPLC chromatogram demonstrated purity) was obtained from Dr. M. Carey (see Acknowledgments). The toxin was activated and isolated from a *crylAb* clone expressed in *E. coli*. It was then desalted, freeze-dried, and shipped overnight to the USDA-ARS laboratory. When not in use, the toxin was stored in a −20°C freezer.

**Experimental Design.** A completely randomized design was used for this study, and treatments were replicated three to four times over time depending on treatment combination. Treatment design was a 2x8 factorial. The two levels of the first factor were *Nosema*-
infected and uninfected larvae, respectively, and the eight levels of the second factor corresponded to concentrations of Cry1Ab in meridic diet. Thus, 16 treatment combinations (*Nosema* infection by toxin concentration in diet) were evaluated.

**Diet Preparation.** Standard meridic diet of wheat germ was prepared using methodology similar to Lewis and Lynch (1969) for each replication except Fumidil B (*Nosema* growth inhibitor) was omitted. A stock solution of Cry1Ab was serially diluted with PBS for each respective toxin concentration. Aliquots of diet were mixed with one dilution of purified Cry1Ab in a blender for about 30 s, giving seven individual concentrations and a control with no Cry1Ab. The eight toxin concentrations used were 370, 185, 92, 46, 23, 11, 5, and 0 (control) ng/ml. About five milliliters of diet from each mixture was poured into 18.5-ml plastic diet cups (Fill Rite, Newark, New Jersey), and the diet cups were covered with a plastic sheet. The diet solidified at room temperature for about 6 hr. Half of the diet cups at each concentration received 250 µl of *Nosema* spores (4.96 x 10^6 spores per milliliter) topically, and the *Nosema* treatments were dried at room temperature for an additional 6–12 hr before larvae were applied.

**Dose-Response Bioassay.** Only neonate larvae (<24 hr post eclosion) were used. A single larva was placed onto the surface of diet within each diet cup. For each treatment combination, about 20 larvae were tested; about 320 larvae were used in each replication. Diet cups were held in an environmentally controlled room at 27°C, 24:0 (L:D), and 80% RH. After 10 d, growth, development, and mortality of the larvae was determined. At least 10 surviving larvae from each treatment were weighed and assessed for instar based on body length, prothoracic shield size (Dewitt and Stockdale 1983), and the number of head capsule
exuvia found in each diet cup. Instar was used to quantify development, and weight was used to measure growth. Larvae were destructively sampled to confirm presence or absence of Nosema infection. For analyses that included mortality, living first instars were recorded as dead because they effectively have negligible contribution to plant damage and subsequent O. nubilalis populations.

**Statistical Analyses.** Distributions of the five larval instars for each level of infection were analyzed in an 8x5 contingency table for independence between Cry1Ab concentration in diet and larval development (CHISQ option of the SAS procedure PROC FREQ; SAS Institute 1988). Instar data were pooled over replications because visual inspection of the data failed to indicate a trend temporally.

The influences of Nosema infection, toxin concentration, replication, and main-effect interactions on transformed larval weight were assessed by using analysis of variance, and means were separated using the Tukey multiple range test (PROC GLM, SAS Institute 1988). Weight data were transformed for ANOVA by using a natural logarithm to meet the assumptions of homogeneity of variance and normality. Additionally, the relationship among Nosema infection, toxin concentration, and larval weights was described by using an exponential function \( Y = \beta_0 + \exp[\beta_1 - (\beta_2 \times \text{TOXIN}) - (\beta_3 \times \text{INFECTION})] \), where \( Y \) is larval weight, \( \text{TOXIN} \) is the concentration of Cry1Ab in diet, \( \text{INFECTION} \) is the presence or absence of \( N. pyrausta \), and \( \beta_0, \beta_1, \beta_2, \) and \( \beta_3 \) are estimated. The exponential function was fitted to the larval weight data by using non-linear regression using the SAS procedure PROC NLIN (SAS Institute 1988).
Survival data were subjected to Probit analysis to calculate LC$_{50}$ values for infected and uninfected larvae, respectively, on a logarithmic base 10 scale for each replication. The transformed LC$_{50}$ values were subsequently analyzed by using ANOVA and Tukey multiple range test (PROC GLM, SAS Institute 1988).

**Results and Discussion**

The results of the contingency table indicated that the number of uninfected larvae in each stadium was dependent on Cry1Ab concentration in diet ($\chi^2 = 822.09$, df = 28, $P < 0.0001$; $n = 567$). Similarly, the number of infected larvae in each stadium was dependent on toxin concentration in diet ($\chi^2 = 796.37$, df = 28, $P < 0.0001$; $n = 760$). However, in the case of the infected larvae, the effect was more pronounced and higher numbers of larvae remained in earlier stadia. Overall, larval development was reduced when toxin concentration in diet increased, and the reduction in development was amplified by *Nosema* infection (Fig. 1).

The ANOVA indicated a significant relationship among transformed larval weight, *Nosema* infection, Cry1Ab concentration in diet, and replication ($F = 43.69$, df = 53, 441; $P < 0.0001$). As Cry1Ab concentration increased, the difference between the weights of infected and uninfected larvae decreased (Fig. 2), which is demonstrated by the significant interaction term ($F = 2.53$, df = 7, 441; $P = 0.0147$). The reduction in larval weight associated with Cry1Ab in diet was increased by *Nosema* infection. Although replication was significant in the model ($P < 0.0001$), differences in larval weight among replications were not chronological, suggesting that the viability of the *Nosema* spores and the toxicity of Cry1Ab were not progressively changing temporally. Non-linear regression analysis indicated a
significant exponential relationship among larval weight, toxin concentration in diet, and infection ($F = 13.80; \text{df} = 4, 76; P < 0.0001; r^2 = 0.61$) ($n = 495$). The overall model was:

$$\text{Larval Weight} = 3.9 + \exp[3.8 - (25437 \times \text{TOXIN}) - (0.5 \times \text{INFECTION})]$$

The Probit analysis used to obtain LC$_{50}$ values for infected and uninfected *O. nubilalis* was significant (Table 1). The LC$_{50}$ for infected larvae was significantly lower than uninfected larvac ($F = 8.48; \text{df} = 1, 5; P = 0.0333$; Fig. 3). For infected larvae and uninfected larvae, the LC$_{50}$ was 76 and 245 ng/ml, respectively. Thus, uninfected larvae were over three times as tolerant of Cry1Ab in diet than infected larvae.

Dipel® ES may be more pathogenic to *O. nubilalis* than purified Cry1Ab. When a corresponding LC$_{50}$ calculated by Pierce et al. (2001) was compared with the LC$_{50}$ for infected larvae derived from the current study, the LC$_{50}$ associated with Dipel® ES ($=0.01$ ng/ml) was 7,600 times less than that of the LC$_{50}$ associated with purified Cry1Ab (76 ng/ml). This suggests that larvae respond differently to Dipel® ES than purified Cry1Ab. Further, the differential response of larvae to purified Cry1Ab and Dipel® ES demonstrates the synergism among endotoxins and bacterial spores (Mohd-Salleh and Lewis 1982). This observation, however, may be confounded with differences in the *Nosema* spores and larvae used and the methodology of diet preparation used in the two studies.

Our findings may have implications for management of *O. nubilalis* resistance to transgenic corn hybrids. *Nosema*-infected larvae that are resistant to transgenic corn hybrids may be overlooked during screening processes that rely on survivorship or growth, and such
larvae could be misidentified. In such a case, without intervention a resistance trait may be propagated through an insect population. Other pathogens—not examined in this study—may similarly cause an increase in errors when conducting bioassays.

Acknowledgments

We are indebted to Jean Dyer, Bob Gunnarson, and Miriam Lopez of the Corn Insects and Crop Genetics Research Unit, USDA-ARS, for technical assistance, and Dr. Marianne Carey, Department of Biochemistry, Case Western Reserve University, for supplying Cry1Ab. We also thank Dr. Leellen Solter, Insect Pathology, Illinois Natural History Survey, Dr. Stefan Jaronski, Pest Management Research Unit, USDA-ARS, and Dr. Blair Siegfried, Department of Entomology, University of Nebraska-Lincoln, for their critical reviews of this manuscript. Finally, we thank Dr. Thomas Sappington, Corn Insects and Crop Genetics Research Unit, USDA-ARS, for his insightful suggestions. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

References Cited


Table 1. Toxicity of Cry1Ab to *Nosema pyrausta*-infected and uninfected *Ostrinia nubilalis*.

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>Slope (SE)$^a$</th>
<th>LC$_{50}$ (95% FL)$^b$</th>
<th>df</th>
<th>$\chi^2$$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>684</td>
<td>-1.73 (0.19)</td>
<td>76 (58–101)</td>
<td>1</td>
<td>84.35</td>
</tr>
<tr>
<td>Uninfected</td>
<td>512</td>
<td>-1.68 (0.24)</td>
<td>245 (176–394)</td>
<td>1</td>
<td>51.11</td>
</tr>
</tbody>
</table>

$^a$ Transformed to logarithm base 10 scale.

$^b$ Units are nanograms of toxin per milliliter of meridic diet.

$^c$ $P < 0.01$ for all Chi-square values.
Fig. 1. Percent of both first and second, third, and both fourth and fifth instars of *Nosema pyrausta*-infected and uninfected *Ostrinia nubilalis* at different concentrations of purified Cry1Ab in meridic diet after 10 d.
Fig. 2. Mean (±SE) larval weights of Nosema pyrausta-infected and uninfected Ostrinia nubilalis fed different concentrations of purified Cry1Ab in meridic diet for 10 d.
Fig. 3. Percent survival (±SE) of *Nosema pyrausta*-infected and uninfected *Ostrinia nubilalis* fed different concentrations of purified Cry1Ab in meridic diet for 10 d.
CHAPTER 3. IMPACT OF TRAP DESIGN, WINDBREAKS, AND WEATHER ON CAPTURES OF THE EUROPEAN CORN BORER (LEPIDOPTERA: CRAMBIDAE) IN PHEROMONE-BAITED TRAPS

A paper to be submitted to the Journal of Economic Entomology

Brendon J. Reardon, Douglas V. Sumerford, and Thomas W. Sappington

Abstract

Pheromone-baited traps are often used in ecological studies of the European corn borer, *Ostrinia nubilalis* (Hübner). However, differences in trap captures may be confounded by trap design, trap location relative to a windbreak, and changes in local weather. The objectives of this experiment were, first, to examine differences in *O. nubilalis*-moth captures among the Intercept™ wing trap, the Intercept™ bucket/funnel UNI trap, the Hartstack wire-mesh, 75-cm diameter cone trap (large metal cone trap), as well as among three cone trap designs. Second, we examined the influence of the location of the large metal cone trap relative to a windbreak on the number of moths captured. Third, we examined the relationship between nightly mean air temperature, relative humidity, wind speed, precipitation, and the numbers of moths captured in the large metal cone traps. The number of moths captured was significantly influenced by trap design, with the large metal cone traps capturing the most moths. Wing and bucket traps were ineffective. Differences among trap captures were significant among the trap locations relative to a windbreak. Under strong (>14 kph) or moderate (7<14 kph) wind speeds, traps located leeward of the windbreak captured the most moths, but when wind speeds were light (<7 kph), traps not associated with the windbreaks captured the most moths. The multiple regression models fitted to the
relationship between the numbers of moths captured daily and the nightly weather patterns were significant. Nightly mean air temperature was consistently the most influential parameter in the models, and its relationship with moth capture was positive.

Introduction

The European corn borer, *Ostrinia nubilalis* (Hübner), is a major pest of corn, *Zea mays* L., in most of North America east of the Rocky Mountains. Understanding its biology and ecology is pivotal for its management because control tactics are often predicated on such (Mason et al. 1996). Insect traps are an easy-to-use device for detection and monitoring populations of some insects, including *O. nubilalis*. However, fluctuations in *O. nubilalis* populations may not be correlated necessarily with changes in the numbers of moths captured in traps, and trap performance may not be consistent across trap designs or trap locations.

Many pheromone-trap designs have been used in studies of *O. nubilalis*, including aerial water-pans (Webster et al. 1986, Thompson et al. 1987, Bartels and Hutchison 1998), aluminum-screen petri-dish cages (Showers et al. 1974), wire-mesh cone traps (Webster et al. 1986, Mason et al. 1997, Bartels and Hutchison 1998, Showers et al. 2001, Sorenson et al. 2005), nylon-mesh cone traps (Webster et al. 1986, Bartels et al. 1997), and sticky traps (Oloumi-Sadeghi et al. 1975, Kennedy and Anderson 1980, Webster et al. 1986). Pheromone-baited traps are advantageous because they are species-specific, sex-specific, portable, relatively cheap, do not require a power source, and easy to operate and maintain. However, the relative efficiency among trap designs may differ.

The location or surroundings of insect traps influences their efficiency (Wellington and Trimble 1984, Lee 1988, Mason et al. 1997, Sappington and Spurgeon 2000,
Kavallieratos et al. 2005), and insect responsiveness to traps is often a function of weather (Davidson and Andrewartha 1948, Vogt 1986, Gregg et al. 1994, Mohamed-Ahmed and Wynholds 1997, Butler et al. 1999). Windbreaks such as tree lines modulate air movement, airborne chemicals, and airborne insects (Lewis and Dibley 1970), and an insect’s ability to control flight is a function of wind speed. Thus, determining the sensitivity or ability of a trap to detect insect populations and their ability to monitor population changes requires knowledge of trap efficiency in different environments and conditions.

The objectives of this study were to examine differences in O. nubilalis-moth captures among the Intercept™ wing trap, the Intercept™ bucket/funnel UNI trap, and the Hartstack wire-mesh cone trap (large metal cone trap). In addition, we compared capture efficiency among three cone trap designs, including the large metal, small metal, and small nylon cone traps. All of these traps are advertised by the manufacturers and vendors as effective for trapping O. nubilalis. Second, we examined the influence of the location of the large metal cone trap relative to a windbreak on the number of moths captured. Third, we examined the relationship between the numbers of moths captured daily in large metal cone traps and nightly mean air temperature, relative humidity, wind speed, and precipitation.

**Materials and Methods**

**Experimental Design.** Traps were established at sites in Story and Boone Counties, IA, in 2003, 2004, and 2005, and were maintained throughout the summer over both moth flights. Ostrinia nubilalis is bivoltine in much of the Corn Belt, including Iowa (Mason et al. 1996). The first flight usually begins in mid-May and lasts 3–4 wk, and the second flight in mid-July and typically lasts 4–6 wk. In 2003, three trap designs were deployed on 20 May at
each trap site. The three trap designs used at a site were the Intercept™ wing trap (hereafter wing trap) (IPM Tech, Inc., Portland, OR), the Intercept™ bucket/funnel UNI trap (hereafter bucket trap) (IPM Tech, Inc., Portland, OR), and the Hartstack wire-mesh, 75-cm diameter cone trap (hereafter large metal cone trap) (Hartstack et al. 1979) (Figs. 1a–1c). At each site, the traps were placed 30 m apart from one another in a line that ran east to west. The wing and bucket traps were each suspended from a 1.5 cm-diameter metal pole that was hammered into the ground. The large metal cone traps were mounted on top of the poles. Traps were equipped with a pheromone lure (Trécé Inc., Adair, OK) impregnated with a mixture of 97% cis-11-tetradecenyl acetate and 3% trans-11-tetradecenyl acetate, attracting males of the Z- or cis-strain of *O. nubilalis*, which is the strain inhabiting Iowa (Klun 1968, Klun and Brindley 1970). The lures were replaced biweekly and suspended 1 m above the ground. The sticky bottoms of the wing traps were replaced at least weekly. Traps were serviced 5 d per wk, and the order of the trap designs in a trap line at a site was randomly selected daily during each moth flight, so that each trap design had the same chance to capture moths at a given site (Sappington 2002). All of the traps were within 100 m of corn, and the native grasses’ heights around the traps were generally <1 m.

To test the effect of windbreaks on the number of moths captured in traps, trap lines were placed away from, on the north side, or on the south side of the windbreaks. All windbreaks at the trap sites ran east to west. Lines of mature trees >100 m long and >10 m deep constituted a windbreak, and associated trap lines were placed within 5 m of the windbreak. In 2003, two trap lines were located in open fields away from windbreaks, three
trap lines were located north of windbreaks, and four trap lines were located south of windbreaks.

The experimental design used in 2004 was similar to 2003. The traps were deployed 17 May, and the order of the trap design in a trap line at a site was randomly selected about thrice weekly. Two trap lines were located in open fields away from a windbreak, three trap lines were located north of windbreaks, and three trap lines were located south of windbreaks.

In 2005, the experimental design for testing the effects of windbreaks on moth captures was akin to 2003 and 2004. However, only the large metal cone traps were deployed because data from the previous years showed that the wing and bucket traps collected few moths, as will be described in the Results sections. The large cone traps were deployed 26 April. Three trap sites were located in open fields away from windbreaks, three sites were located north of windbreaks, and three sites were located south of windbreaks.

In 2005, numerous trap sites were set up along two transects across Iowa and into adjoining states to collect moths for a population genetics study. Because we recorded the numbers of moths collected per trap per location, and because three types of cone traps were used, the data provided an opportunity to test different cone trap designs on capture efficiency. The trap designs evaluated in this experiment were the large metal cone trap (Fig. 1c), a modified Hartstack wire-mesh, 35-cm diameter cone trap (hereafter small metal cone trap) (Fig. 1d), and the nylon-mesh Heliothis, 35-cm diameter cone trap (hereafter small nylon cone trap) (Gemplers®, Madison, WI) (Fig. 1e). Traps sites were established in late June and early July before the onset of the second flight. All traps were positioned along
roadsides within 5 m of a corn field. At each site there were five traps, located within about 2 km of each other. The spatial arrangement of the trap designs deployed at a site was arbitrarily chosen, and the number of each trap design at a site varied. The trap sites were spaced at 80-km intervals along two transects in the cardinal directions centered on Ames, IA. Nested within two of the 80-km intervals along the east-west transect in central and eastern Iowa were trap sites spaced at 16-km intervals. The traps were serviced about twice weekly, and lures were replaced biweekly.

We examined the effects of windbreaks and weather on moths captured in the large metal cone traps. A weather station (Campbell Scientific, Inc., Logan, UT) was used to monitor weather parameters during the moth flights, and was located within 9 km of the traps. Air temperature (°C), dew point (°C), precipitation (mm), relative humidity (%), wind direction (°), and wind speed (kph) were measured every 60 sec, and a mean output for each variable was calculated hourly.

The relative location of trap lines associated with windbreaks (i.e., windward or leeward) was assigned daily based on mean direction of wind relative to windbreaks during the night of capture. Nightly mean wind directions between 337.5° and 22.5° were considered north winds, and mean wind directions between 157.5° and 202.5° were deemed south winds, where cardinal north equals 0° or 360°. Data from nights with average winds outside the indicated limits were omitted from the analyses to reduce possible confounding effects of interference among traps in a trap line at a site (Sappington 2002), and to increase the dampening effect of a windbreak on wind speed. The trap sites not associated with windbreaks were always considered as such regardless of wind direction. However, dates
that were omitted from the leeward-windward sites because the wind direction was outside the indicated limits were also omitted from the no-windbreak sites, so that the comparisons were more similar. Because the moths are nocturnal, only weather data collected between 21:00 and 06:00 were used to determine the predominant nightly wind direction for assigning trap locations relative to windbreaks.

Mean nightly measurements of the weather factors also were regressed on the numbers of moths captured in the large metal cone traps. Moth-capture data from Mondays were omitted from all analyses because they represented a 3-d capture. Only data that were collected during the natural moth flights were used for analyses (Fig. 2).

**Statistical Analyses.** An analysis of variance (restricted maximum likelihood estimates; REML-ANOVA) was used to determine if trap designs tested in 2003 and 2004 (wing, bucket, and large metal cone traps) influenced the number of moths captured (PROC MIXED, SAS Institute 2001). The dependent variable of the model was the mean number of male moths collected daily, and trap design was the fixed effect. The random effects in the model were dates nested in year and trap site. Treatment means were separated using the LSMEANS option with the Tukey-Kramer adjustment, and the degrees of freedom were estimated using the Satterthwaite’s approximation (SAS Institute 2001). A similar model was used to assess the influence of the large metal cone trap, the small metal cone trap, and the small nylon cone trap on the number of moths captured. The dependent variable in the model was the mean number of moths collected daily, and the independent variable was trap design. The random factors were date, trap site, and their interaction.
A REML-ANOVA was used to determine if trap location relative to a windbreak influenced the number of moths captured in the large metal cone traps (PROC MIXED, SAS Institute 2001). Separate models were fitted to moth-capture data under various wind speed categories because the difference in speed between windward and leeward locations presumably increases as wind speed increases (e.g., Sappington and Spurgeon 2000). Winds were considered strong, moderate, or light when nightly mean wind speeds were >14, 7–14, or <7 kph, respectively. The dependent variable of the model was the mean number of moths captured, and trap location relative to a windbreak was the fixed effect. The random effect in the model was date. Treatment means were separated using the LSMEANS option with the Tukey-Kramer adjustment, and the degrees of freedom were estimated using Satterthwaite’s approximation (SAS Institute 2001).

The relationship between the numbers of moths collected in the large metal cone traps and weather was assessed by multiple regression for each flight of each year of the experiment (PROC REG, SAS Institute 2001). The dependent variable in the models was the numbers of moths collected, and the fixed effects were a linear term for date, a quadratic term for date, a cubic term for date, nightly mean air temperature (°C), nightly mean relative humidity (%), nightly mean wind speed (kph), and nightly precipitation (mm). The orthogonal polynomial functions of Julian date helped model the natural rise and fall cycle of moth populations over time (Davidson and Andrewartha 1948). Model construction was conducted in a stepwise fashion. Only data from the windward cone traps and the cone traps not associated with windbreaks were used because the leeward cone traps presumably experienced wind speeds less than those recorded by the weather station.
The dependent data used to analyze trap design and in multiple regressions were transformed to ln (X + 0.5) to meet model assumptions of homoscedasticity and normality (Fry 1993, Ott and Longnecker 2001), and the results are presented as untransformed data. All REML-ANOVA and multiple-regression model assumptions and fits were assessed with residual and normal plots.

Results

The REML-ANOVA model used to examine the relationship between 2003-2004 moth captures and trap design was significant ($F = 635.21; \text{df} = 2, 1612; P < 0.0001$). Least-squares differences of means between the bucket trap and the large metal cone trap, and between the wing trap and the large metal cone trap, were significant ($P < 0.0001$). However, the least-squares difference of means between the wing trap and the bucket trap was not significant ($P = 0.1279$). The large cone traps averaged 5.3 moths daily over the two natural moth flights of both years of the study ($n = 564$; Fig. 3a). The average daily trap counts of the wing traps ($n = 564$) and the bucket traps ($n = 564$) was less than 0.1 daily (Fig. 3a), and on most dates these trap counts were zero.

The REML-ANOVA model used to examine the relationship between moth captures and cone trap design was significant ($F = 14.47; \text{df} = 2, 131; P < 0.0001$) in 2005. Least-squares differences of means between the large metal cone trap and the small nylon cone trap, and between the large metal cone trap and the small metal cone trap, were significant ($P < 0.006$). However, the least-squares difference of means between the small cone trap and the nylon cone trap was not significant ($P = 0.0588$). In this case, the large metal cone traps averaged 21.9 moths daily from mid-July through mid-August of 2005 ($n = 67$; Fig. 3b). The
average daily trap count of the small metal cone traps \((n = 132)\) was 10.7, and for the small nylon cone traps \((n = 171)\) was 11.7 daily (Fig. 3b).

The location of the large metal cone traps relative to windbreaks significantly influenced the number of moths captured under all wind conditions in 2003 and 2004. Under strong winds (>14 kph), the location of the traps significantly influenced moth capture \((F = 6.01; \text{df} = 2, 127; P = 0.0032)\). The same was true under moderate winds \((7<14 \text{ kph})\) \((F = 7.43; \text{df} = 2, 370; P = 0.0007)\). The traps located leeward of a windbreak captured the most moths at strong or moderate wind speeds (Fig. 4). Conversely, trap location significantly influenced moth captures during light winds \((<7 \text{ kph})\) \((F = 4.69; \text{df} = 2, 199; P = 0.0102)\), but the traps not associated with windbreaks captured the most moths (Fig. 4).

When the numbers of moths captured in the large metal cone traps were regressed on the weather parameters in 2003 and 2004, all the fitted models were significant (Table 1). The range of the nightly means for the air temperature was 9.7–25.4°C, for the relative humidity was 44.1–100%, and for the wind speed was 1.6–22.7 kph. The range of the nightly precipitation sum was 0–15.2 mm. The influence of all weather parameters on the numbers of moths captured daily in the large metal cone traps was positive except for wind speed, which was negative (Table 2). The nightly mean air temperature was consistently the most influential weather parameter in the multiple regression models (Table 3).

**Discussion**

In our experience, the large metal Hartstack cone pheromone traps have consistently performed well for collecting *O. nubilalis*. However, these traps are expensive to build, and to our knowledge are no longer available on the open market. So, we were interested in
testing different traps in a search for a suitable and affordable substitute. Wing traps and bucket traps are relatively inexpensive, and commonly advertised as being effective for capturing *O. nubilalis*. Similarly, the modified smaller Hartstack cone traps, both metal and nylon designs, are advertised as less-expensive substitutes for the conventional large metal cone traps.

In this study, trap design significantly influenced the number of *O. nubilalis* moths collected in pheromone-baited traps. The large metal cone traps assessed in 2003-2004 captured the most moths consistently throughout the experiment (Figs. 2a and 2b). In contrast, the wing and bucket traps captured very few moths during the same period. Because of the paucity of moths captured with the wing and bucket traps during the same period that the large metal cone traps captured moths, we conclude that the former two trap designs are not effective detection or monitoring devices of *O. nubilalis*, manufacturers’ and distributors’ claims notwithstanding. Although the pheromone in the wing and bucket traps presumably attracts males to the vicinity, the traps are ineffective at collecting and holding moths of *O. nubilalis*. Similarly, previous studies showed that sticky wing traps baited with pheromone are inefficient and not adequate for monitoring *O. nubilalis* (Oloumi-Sadeghi et al. 1975, Kennedy and Anderson 1980, Webster et al. 1986, Athanassiou et al. 2004). Goodenough et al. (1989) found, conversely, bucket traps to be superior over large metal cone traps when collecting southwestern corn borer, *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae). In that study, most of the moths captured were still alive when the traps were serviced, but kill strips impregnated with dichlorvos were placed in the base of the trap and may have
improved retention of captured moths. However, Ngollo et al (2000) found that the small nylon cone traps captured more *O. nubilalis* than bucket traps containing a kill strip.

Though not compared directly, the small metal and small nylon cone traps captured relatively more moths in 2005 than the wing and bucket traps in 2003 and 2004. Nonetheless, the small metal and small nylon cone traps consistently captured fewer moths than the large metal cone traps in direct comparisons in 2005 (Fig. 2b). The discrepancy among the moth captures in the 2005 cone traps may be a result of the differences in the diameter at the base of the cone, which is more than twice as great in the large metal cone trap. Moths tend to fly upward when disturbed, so larger trap openings will lead to greater captures of moths. Bartels and Hutchison (1998) similarly found that the large metal cone traps were superior to other pheromone-baited traps tested, including the small nylon cone trap. Moreover, although the large metal cone traps began to capture moths at the onset of moth flights, as did blacklight traps, the latter were able to detect the peak flight of moths about two weeks before the large cone traps (Bartels and Hutchison 1998). Blacklight traps are indiscriminant trapping devices, whereas the pheromone-baited traps mimic female-produced pheromone. During peak flight, females are most bountiful, and may be superior competitors over the pheromone-baited traps (Thompson et al. 1987). It is important to accurately know when peak flight occurs when scouting fields for management decisions. When it is important to sample *O. nubilalis* moth populations, particularly when the population levels are low, using traps that are sensitive enough to detect moths is imperative. The large metal cone traps were the most efficient traps assessed in this experiment because they consistently captured the most moths at both low and high population levels.
The location of the large metal cone traps relative to a windbreak significantly influenced the number of moths captured. When winds were strong or moderate, most moths were collected in traps located leeward of a windbreak, and there were no significant differences between the windward cone traps and traps located away from windbreaks (Fig. 4). However, under light winds, the most moths were captured in traps located away from windbreaks. Likewise, Sappington and Spurgeon (2000) showed that when winds were >10 kph captures of boll weevils (Coleoptera: Curculionidae) were greater in pheromone-baited traps located leeward of a windbreak compared with windward traps. When wind speeds were light, there was no difference in captures in leeward and windward traps. Because windbreaks alter wind speeds, the large metal cone traps located leeward of windbreaks were presumably exposed to the calmest winds. Under strong and moderate wind speed conditions, male moths following the pheromone plume may have better control of their flight at traps located leeward of the windbreak, which may result in an increased number of moths captured. Alternatively, the pattern of the winds near a windbreak may direct airborne insects to the leeward side of a windbreak, increasing the number of moths flying in the vicinity of a leeward trap. For example, Lewis and Dibley (1970) showed that windbreaks create a sheltered zone leeward of the windbreak where flying insects tend to accumulate. The magnitude of this influence is proportional to the angle and speed of the incident wind and the permeability and dimensions of the windbreak (Lewis and Dibley 1970). Although we did not directly quantify the effect of the windbreaks on wind movement, we chose tree lines typical of the Iowa landscape. They were tall and thick enough to reasonably assume
they had a substantial dampening effect on winds striking it at an angle <22.5° of perpendicular.

Conversely, the traps not associated with windbreaks captured the most moths when the wind speeds were light, but they did not differ significantly from the number captured in leeward traps (Fig. 4). The windward traps captured the fewest number of moths when wind speeds were light. Under light winds, the windbreaks have the least effect on wind speed. The pheromone plumes emitted from traps located at sites not associated with windbreaks are free of obstruction in all directions, whereas traps associated with windbreaks may be effective only on one side of the windbreak, limiting the area from which the trap is attracting moths. Early in the moth flight, Ngollo et al (2000) found that more *O. nubilalis* moths were collected in cone traps located at cornfield borders and in bordering grasses, whereas traps in the middle of the cornfield captured the most moths during peak flight. Mason et al. (1997) indicated that more moths were collected in large metal cone traps when the openings were placed within the grass canopy instead of above the canopy, perhaps because a mixture of pheromone with plant volatiles is more attractive. Further, the moth counts from traps embedded in the grass canopy were less variable, which may improve the use of traps in estimating moth population changes.

The numbers of *O. nubilalis* moths captured in the large metal cone traps were significantly influenced by weather, and the fitted models accounted for 10–40% of the observed variation in nightly captures (Table 1). The nightly mean air temperature was included in five of the six fitted regression models and was positively related to moth capture (Table 2). Further, it accounted for most of the observed variability (Table 3). The model
that did not include temperature (i.e., 2003, second flight) was fitted to moth counts that were collected during a flight where the mean nightly temperature was the warmest, and the range in nightly temperatures was the smallest. The small range is likely why temperature was not included in the model. Other studies have indicated that air temperature is the most important weather parameter to describe moth captures in traps. Butler et al. (1999) collected the most Lepidoptera in blacklight traps on warm nights compared with cool nights, and noted that there was a negative relationship between precipitation and moth captures in blacklight traps. Mohamed-Ahmed and Wynholds (1997) found that the temperature was the most important weather variable that influenced (positive) tsetse fly, Glossina fuscipes fuscipes Newstead (Diptera: Glossinidae), captures in traps. Vogt (1986) noted that temperature was the most influential, and positive, factor in captures of the brush fly, Musca vetustissima Walker (Diptera: Muscidae), in traps.

Relative humidity was included in three of the six multiple regression models, and its relationship with moth capture was positive (Tables 1 and 2). However, mean nightly relative humidity accounted for <5% of the observed variation (Table 3). Wind speed and precipitation were each included in separate models once only (Table 1). The total nightly precipitation was related (positive) to moth captures, but it accounted for only 2% of the model variation (Tables 2 and 3). Sappington and Spurgeon (2000) demonstrated a negative relationship between wind speed and captures of boll weevils in pheromone-baited traps. During the first flight of 2004 when wind speed was a significant factor in the regression model, the mean nightly wind speeds were the highest and most variable. The negative relationship between wind speed and moth captures in the large metal cone traps was likely a
result of the strong and variable winds. However, the wind speed factor in this case accounted for only 6% of the model variation (Table 3). Unlike air temperature and relative humidity, which are consistent spatially at a local scale, wind speed and precipitation may be more variable spatially and not as accurately estimated for each trap site, leading to spurious estimates for the regression coefficients.

Although the effect of weather on trap captures seems intuitively to be important, identifying and quantifying such effects is notoriously difficult to accomplish. Sappington and Showers (1983) explored the effect of a number of weather variables on *O. nubilalis* captures in blacklight traps. When wind speeds were greater than 30 kph, blacklight captures of *O. nubilalis* decreased, and the size of the reduction may be further increased by precipitation. Similarly, when nightly air temperatures fell below about 10°C, blacklight trap captures decreased. There are many potential factors that can hinder detection of any relationships between moth captures and weather parameters, such as crop phenology, moth immigration and emigration, predation, parasitization, pathogen load, local farming practices, and microclimatic differences not reflected by measurements at weather stations located outside the trap sites.

If pheromone-baited traps are to be used to detect and monitor *O. nubilalis* populations, it is important to recognize that trap design, surroundings, and the weather influence the numbers of moths captured on any given night, in addition to changes in the population numbers themselves. How numbers of moths captured in traps relates to the actual or relative size of *O. nubilalis* populations remains an elusive goal. However, when moth population levels are low, it is essential that devices used to detect and monitor their
presence are as sensitive as possible to reduce false-negative measurements. In this study, we
learned that the large metal cone traps were the most sensitive pheromone-baited trap
evaluated. But because they are no longer commercially available, the small metal or nylon
cone traps are the next best alternative, though clearly inferior, being about 2-fold less
sensitive. Dependence on wing traps or bucket traps for detection, despite the attraction of
their low cost, is a false economy and is not suggested.

Trap location influences the number of moths collected, so it is best to use a
combination of trap locations near and away from windbreaks given the difference in
sensitivity depending on wind speed on a given night. Apart from air temperature, easily
measured weather parameters seem to be overwhelmed by other unknown factors, potentially
both abiotic and biotic in determining fluctuations in nightly trap captures, and are not likely
to be of much aid in improving their interpretation.

Acknowledgments

We are grateful for the field assistance that was received from N. Passolano, J.
Gibson, R. Ritland, M. Fiscus, D. Starret, K. Reardon, and A. Kronback. This article reports
the results of research only. Mention of a proprietary product does not constitute an
endorsement or a recommendation by the USDA for its use.

References Cited

Athanassiou. C. G., N. G. Kavallieratos, and B. E. Mazomenos. 2004. Effect of trap type,
trap color, trapping location, and pheromone dispenser on captures of male *Palpita


Mason, C. E., M. E. Rice, D. D. Calvin, J. W. Van Duyn, W. B. Showers, W. D.


Table 1. Model statistics for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather.

<table>
<thead>
<tr>
<th>Year</th>
<th>Flight</th>
<th>F</th>
<th>df</th>
<th>P</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>1</td>
<td>13.32</td>
<td>4, 135</td>
<td>&lt;0.0001</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.69</td>
<td>5, 130</td>
<td>&lt;0.0001</td>
<td>0.27</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>10.47</td>
<td>6, 152</td>
<td>&lt;0.0001</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.58</td>
<td>5, 52</td>
<td>&lt;0.0001</td>
<td>0.39</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>10.44</td>
<td>4, 160</td>
<td>&lt;0.0001</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.16</td>
<td>3, 158</td>
<td>0.0006</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Table 2. Parameter coefficients for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather.

<table>
<thead>
<tr>
<th>Year</th>
<th>Flight</th>
<th>Intercept</th>
<th>Lin$^b$</th>
<th>Quad$^b$</th>
<th>Cubic$^b$</th>
<th>temp.</th>
<th>RH</th>
<th>speed</th>
<th>Rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>1</td>
<td>-2.38</td>
<td>0.02</td>
<td>-1.78</td>
<td>-0.70</td>
<td>0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.64)</td>
<td>(0.51)</td>
<td>(0.42)</td>
<td>(0.42)</td>
<td>(0.04)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-6.83</td>
<td>-0.79</td>
<td>-2.81</td>
<td>-2.01</td>
<td>-</td>
<td>0.08</td>
<td>-</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2.25)</td>
<td>(0.57)</td>
<td>(0.69)</td>
<td>(0.72)</td>
<td>(0.03)</td>
<td>(0.09)</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>-4.74</td>
<td>0.96</td>
<td>-1.15</td>
<td>-0.06</td>
<td>0.20</td>
<td>0.04</td>
<td>-0.10</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.25)</td>
<td>(0.48)</td>
<td>(0.50)</td>
<td>(0.55)</td>
<td>(0.04)</td>
<td>(0.01)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-11.13</td>
<td>1.55</td>
<td>-1.87</td>
<td>0.81</td>
<td>0.26</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4.56)</td>
<td>(0.64)</td>
<td>(0.67)</td>
<td>(0.53)</td>
<td>(0.07)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>-2.02</td>
<td>-0.39</td>
<td>-1.42</td>
<td>-0.16</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.62)</td>
<td>(0.57)</td>
<td>(0.45)</td>
<td>(0.44)</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-1.83</td>
<td>-0.16</td>
<td>-1.99</td>
<td>1.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.59)</td>
<td>(0.42)</td>
<td>(0.42)</td>
<td>(0.42)</td>
<td>(0.03)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Rows with a hyphen indicate that the weather parameter was not significant ($P > 0.05$).

$^b$ Orthogonal polynomial functions of Julian date to model the natural rise and fall cycle of moth populations over time (Davidson and Andrewartha 1948).
**Table 3.** Partial $r^2$ values for multiple regressions fitted to the relationship between the numbers of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps and weather.

<table>
<thead>
<tr>
<th>Year</th>
<th>Flight</th>
<th>Air temp.</th>
<th>RH</th>
<th>Wind speed</th>
<th>Rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>1</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.24</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Rows with a hyphen indicate that the weather parameter was not significant ($P > 0.05$).
**Fig. 1.** Three trap designs used to examine the relative efficiency of traps in capturing *Ostrinia nubilalis* moths in 2003 and 2004: a) the Intercept™ wing trap (wing trap), b) the Intercept™ bucket/funnel UNI trap (bucket trap), and c) the Hartstack wire-mesh, 75-cm diameter cone trap (large metal cone trap) (Hartstack et al. 1979). In 2005, the large metal cone trap was compared to: d) a modified Hartstack wire-mesh, 35-cm diameter cone trap (small metal cone trap), and e) the *Heliothis*, 35-cm diameter cone trap (small nylon cone trap). All traps were baited with an Iowa-strain pheromone lure, changed biweekly.
Fig. 2. Mean number of *Ostrinia nubilalis* moths collected daily in Hartstack wire-mesh, 75-cm diameter cone traps per week.
Fig. 3. Mean (±SE) number of *Ostrinia nubilalis* moths collected daily with different pheromone-baited trap designs in a) 2003 and 2004 (pooled), and b) 2005.
Fig. 4. Mean (±SE) number of *Ostrinia nubilalis* moths collected daily in pheromone-baited, Hartstack wire-mesh, 75-cm diameter cone traps in different locations relative to a windbreak at different wind speeds in 2003, 2004, and 2005. Captures at different trap locations within wind speed category with different letters were significantly different based on least-squares differences of means ($P < 0.05$).
CHAPTER 4. DISPER S A L OF NE WLY-ECLOSED EUROPEAN CORN BORER
MOTHS (LEPIDOPTERA: CRAM B IDAE) FROM CORN INTO SMALL-GRAIN
AGGREGATION PLOTS

A paper submitted to the Journal of Economic Entomology

Brendon J. Reardon, Douglas V. Sumerford, and Thomas W. Sappington

Abstract

Genetically-modified, insecticidal corn hybrids (Bt corn) are used throughout the US Corn Belt for European corn borer, Ostrinia nubilalis (Hübner), control. To slow development of Bt-corn resistance, the EPA requires provision of nearby refugia. Determining the appropriate distance between a refuge and Bt corn, and development of mitigation-remediation strategies such as mass releases of susceptible moths, requires an understanding of adult dispersal and mating behavior. However, much remains unknown about these behaviors. Because mating often occurs in grass near cornfields where moths aggregate, we planted small-grain plots as aggregation sites in an attempt to retain mass-released moths. The objectives of this study were to examine influences of pheromone lure, plant density, and plant species on distributions of feral and newly-emerged, laboratory-reared O. nubilalis among small-grain aggregation plots. Feral moths were collected in aggregation plots in relative abundance, indicating that small-grain plots were acceptable aggregation sites. In contrast, newly-emerged moths that were released weekly as dye-marked pupae were rarely found in aggregation plots, with ≈150–1,500-fold fewer moths captured than expected if all released moths had occupied the plots for ≥1 d. The majority of newly-emerged adults did not colonize the aggregation plots, suggesting that recently-eclosed adults leave their natal
field and do not colonize the first aggregation sites encountered. Plant species significantly influenced moth distributions among aggregation plots. Mass releases of laboratory-reared pupae in the field may not be a viable remediation tactic because almost all of the newly-emerged moths dispersed beyond 300 m of the release point.

**Introduction**

The European corn borer, *Ostrinia nubilalis* (Hübner), is a major pest of corn, *Zea mays* L., in most of North America east of the Rocky Mountains. Yield losses and control measures associated with *O. nubilalis* in the US have exceeded one billion dollars annually (Mason et al. 1996). Genetically-modified corn hybrids that express crystal protein endotoxin genes from *Bacillus thuringiensis* Berliner (*Bt* corn) (Koziel et al. 1993) for the control of *O. nubilalis* have been available commercially in the US since 1996, and are an increasingly popular and efficacious management tool (Pilcher et al. 2002). However, there is concern that resistance to *Bt* corn may develop in *O. nubilalis*, which would reduce the effective life of this transgenic technology.

The insect resistance management (IRM) strategy for *O. nubilalis* on *Bt* corn is predicated on the expression of a high dose of *Bt* toxin in the plants, and the provision of untreated, non-transgenic refugia planted within a prescribed distance from stands of *Bt* corn (EPA 1998, 2001). High doses of *Bt* toxin increase the likelihood of mortality of larvae carrying a single resistance allele. The rationale for the refuge requirement is to facilitate mating between resistant moths that might be emerging from a *Bt* cornfield and susceptible moths emerging from a nearby refuge, thus diluting the frequency of resistance alleles in the *O. nubilalis* population and promoting the production of heterozygous progeny, which are
susceptible to *Bt* corn (Taylor and Georghiou 1979, Alstad and Andow 1995, Ostlie et al. 1997, Onstad and Gould 1998, Caprio 2001). The appropriate distance between a refuge and *Bt* corn depends on dispersal behavior and the relative timing of mating behavior of *O. nubilalis* adults, but much remains unknown about these behaviors. If resistance to *Bt* corn becomes evident in an *O. nubilalis* population, manipulation of the spatial distribution of susceptible moths (wild or laboratory-reared) and resistant moths to promote interbreeding may reduce the frequency of resistance alleles and slow the spread of resistance in other populations. Further, knowledge of dispersal and mating dynamics of *O. nubilalis* will allow more realistic parameterization of IRM and integrated pest management (IPM) models.

Caffrey and Worthley (1927) first reported that *O. nubilalis* moths were found in dense vegetation outside agricultural crops during the day. Although *O. nubilalis* adults may prefer to remain in irrigated cornfields in the western Corn Belt (Hunt et al. 2001), they usually occupy areas of dense grass in the Midwest (Showers et al. 1976, DeRozari et al. 1977). These aggregation sites are where mating behaviors occur, such as emission of pheromone and copulation (Klun 1968, DeRozari et al. 1977). Showers et al. (1976, 1980) proposed a scenario for *O. nubilalis* mating dynamics. Once *O. nubilalis* females emerge, they leave the cornfield and fly to nearby aggregation sites to imbibe freestanding water and seek mates. On the nights after mating, the females move between cornfields to oviposit and aggregation sites to rest. Conversely, according to the paradigm, males do not move as frequently as females from aggregation sites. After emerging and leaving corn, males go to nearby aggregation sites to mate, and remain there until the female population in that site is sparse. Males then disperse to another aggregation site to seek mates.
A variety of factors have been proposed to govern the selection of aggregation sites, including presence of dew droplets, temperature, humidity, distance from corn stands, plant species, and plant architecture (Showers et al. 1976, DeRozari et al. 1977, Hellmich et al. 1998, Pleasants and Bitzer 1999, Showers et al. 2001, Anderson et al. 2003, Sappington 2005). Hellmich et al. (1998) found that several grain crops including oats (Avena spp.) attracted moths, particularly when the grains were planted at high densities. Anderson et al. (2003) compared the preference of O. nubilalis to several millets and reported that proso millet, Panicum miliaceum L., was the most preferred by all life stages, but Siberian foxtail millet, Setaria italica (L.), harbored the greatest number of adults. Sappington (2005) found that aggregation sites proximal to corn harbored greater moth densities than sites not adjacent to corn. Showers et al. (2001) recovered the greatest number of marked moths from habitats consisting of a mix of brome grass (Bromus spp.) and giant foxtail grass (Setaria spp.) that was 65–70 cm in height and 5–20 m from the site of release. Although brome grass is an important component of aggregation sites for adults during the first flight, which occurs in spring and early summer, it senesces during midsummer and is relatively unattractive to moths of the second flight (Showers et al. 2001). Typically, giant foxtail grass is attractive to moths later in the summer (Pleasants and Bitzer 1999).

Learning enough about O. nubilalis dispersal and aggregation behavior so that it can be manipulated for IPM and IRM goals will require more thorough experimental probing. In this study, we provided a variety of potential aggregation sites to newly-emerged moths by planting plots of various small-grains (Cyperales) near the natal corn plot. Our goal was to determine if we could create conditions suitable to retain laboratory-reared moths near their
emergence site when taken to the field as pupae. Success would provide a potential means of introducing large numbers of unmated, Bt-susceptible moths to an area where resistance alleles are increasing in frequency. We also expected that the results would provide insight into *O. nubilalis* dispersal behavior in general. The specific objectives of this study were to examine the influences of pheromone lure, aggregation-site plant density, and aggregation-site plant species on the distributions of released and feral *O. nubilalis* adults among aggregation plots of small grains.

**Materials and Methods**

**Insect Culture.** Adult *O. nubilalis* collected from light traps during the summers of 2002 and 2003 were used to establish laboratory colonies at the Corn Insects and Crop Genetics Research Unit, Ames, IA, which were the source of marked insects employed in experiments in 2003 and 2004, respectively. Moths were reared following procedures similar to Raun (1961) and Guthrie et al. (1965). Standard meridic diet of wheat germ was prepared using methodology similar to Lewis and Lynch (1969). The larvae were allowed to feed ≈21 d on diet impregnated with either Solvent Blue 35 (Sudan Blue II, C.I. 306436; Sigma-Aldrich, Milwaukee, WI), to produce blue-marked moths, or Fat Red 7B (Sudan Red 7B, C.I. 201618), to produce red-marked moths. Consumption of the dye-containing diet stains the fat bodies of *O. nubilalis* larvae, and the mark is retained through adulthood. Using two dyes allowed simultaneous moth releases in overlapping study arenas. Ostlie et al. (1984) and Hunt et al. (2000) reported that these two dyes had negligible effects on moth biology. Each dish containing the diet and larvae was overlaid with a ring of corrugated cardboard. After the ultimate larval molt, larvae colonized the cells in the cardboard rings for pupation and
adult eclosion. A total of 600–800 pupae per ring is typical, and the sex ratio of females to males is about 1:1 (Guthrie et al. 1985). Larvae were reared in an environmentally controlled room at 27°C, 16:8 (L:D), and 80% RH until pupation. Rings of pupae were placed in experimental corn plots from which marked moths emerged and dispersed. Releases were conducted during the natural moth flights. *Ostrinia nubilalis* is bivoltine in much of the Corn Belt, including Iowa (Mason et al. 1996). The first flight usually begins in mid-May, and the second flight in mid-July. Each flight typically lasts 3–6 wk, but the second flight is longer generally.

**2003 Experimental Design.** Study arenas were established in Boone Co., IA, on two different farms. Using recommendations for tillage and fertilization regimes for Iowa producers (Hansen 1992, Farnham 2001, Lang 2001), two 0.81-ha. corn plots were planted to ‘Pioneer 34B62RR’ on each farm on 19 May. The density of plants in each corn plot was 69,160 plants per ha. In 2003, both farms received pre-emergence applications of herbicides (dicamba, glyphosate, isoxaflutole, and metolachlor) to eliminate weeds and potential moth aggregation sites from the corn plots, and a soil insecticide (chlorpyrifos) to control corn rootworms (*Diabrotica spp.*) (Coleoptera: Chrysomelidae). The corn plots were flanked on two sides by three tiers of aggregation plots, and the distance from the center of a given corn plot to the proximal edge of each flanking tier was 37, 87, and 137, or >137 m, respectively (Fig. 1); the >137-m distance corresponds to all tiers at a farm beyond the second corn plot. Tiers were planted at different distances to examine the influence of distance from point of emergence on moth propensity to colonize aggregation plots. Each set of three tiers constituted a block, with a tier comprised of four 20x20-m aggregation plots separated by
alleyways that were 14–20 m wide (Fig. 1). Weeds in the alleyways were kept to a minimum by tilling and mowing. Within a tier, the four aggregation plots were planted to ‘Oxen’ spring wheat, _Triticum aestivum_ L., at either the standard rate (75 kg per ha.) or twice the standard rate (150 kg per ha.). The tiers of aggregation plots at one farm were planted to wheat on 3 April, and the tiers at the second farm were planted on 14 April. These plots were designed to be used as aggregation sites during the time of the natural first-flight of moths. Half of the aggregation plots were spiked with a pheromone lure (Trécé Inc., Adair, OK) impregnated with a mixture of 97% cis-11-tetradecenyl acetate and 3% trans-11-tetradecenyl acetate, which attracts the Z- or cis-strain of _O. nubilalis_, the strain inhabiting Iowa. The pheromone lures were placed in the middle of the aggregation plots and suspended from a metal rod 20 cm above the ground. Pheromone lures were replaced biweekly, and plots within each tier were assigned randomly to plant density and pheromone lure treatments.

Unfavorable weather conditions prevented timely planting of small-grains for the second _O. nubilalis_ flight of 2003. Visual appearance of the plots suggested they would not be suitable for moth aggregation because the plots were contaminated with native weed species and the small-grain stands were not homogenous within a plot. Few to zero marked and feral moths were collected from the second-flight aggregation plots, confirming that the condition of the plots at the time of our releases was considered unfavorable for _O. nubilalis_ aggregation. Therefore, data from the second _O. nubilalis_ flight of 2003 were not included in any of the analyses.

During the first flight of 2003, eight cardboard rings infested with marked pupae of one color were placed weekly in each corn plot on a given farm. The other corn plot at that
farm received eight rings of pupae of the other color (Fig. 1). The rings were placed near the center of the corn plots in the late afternoon. The number of pupae per ring was estimated by counting the number of pupae in two rings per color per farm on three release dates. The proportion of moths that successfully emerged during a release was estimated as the number of empty pupal cases per total number of pupae in a ring, and for releases where no emergence or pupae-per-ring data were taken, the mean of the three release dates was used. The rings were suspended ≈20 cm from the surface of the ground in a 61x61x61-cm wire-mesh cage, which had two 20x58-cm openings in the top of the sides to allow emergence of moths. Cage tops were covered with plywood or rubber mats to reduce the potential of thermal damage and desiccation of the moths induced by direct sunlight. The cages remained in the corn plots for 7 d, and five releases were made in 2003.

2004 Experimental Design. Study arenas were established in Story Co., IA, on two different farms. Corn plots were planted to ‘Dekalb DKC63-80AR RR’ on each farm on 26 April. Both farms received a pre-emergence application of herbicides (dicamba, glyphosate, isoxaflutole, and metolachlor). The corn plots were flanked on both sides by three tiers of aggregation plots, similar to the arrangement used in 2003 (Fig. 1). However, for the second flight of 2004, the aggregation-plot dimensions were 20x10-m, and they were situated between the 20x20-m aggregation plots used for the first flight. The difference in design improved planting logistics associated with preparing second-flight aggregation plots. Two 20x20-m plots per tier on both farms were planted on 14–15 April to ‘Oxen’ spring wheat at twice the standard rate (150 kg per ha.). The other two aggregation plots per tier were planted on both farms on 14–15 April to ‘Jerry’ oats, *Avena sativa* (L.), at twice the standard
density (180 kg per ha.). During the week of 27 June, the first-flight aggregation plots of
senescing wheat and oats were cut and baled. For the second flight, two 20x10-m wheat
plots per tier on both farms were planted at twice the standard density on 9 June. The other
two aggregation plots per tier on both farms were planted on 9 June to ‘Manta’ Siberian
foxtail hay millet, *Setaria italica* L., at twice the standard density (45 kg per ha.). Half of the
aggregation plots per moth flight per plant species were spiked with pheromone lure.
Aggregation plots within each tier were assigned randomly to plant species and pheromone
lure treatments per moth flight.

During the first and second natural moth flights of 2004, 15 cardboard rings infested
with marked pupae were placed in each corn plot weekly as described for 2003. The number
of pupae per ring was estimated on four release dates, and the number of rings was increased
in 2004 to increase the number of released moths, and therefore improve collection rates.
The proportion of moths that successfully emerged during a release was estimated for four
releases. For releases where no emergence or pupae-per-ring data were taken, the mean of
the four releases was used. The wire-mesh cages used to house the rings of pupae were left
in the corn plots for 3 d instead of 7 d so that the released cohorts were more homogenous in
age. Seven moth releases were made in 2004.

**Data Collection.** The aggregation plots were sampled five days per week, beginning
the day marked pupae were placed in the corn plots. Each aggregation plot was sampled with
two sampling techniques: A heavy-duty, 0.38-m-diameter sweep net (Bioquip Products, Inc.,
Rancho Dominguez, CA) was used to collect moths so that their origin (laboratory or feral),
sex, and the mating status of females (Showers et al. 1974) could be determined. Counts of
feral moths were important in determining which plots were acceptable to *O. nubilalis* as aggregation sites. On each sample date, each aggregation plot was swept 30 times over a distance of about 30 m before the contents of the net were assessed. Different paths were taken through the plots during sweep sampling each day to reduce damage to the grass.

Second, a flush bar technique (Sappington and Showers 1983a) was used to increase the area of aggregation plots sampled, and to facilitate the determination of the suitability of the small-grain plots as aggregation sites. A 1.5-m plastic rod was used to disturb the grass along a randomly-selected, 20-m edge of each aggregation plot on each sample date. Flushed moths were counted on the wing, although moth sex and origin could not be determined.

Observations of moths remaining in the corn plots were taken about twice per release in 2004 (*n* ≈ 14 per farm). Along the edge of each corn plot that flanked the aggregation plots, and along the middle rows of each corn plot, 50-m lengths of row were visually inspected to confirm that moths were dispersing and not colonizing the corn plots at each farm. An additional 50-m section of native grasses along the edge of each farm in 2004 was swept for moths to determine the density of moths outside of the immediate vicinity of the study arena.

**Statistical Analyses.** The number of marked moths of each color dispersing per release was estimated as the product of the number of rings placed at a farm (*n* = 8 or 15), the proportion of estimated emergence, and the estimated number of pupae per ring. The expected number of moths per aggregation plot was calculated under the scenario that all marked moths that dispersed from the pupal rings occupied only the aggregation plots and were distributed uniformly within them. Thus, the expected mean number of released moths
occupying each plot was calculated by dividing the number of marked moths of a given color released at a farm by the number of aggregation plots at the farm (n = 36). That quotient was multiplied by the fraction of area of an aggregation plot that was sampled with the sweep net on a sample date (=12%) to give the number of marked moths expected to be captured by the sweep net. An analysis of variance (restricted maximum likelihood estimates; REML-ANOVA) was used to determine if the observed mean number of marked moths swept per plot per release was different than the number of marked moths expected to be captured per plot (PROC MIXED, SAS Institute 2001). The dependent variable in the model was the mean number of moths swept, and the fixed effect was moth category (expected, observed). The random effects in the model were year, release date, sample date, block, and treatment (plant density, plant species, and pheromone lure). The moth-category means were separated using the LSMEANS option (SAS Institute 2001).

To test the effect of distance of aggregation plot from point of moth release on the mean number of marked moths recovered, a mixed model was used (PROC MIXED, SAS Institute, 2001). The analyses were applied to both sexes combined and to each sex separately, and the four levels of the fixed factor corresponded to the four distances of the tiers of aggregation plots from the point of moth emergence in the corn plots (distance: 37, 87, 137, and >137 m). Year, flight, and field were the random parameters in the models, and fixed-effect means were separated with the LSMEANS option (SAS Institute 2001).

The influence of plant density and pheromone lure on the combined mean number of marked and feral moths collected with the sweep net in wheat plots during the first flight in 2003 was assessed with REML-ANOVA (PROC MIXED, SAS Institute 2001). The
dependent variable in the model was the mean number of total moths collected per aggregation plot, and the fixed effect was treatment (standard plant density with lure, standard plant density without lure, double plant density with lure, and double plant density without lure). Block and release date were random effects in the model. The treatment means were separated using the LSMEANS option (SAS Institute 2001). Linear contrast statements were used to examine the impact of plant density, pheromone lure, and the interaction of plant density and pheromone lure. Similar models were assessed for the mean number of male moths swept per aggregation plot, mean number of female moths swept per aggregation plot, and mean number of moths flushed per aggregation plot.

The REML-ANOVA models applied to the 2004 data were akin to the models used in the analyses of the first-flight data of 2003. However, for the first flight of 2004, the levels of the fixed (treatment) effect were wheat with lure, wheat without lure, oats with lure, and oats without lure. The treatment levels of the second flight of 2004 were millet with lure, millet without lure, wheat with lure, and wheat without lure. For the data of each flight of 2004, linear contrasts were used to separate the influence of plant species, pheromone lure, and their interaction.

The relationship between the mean number of moths swept and sex was examined using REML-ANOVA (PROC MIXED, SAS Institute 2001). The dependent variable in the model was the combined mean number of marked and feral moths swept per aggregation plot, and the fixed effect was sex. The random effects in the model were year, release date, block, and treatment (plant species, plant density, and pheromone lure). The sex means were separated using the LSMEANS option (SAS Institute 2001). A similar model was used to
assess the interaction between sex and treatment (plant species, plant density, and pheromone lure). The fixed effects in this case, however, were sex, treatment, and the interaction of sex and treatment. The random effects were year, release date, and block.

All of the data were transformed to ln (X + 0.5) for all of the analyses to meet the assumptions of homoscedasticity and normality (Ott and Longnecker 2001), but the results are presented as back-transformed data. Assumptions were assessed with residual plots and normal plots (PROC UNIVARIATE, SAS Institute 2001).

**Results**

An estimated total of ≈215,900 artificially-marked adults were released over the two years of the experiment. Of these, 103 marked adults (0.048%) were recovered in the aggregation plots with the sweep net. Of the estimated ≈102,500 red-marked moths released, 42 (0.041%) were recovered with the sweep net. Nearly 93% of the recovered red adults were male. One of the three red females recovered had not mated and two had mated within 48 h of capture, according to the presence and melanization of the spermatophore (Showers et al. 1974). About 113,437 blue-marked moths were released, and 61 (0.054%) were recovered with the sweep net. Eighty-four percent of recovered blue adults were male. Two of the females recovered were unmated, and eight had mated within 48 h of collection.

Observations in 2004 in the corn plots and sweep net samples in the native grasses at the periphery of the study arenas revealed no moths, suggesting that few in any of the marked moths colonized those areas.

During the first flight of 2003 and the flights of 2004, feral moths were found in the small-grain aggregation plots, outnumbering marked moths by about 10:1. Over the course
of the study, 1,033 feral moths were collected with the sweep net, 61% of which were males. Of the 408 females collected, 52 were unmated (13%), 348 had mated within 2 d of capture (85%), and the remaining eight (<1%) had mated more than 2 d previously. The presence of feral moths served as a positive control, demonstrating that the aggregation plots were adequate to function as such. The densities of moths flushed from the grassy plots (Figs. 3–5) were within a factor of 10 of densities documented in natural aggregation sites in other studies (Showers et al. 1976, Sappington and Showers 1983a, Sappington and Showers 1983b, Hellmich et al. 1998, Sappington 2005), so we conclude that the small-grain aggregation plots were generally suitable as aggregation areas.

The difference between the mean number of marked moths collected with the sweep net per aggregation plot and the expected number of moths recovered per aggregation plot was significant \( F = 53,458.6; \) \( \text{df} = 1, 2077; \) \( P < 0.0001 \). Fewer marked moths were collected by sweep net than were expected during every release, with the difference ranging from 154–1,580 fold (Table 1), indicating that much less than 1% of the newly-emerged moths colonized the nearby aggregation plots for even one day.

The relationship between distance from point of eclosion and the mean number of marked moths recovered was not significant (Fig. 2) for sexes combined \( F = 0.71; \text{df} = 3, 13.8; \) \( P = 0.5643 \), males only \( F = 1.16; \text{df} = 3, 12.8; \) \( P = 0.3612 \), or females only \( F = 1.39; \text{df} = 3, 14.4; \) \( P = 0.2848 \).

Over the first flight of 2003, the influence of treatment (plant density and pheromone lure) on the mean number of males collected with the sweep net was not significant \( F = 1.18; \text{df} = 3, 107; \) \( P = 0.3201 \) (Fig. 3). The mean number of females collected with the
sweep net was not statistically related to treatment ($F = 1.46; \text{df} = 3, 107; P = 0.2307$) (Fig. 3). When the male data and female data were combined, the relationship between treatment and the mean number of moths collected in the sweep net was not significant ($F = 2.35; \text{df} = 3, 107; P = 0.0763$) (Fig. 3). Similarly, the relationship between the mean number of moths flushed and treatment was not significant during the first flight of 2003 ($F = 2.07; \text{df} = 3, 84; P = 0.1098$) (Fig. 3).

In 2004, the relationship between the mean number of male moths collected with the sweep net and treatment (plant species and pheromone lure) was significant during the first flight ($F = 5.46; \text{df} = 3, 84; P = 0.0018$) (Fig. 4). Linear contrasts of the first-flight data indicated that both plant species ($F = 10.08; \text{df} = 1, 84; P = 0.0021$) and pheromone lure ($F = 5.10; \text{df} = 1, 84; P = 0.0265$) significantly influenced the number of male moths collected, although the interaction between the two factors was not significant ($P = 0.2760$). More males were swept from plots of oats than spring wheat plots, and pheromone-spiked plots harbored more males than plots without lure. The relationship between the mean number of females swept and treatment was not significant ($F = 2.16; \text{df} = 3, 89; P = 0.0984$) (Fig. 4). When the male and female data of first flight of 2004 were combined, the relationship between treatment and the mean number of moths collected was significant ($F = 4.87; \text{df} = 3, 89; P = 0.0035$) (Fig. 4). Linear contrasts indicated that the effect of plant species was significant ($F = 12.14; \text{df} = 1, 89; P = 0.0008$), and more moths were collected from oat plots than spring wheat plots. However, neither pheromone lure ($P = 0.1190$) nor the interaction of lure and plant species ($P = 0.9983$) was significant. The relationship between the mean
number of moths flushed and treatment was not significant ($F = 1.50$; $df = 3, 84$; $P = 0.2202$) (Fig. 4).

During the second flight of 2004, the relationship between the mean number of males collected and treatment (plant species and pheromone lure) was significant ($F = 10.04$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). Linear contrasts showed that the effect of plant species was significant ($F = 29.99$; $df = 1, 43$; $P < 0.0001$), with foxtail millet being much more attractive than spring wheat. The effects of pheromone lure ($P = 0.7727$) and the interaction of lure and plant species ($P = 0.8709$) were not significant. The mean number of females was significantly affected by treatment level ($F = 12.31$; $df = 3, 43$; $P < 0.0001$) (Fig. 5), and the linear contrasts indicated significant differences between the plant species ($F = 36.91$; $df = 1, 43$; $P < 0.0001$). More females were collected from plots of foxtail millet than spring wheat plots. However, neither pheromone lure ($P = 0.9064$) nor the interaction between plant species and lure ($P = 0.9145$) were significant. When the male and female data were combined, the influence of treatment on the mean number of moths swept was significant ($F = 20.72$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). Linear contrasts indicated that plant species was significant ($F = 62.10$; $df = 1, 43$; $P < 0.0001$) and the moths favored foxtail millet plots over spring wheat plots, but pheromone lure ($P = 0.9948$) and the interaction of the two factors ($P = 0.7918$) were not significant. The relationship between the mean number of moths flushed and treatment was significant ($F = 16.27$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). The linear contrast indicated that plant species was significant ($F = 48.57$; $df = 1, 43$; $P < 0.0001$), and the moths preferentially colonized foxtail millet plots over spring wheat plots. However, the mean
number of moths flushed was neither affected by pheromone lure \((P = 0.8021)\) nor the interaction between plant species and lure \((P = 0.6854)\).

Overall, about two-fold more male than female moths were collected with the sweep net \((F = 27.42; \text{df} = 1, 505; P < 0.0001)\). When the interaction between sex and pheromone lure was examined with a type-three test of fixed effects, no significant relationship was noted \((F = 0.83; \text{df} = 9, 498; P = 0.5880)\), notwithstanding that the pheromone lure mimics female-produced pheromone (Klun and Brindley 1970), and is attractive to males only.

**Discussion**

Dispersal behavior, and its relation to timing of mating, has important implications for IRM modeling and strategies because patterns of dispersal directly affect spatial patterns of gene flow (Mallet 2001). The current Bt-IRM strategy for *O. nubilalis* depends critically on the assumption that any Bt-resistant moths emerging from Bt corn are likely to encounter and mate with susceptible moths emerging from nearby refugia, which growers are required to plant within 800 m of the Bt corn (EPA 2001). Despite the importance of understanding *O. nubilalis* dispersal behavior in this context, very little is known about it. *Ostrinia nubilalis* moths have been documented traveling nearly 45 km across Lake Erie (Caffrey and Worthley 1927). Extrapolation from range expansion data suggests that moths can move 80 km annually (Chiang 1972) or 34 km per generation (Showers 1979). Likewise, Showers et al. (2001) recovered marked moths 23–49 km from their point of release, and found that some moths moved more than 14 km within only 100 min. Yet the proportion of a population that disperses such distances is unknown, as are the average dispersal distance per generation and its timing. Our experiments were designed based on the same assumption
inherent in the current IRM strategy for *O. nubilalis*, namely that newly-emerged moths aggregate and mate in grassy sites near the natal cornfield. However, our results were unexpected in that only a small fraction of newly-emerged moths colonized nearby sites, and this raises new questions about *O. nubilalis* dispersal behavior. The small-grain species used in this study, and the dates and rates at which the aggregation plots were planted, resulted in generally suitable aggregation sites for *O. nubilalis* adults. This is evidenced by the relatively large number of feral moths collected from these sites and by the numbers of moths flushed from them, which were comparable within an order of magnitude to the numbers reported in previous studies (Showers et al. 1976, Sappington and Showers 1983a, Sappington and Showers 1983b, Hellmich et al. 1998, Sappington 2005). However, the natal fields from which the feral moths originated, and their distance from the study arenas, cannot be determined. Showers et al. (2001) suggested that the distance from the point of moth release was a significant factor in recapture rate in pheromone traps, and that male moths were often recovered ≥800 m from the release point. Conversely, Hunt et al (2001) found no differences in the number of moths captured in pheromone-baited traps at different distances within 200 m of the moth-release site. We also found no differences in the number of moths recovered in the grass at different distances within 300 m of the emergence site (Fig. 2).

Despite their suitability as aggregation areas, many-fold fewer marked moths than hypothesized were recovered from the aggregation plots (Table 1). This suggests that as newly-emerged moths disperse from their natal field, they do not colonize the first grassy sites encountered that are suitable for aggregation. Rather, our data indicate that most newly-eclosed adults disperse beyond at least 300 m before colonizing grassy sites and mating.
Showers et al. (1976) found that within 1 d adult moths dispersed from grass plots where they were released. Similarly, Showers et al. (2001) noted that a small proportion of the moths released in their study remained near the point of release, but they dispersed within 1–2 d. Surveys of first-flight moth distribution among grassy ditches in central Iowa suggest that although some moths may colonize the grass adjacent to their presumed natal cornfields, they do not remain there long (Sappington 2005). Our findings, along with evidence from previous studies (Showers et al. 1976, Showers et al. 2001, Sappington 2005), imply that *O. nubilalis* may undergo an obligate dispersal phase soon after emergence, as is the case for many insects (Johnson 1969). The existence and nature of such a phase will require more direct experimentation.

Aggregation sites are an important component of *O. nubilalis* ecology, and it is important to understand them in an IRM context because mating often occurs there (DeRozari et al. 1977). Although few marked moths were recovered in this experiment, many feral moths were collected and differences in moth spatial distributions were noted among the aggregation plots. The initial hypothesis was that within the confines of less than 300 m we could manipulate moth aggregations nearby the natal cornfield to ultimately foster allele flow, but we found that the vast majority of the newly-emerged moths dispersed beyond the immediate vicinity of the natal field. We were, however, able to successfully manipulate where feral moths aggregate by using different plant densities and plant species. High-density grass plots harbored the most moths numerically during the first flight of 2003 (Fig. 3). Although the influence of plant species on the number of moths swept from aggregation plots was not compared across flights because of confounding and changing
moth population sizes, the effect of plant species was significant within a flight, and oats and foxtail millet were preferred as aggregation sites over spring wheat (Figs. 4 and 5). Hellmich et al. (1998) found that double density oats harbored the highest number of adult moths when compared to regular density plots of oats and high and low densities of other small-grains. It is likely that the higher-density sites’ microclimate is more attractive to discerning moths, and that some plant species at certain densities are more suited to fostering ideal microclimatic conditions than others.

Because the reproduction strategy of *O. nubilalis*, like most Lepidoptera, is sexual (Normark 2003), the flight behavior of males and females may differ. Both sexual dimorphism and behavioral differences between the sexes may lead to differences in the spatial distribution of sexes. Showers et al. (1976, 1980) proposed that male moths spend less time in cornfields than females, and that gravid, mated females leave aggregation sites to oviposit, whereas males move among aggregation sites to find females. We found that more marked males than females colonized aggregation sites, even though we released approximately equal proportions of each sex. A similar phenomenon was observed with feral moths. Among the feral moths collected, about 61% were male, and 84–92% of the marked moths recovered were male. The findings of Showers et al. (1976, 1980) and Sappington and Showers (1983c) parallel our findings in concluding that few females reside within aggregation sites more than 2 d post-mating. Conversely, <1% of the females that we found in aggregation plots had been mated more than 48 h at the time of collection. The paucity of older females (classes 3 and 4) in the aggregation sites suggests that females leave soon after they have mated, presumably to oviposit, and that few return to the aggregation sites.
If Bt-resistant moths are ever detected, mitigation-remediation tactics must be implemented to sustain the efficacy of the insecticidal, transgenic plants. Andow and Ives (2002) suggested possible tactics for reducing the frequency of resistance alleles in the field, including creating super-refugia for susceptible moths, releasing artificially-reared moths into the field, manipulating refugia spatial patterns relative to Bt corn, and using pheromone lures to modulate susceptible male movements in and around Bt cornfields. If resistance is detected in the field and laboratory-reared susceptible moths are mass-released in that area to mate with the resistant moths, it is imperative that the former remain in the area long enough to mate. Our results indicate that mass releases of laboratory-reared, susceptible O. nubilalis via placement of pupal rings in the field may not be a viable remediation tactic because almost all of the moths likely dispersed beyond the immediate vicinity (i.e., \( \leq 300 \text{ m} \)) of the release point. It will be important to more accurately clarify the spatial and temporal distribution of moths relative to the natal cornfield. Furthermore, pheromone lures did not consistently increase male concentrations in aggregation sites, as was originally hypothesized (Figs. 3–5).

Moth age or reproductive development may influence the likelihood or timing of dispersal (Johnson 1969). Although O. nubilalis females generally are not polyandrous, males are promiscuous (Pesho 1961, Loughner 1971, Showers et al. 1974, Elliott 1977, Royer and McNeil 1993, XiangQuan et al. 2004). Laboratory-reared, mated males may not be as prone to disperse from their point of release as were the newly-emerged, unmated males in this study. If true, this would make mated males better candidates for release into an area where Bt resistance has been detected in a local O. nubilalis population. Releasing females
that have mated may not be a viable option as a mitigation-remediation strategy because females generally will not mate again, although 20–30% multiple-mated females have been reported from light trap captures in Ontario and Pennsylvania (Elliott 1977, XiangQuan et al. 2004). Because moth age is correlated to their sexual maturation (XiangQuan et al. 2004), older moths may be more likely to remain in the area of release than younger moths because they are sexually mature and prepared to mate. Studies are underway to examine the influences of age and mating status on dispersal of released moths.

Acknowledgments

We are grateful for the field assistance received from Dr. E. Berry, N. Passolano, J. Gibson, R. Ritland, M. Fiscus, D. Starret, K. Reardon, A. Kronback, D. Niedermann, D. Crosman, and B. Hibbing. Further, we are indebted to J. Dyer for her assistance in the laboratory. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

References Cited


Table 1. Expected and observed mean number of marked *Ostrinia nubilalis* adults recovered from aggregation plots with a sweep net within five days of moth release.

<table>
<thead>
<tr>
<th>Year</th>
<th>Flight</th>
<th>Release Date</th>
<th>Expected $\bar{X}$ per Aggregation Plot</th>
<th>Observed $\bar{X}$ (SE) per Aggregation Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>1</td>
<td>2 June</td>
<td>6.70</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9 June</td>
<td>6.70</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>17 June</td>
<td>6.55</td>
<td>0.04 (0.02)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>24 June</td>
<td>5.50</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2 July</td>
<td>8.23</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>20 May</td>
<td>8.15</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3 June</td>
<td>9.25</td>
<td>0.06 (0.01)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8 June</td>
<td>6.35</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15 June</td>
<td>8.15</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>27 July</td>
<td>16.20</td>
<td>0.09 (0.02)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5 August</td>
<td>11.63</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10 August</td>
<td>15.80</td>
<td>0.01 (0.02)</td>
</tr>
</tbody>
</table>
Fig. 1. Schematic of the study arena at each farm for release of marked *Ostrinia nubilalis* moths via pupal rings. The numbers within the squares correspond to randomly-assigned treatments in small-grain aggregation plots. Areas between aggregation plots were fallow alleyways.
Fig. 2. Mean (±SE) number of marked *Ostrinia nubilalis* adults collected by sweep net per aggregation plot per sample date at four distances from the point of moth emergence from pupal rings.
Fig. 3. Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the first flight of 2003.
Fig. 4. Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the first flight of 2004.
Fig. 5. Mean (±SE) number of *Ostrinia nubilalis* collected per aggregation plot per sample date during the second flight of 2004.
CHAPTER 5. SUMMARY AND GENERAL CONCLUSIONS

The European corn borer, *Ostrinia nubilalis* (Hübner), injures corn leaves, stalks, shanks, and ears when it is in the larval stage. Genetically-modified corn hybrids that express a gene from a toxic bacterium are used to manage *O. nubilalis* in North America. *Ostrinia nubilalis* also are vulnerable to a common pathogen, *Nosema pyrausta* (Paillot). We examined the effect of feeding artificial diet and the toxin found in genetically modified corn on growth, development, and survival of *Nosema*-infected and uninfected *O. nubilalis* larvae. Infected larvae developed more slowly than uninfected larvae. Increasing the concentration of toxin in diet reduced larval development, and the reduction in development was increased by disease. Infected larvae weighed significantly less than uninfected larvae. Infected larvae have a higher level of mortality to toxin than uninfected larvae. This work has implications for resistance management of *O. nubilalis* to genetically modified corn, particularly during monitoring, and demonstrates that it is important to determine whether disease-causing agents are present when testing the susceptibility of larvae to genetically modified corn hybrids. These results also provide a better understanding of the interactions of *O. nubilalis*, disease, and the toxin found in genetically modified corn.

Pheromone-baited traps are frequently used in ecological studies of *O. nubilalis.* However, interpretation of trap data is often confounded by factors other than natural changes in the size of the population. We examined the impact of trap design, trap location, and weather on moth captures in pheromone-baited traps. First, we examined the differences in *O. nubilalis*-moth captures among the Intercept™ wing trap, the Intercept™ bucket/funnel UNI trap, the Hartstack wire-mesh, 75-cm diameter cone trap (large metal cone trap), a
modified Hartstack wire-mesh, 35-cm diameter cone trap, and the nylon-mesh Heliothis, 35-cm diameter cone trap. Second, we examined the influence of the location of the large metal cone trap relative to a windbreak on the number of moths captured. And third, we examined the relationship between nightly mean air temperature, relative humidity, wind speed, precipitation, and the numbers of moths captured in the large metal cone traps. The large metal cone traps proved to be the most efficient trap design assessed because it captured the most moths consistently throughout the studies regardless of the moth population size. The location of the large metal cone trap relative to a windbreak influenced moth capture, and we found that most moths were collected from traps located leeward of a windbreak when nightly mean wind speeds were >7 kph. When winds were slower, most moths were collected from traps not located near a windbreak. Weather also influenced moth captures in the large metal cone traps. Nightly mean air temperature was the most influential factor in the multiple regression models, and positively influenced moth capture in traps. Nightly mean relative humidity was included in half of the multiple regressions. It too was positively related to moth capture. Nightly mean wind speed and nightly precipitation were included each once in separate regression models, and their impact on the number of moths collected in the large metal cone traps was negative and positive, respectively. It is critical that traps used to detect and monitor O. nubilalis are as sensitive as possible. Based on our findings, the large metal cone traps are recommended for this purpose because they were the most sensitive pheromone-baited trap evaluated. Although the large metal cone traps are no longer commercially available, the small metal and small nylon cone traps are the next logical alternative; however, they captured about 2-fold less than the large metal cone traps. The
wing traps and bucket traps are not recommended for *O. nubilalis* monitoring and trapping because they captured moths infrequently while nearby large metal cone traps captured many moths. We also found that trap location influenced the number of moths collected. As such, we recommend using a combination of trap locations near and away from windbreaks given the difference in sensitivity depending on wind speed on a given night. Changes in the numbers of moths captured on a given night were related to weather, but the relationship was generally weak, possibly because moth captures are influenced by variables not accounted for in the fitted multiple regressions.

There is concern that *O. nubilalis* may become resistant to *Bt* toxin, making *Bt* corn ineffective. To prevent this, a common non-*Bt* corn hybrid is planted as a refuge within a prescribed distance of *Bt* corn to allow survival of susceptible moths to mate with resistant moths. Mating often occurs in grassy areas near cornfields where moths aggregate. The appropriate distance between a refuge and *Bt* corn requires an understanding of moth movement and mating behavior, but much remains unknown. We collected wild moths in aggregation sites in relative abundance, indicating the sites were acceptable. In contrast, newly-emerged, artificially-marked moths that were released weekly were rarely found in the aggregation sites, suggesting that young moths fly away from their field of origin and do not land in the first acceptable aggregation sites encountered. The IRM plan for *O. nubilalis* is predicated, in part, on the unproved assumption that a preponderance of mating occurs in grassy areas in proximity to the natal cornfield. It is that presumption which is used to justify the mandated maximum distance between *Bt* cornfields and refugia. Our findings suggest that moths disperse from their natal field soon after or before mating. Subsequently, alleles
are dispersed spatially as the moths disperse. Based on moths recovered in our study, plant species significantly influenced moth distributions among aggregation sites. Understanding adult dispersal behavior is important because it directly affects mating patterns and the rate at which \textit{Bt}-resistance alleles move through a population. These results will help scientists design better refuge plans and better strategies for slowing the spread of resistance once it develops.